Technical report

Volume 2 – Appendixes

Prepared for
National Blood Authority

Project
Guideline for the prophylactic use of Rh D immunoglobulin in pregnancy care

Technical report prepared by
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Note

This volume presents the appendixes (Appendix A to Appendix F) to a systematic literature review on use of Rh D Immunoglobulin (Anti-D) in RhD negative pregnant women. Volume 1 presents the main body of evidence. Together the two volumes cover all research questions developed for this topic.

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Appendix A Literature search results

This appendix documents the literature search strategy for a systematic review on the prophylactic use of Rh D Immunoglobulin (Anti-D) in pregnant women.

A search strategy to address all questions was developed via Ovid for both Embase and MEDLINE. An additional search for studies reporting diagnostic accuracy specific to subquestion 3 was also conducted. Both search strategies were then translated for PubMed (limited to in-process citations and citations not indexed in MEDLINE) and CINHAL.

A1 Questions 1 to 4

A1.1 Embase

Table A.1 Search results Questions 1 to 4: Embase <1974 to 18 July 2018>

Search via Ovid for Level I, Level II and Level III studies conducted 19 July 2018.

#	Searches	Results
1	exp "obstetrics"/ or exp "obstetric care"/ or exp "pregnancy"/ or exp "pregnancy disorder"/ or exp "prenatal disorder"/	1138534
2	(obstetric or obstetrics or pregnancy or maternal).ti,ab,kw.	688903
3	(prepartum or pre partum or pre-partum or intrapartum or intra partum or intra-partum or perinatal or peri natal or peri-natal).ti,ab,kw.	98059
4	(antenatal or ante natal or ante-natal or prenatal or pre natal or pre-natal).ti,ab,kw.	151232
5	(postnatal or post natal or post-natal or postpartum or post partum or post-partum).ti,ab,kw.	194295
6	1 or 2 or 3 or 4 or 5	1434130
7	exp "fetus"/	189819
8	(fetu* or fetal* or f?etu* or f?etal*).ti,ab,kw.	375459
9	7 or 8	428990
10	exp alloimmunization/	4373
11	exp Rh Isoimmunization/	1604
12	(Rh* Isoimmuni?ation or Rh* D Isoimmuni?ation).ti,ab.	719
13	(Rh* alloimmuni?ation or Rh* D alloimmuni?ation).ti,ab.	381
14	(Rh* incompatibility or Rh* D incompatibility or blood group incompatibility).ti,ab.	1102
15	(((Rh* adj3 incompatib*) or Rh* D) adj3 incompatibl*).ti,ab.	203
16	((Rh or RhD or rhesus) adj5 sensiti*).ti,ab.	1325
17	((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) adj2 immuni?ation).ti,ab.	81
18	((rh or RhD or rhesus) adj2 (immuni?ation or autoimmuni?ation)).ti,ab.	862
19	10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18	9257
20	exp rhesus D antigen/	1158
21	rhesus D antigen.ti,ab.	55
22	rh* D antigen.ti,ab.	234
23	(RhD or rhesus D or Rh?D or Rh-?D or Rh D).ti,ab.	7552
24	(Rh-negative or Rh-positive).ti,ab.	1312
25	(Rhesus negative or Rhesus positive).ti,ab.	362
26	((rh or rhesus) adj2 (factor or factors or antigen$ or system or group)).ti,ab.	4806
27	20 or 21 or 22 or 23 or 24 or 25 or 26	12535
28	(Macaca mulatta or Simian Immunodeficiency Virus or zika or macaque?).ti,ab.	38080

29	27 not 28	12360
30	(isoimmuni?ation or alloimmuni?ation).ti,ab,kw.	5921
31	(isoimmuni* or iso-immuni* or isoimmune or iso-immune).ti,ab,kw.	2122
32	(alloimmuni* or allo-immuni* or alloimmune or allo-immune).ti,ab,kw.	11072
33	(unsensiti?ed or un-sensiti?ed or non-sensiti?ed).ti,ab,kw.	2409
34	(sensiti?ation* or sensiti?ed).ti,ab,kw.	119508
35	30 or 31 or 32 or 33 or 34	132314
36	exp Erythroblastosis, Fetal/	11405
37	((erythroblastoses or erythroblastosis) adj2 (fetal* or f?etal*)).ti,ab,kw.	1103
38	(h?emolytic disease* or h?emolytic disorder*).ti,ab,kw.	5204
39	(HDFN or HDN).ti,ab,kw.	1169
40	36 or 37 or 38 or 39	14943
41	6 or 9 or 19 or 29 or 35 or 40	1702755
42	exp Rh D immunoglobulin/	3931
43	exp Rho D Immune Globulin/	3931
44	exp "Rho(D) Immune Globulin"/	3931
45	exp anti-D immunoglobulin/	3931
46	Rh* D Immune Globulin.ti,ab.	93
47	(rh* immunoglobulin or rh* d immunoglobulin).ti,ab.	310
48	(rh* immuni?ation or rh* d immuni?ation).ti,ab.	574
49	42 or 43 or 44 or 45 or 46 or 47 or 48	4584
50	exp rhesus D antibody/	3931
51	rhesus D antibody.ti,ab.	11
52	(rh* D antibody or rh*D antibody).ti,ab.	108
53	(anti-D or anti D or anti?D).ti,ab.	4652
54	50 or 51 or 52 or 53	6309
55	exp rhogam/	3931
56	rhogam.ti,ab.	47
57	exp winrho/	3931
58	winrho.ti,ab.	64
59	exp rhophylac/	3931
60	rhophylac.ti,ab.	21
61	exp MICRhoGam/	3931
62	exp BayRHo-D/	3931
63	exp rhesonativ/	3931
64	'RhD immunoglobulin vf'.ti,ab.	0
65	55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64	3952
66	49 or 54 or 65	6855
67	41 and 66	4895
68	exp meta analysis/ or meta analysis.mp. or exp systematic review/ or systematic review.mp. or pooled analysis.mp. or ((exp review/ or review.mp.) and (systemat* or pool*).mp.)	424029
69	exp comparative study/ or comparative study.mp. or exp clinical trial/ or clinical trial.mp. or randomized controlled trial.mp. or randomi?ed controlled trial.mp. or exp randomized controlled trial/ or exp randomization/ or randomization.mp. or randomi?ation.mp. or exp single blind procedure/ or single blind procedure.mp. or exp double blind procedure/ or double blind procedure.mp. or exp triple blind procedure/ or triple blind procedure.mp. or exp crossover procedure/ or crossover procedure.mp. or exp placebo/ or placebo*.mp. or random*.mp. or rct.mp. or single blind.mp. or single blinded.mp. or double blind.mp. or double blinded.mp. or treble blind.mp. or triple blind.mp. or triple blinded.mp. or exp prospective study/ or prospective study.mp.	3989862
70	exp clinical study/ or exp case control study/ or exp family study/ or exp longitudinal study/ or exp retrospective study/ or exp cohort analysis/ or (cohort adj1 stud*).mp. or (case control adj1 stud*).mp. or	9057811

	(exp prospective study/ not randomi?ed controlled trials.mp.) or (follow up adj1 stud*).mp. or (observational adj1 stud*).mp. or (epidemiologic* adj1 stud*).mp. or (cross sectional adj1 stud*).mp.	
71	"case report"/	2319441
72	(editorial or letter or comment or historical article).pt.	1600355
73	71 or 72	3716272
74	(animals/ or nonhuman/) not humans/	6126128
75	(67 and 68) not (72 or 74)	69
76	(67 and 69) not (73 or 74 or 75)	470
77	(67 and 70) not (73 or 74 or 75 or 76)	990

A1.2 MEDLINE

Table A.2 Search results Questions 1 to 4: Medline <1946 to July 18, 2018>

Ovid MEDLINE(R) Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE and Versions(R) 1946 to July 18, 2018

Search via Ovid for Level I, Level II and Level III studies conducted 19 July 2018

#	Searches	Results
1	exp "obstetrics"/ or exp "obstetric care"/ or exp "pregnancy"/ or exp "pregnancy disorder"/ or exp "prenatal disorder"/	846826
2	(obstetric or obstetrics or pregnancy or maternal).ti,ab,kw.	542671
3	(prepartum or pre partum or pre-partum or intrapartum or intra partum or intra-partum or perinatal or peri natal or peri-natal).ti,ab,kw.	72542
4	(antenatal or ante natal or ante-natal or prenatal or pre natal or pre-natal).ti,ab,kw.	113427
5	(postnatal or post natal or post-natal or postpartum or post partum or post-partum).ti,ab,kw.	152703
6	1 or 2 or 3 or 4 or 5	1127944
7	exp "fetus"/	151495
8	(fetu* or fetal* or f?etu* or f?etal*).ti,ab,kw.	296734
9	7 or 8	368759
10	exp alloimmunization/	0
11	exp Rh Isoimmunization/	1672
12	(Rh* Isoimmuni?ation or Rh* D Isoimmuni?ation).ti,ab.	602
13	(Rh* alloimmuni?ation or Rh* D alloimmuni?ation).ti,ab.	215
14	(Rh* incompatibility or Rh* D incompatibility or blood group incompatibility).ti,ab.	910
15	(((Rh* adj3 incompatib*) or Rh* D) adj3 incompatibl*).ti,ab.	154
16	((Rh or RhD or rhesus) adj5 sensiti*).ti,ab.	1197
17	((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) adj2 immuni?ation).ti,ab.	78
18	((rh or RhD or rhesus) adj2 (immuni?ation or autoimmuni?ation)).ti,ab.	755
19	10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18	4742
20	exp rhesus D antigen/	0
21	rhesus D antigen.ti,ab.	37
22	rh* D antigen.ti,ab.	183
23	(RhD or rhesus D or Rh?D or Rh-?D or Rh D).ti,ab.	4497
24	(Rh-negative or Rh-positive).ti,ab.	951
25	(Rhesus negative or Rhesus positive).ti,ab.	241
26	((rh or rhesus) adj2 (factor or factors or antigen$ or system or group)).ti,ab.	3883
27	20 or 21 or 22 or 23 or 24 or 25 or 26	8684
28	(Macaca mulatta or Simian Immunodeficiency Virus or zika or macaque?).ti,ab.	32787
29	27 not 28	8527

#	Searches	Results
30	(isoimmuni?ation or alloimmuni?ation).ti,ab,kw.	3791
31	(isoimmuni* or iso-immuni* or isoimmune or iso-immune).ti,ab,kw.	2001
32	(alloimmuni* or allo-immuni* or alloimmune or allo-immune).ti,ab,kw.	6618
33	(unsensiti?ed or un-sensiti?ed or non-sensiti?ed).ti,ab,kw.	1631
34	(sensiti?ation* or sensiti?ed).ti,ab,kw.	90235
35	30 or 31 or 32 or 33 or 34	98707
36	exp Erythroblastosis, Fetal/	11582
37	((erythroblastoses or erythroblastosis) adj2 (fetal* or f?etal*)).ti,ab,kw.	858
38	(h?emolytic disease* or h?emolytic disorder*).ti,ab,kw.	4553
39	(HDFN or HDN).ti,ab,kw.	552
40	36 or 37 or 38 or 39	13563
41	6 or 9 or 19 or 29 or 35 or 40	1361580
42	exp Rh D immunoglobulin/	0
43	exp Rho D Immune Globulin/	1271
44	exp "Rho(D) Immune Globulin"/	1271
45	exp anti-D immunoglobulin/	1271
46	Rh* D Immune Globulin.ti,ab.	68
47	(rh* immunoglobulin or rh* d immunoglobulin).ti,ab.	215
48	(rh* immuni?ation or rh* d immuni?ation).ti,ab.	486
49	42 or 43 or 44 or 45 or 46 or 47 or 48	1885
50	exp rhesus D antibody/	0
51	rhesus D antibody.ti,ab.	10
52	(rh* D antibody or rh*D antibody).ti,ab.	86
53	(anti-D or anti D or anti?D).ti,ab.	2820
54	50 or 51 or 52 or 53	2890
55	exp rhogam/	1271
56	rhogam.ti,ab.	32
57	exp winrho/	0
58	winrho.ti,ab.	41
59	exp rhophylac/	1271
60	rhophylac.ti,ab.	8
61	exp MICRhoGam/	1271
62	exp BayRHo-D/	0
63	exp rhesonativ/	0
64	'RhD immunoglobulin vf'.ti,ab.	0
65	55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64	1302
66	49 or 54 or 65	3847
67	41 and 66	2741
68	exp meta analysis/ or meta analysis.mp. or exp systematic review/ or systematic review.mp. or pooled analysis.mp. or ((exp review/ or review.mp.) and (systemat* or pool*).mp.)	278527
69	exp comparative study/ or comparative study.mp. or exp clinical trial/ or clinical trial.mp. or randomized controlled trial.mp. or randomi?ed controlled trial.mp. or exp randomized controlled trial/ or exp randomization/ or randomization.mp. or randomi?ation.mp. or exp single blind procedure/ or single blind procedure.mp. or exp double blind procedure/ or double blind procedure.mp. or exp triple blind procedure/ or triple blind procedure.mp. or exp crossover procedure/ or crossover procedure.mp. or exp placebo/ or placebo*.mp. or random*.mp. or rct.mp. or single blind.mp. or single blinded.mp. or double blind.mp. or double blinded.mp. or treble blind.mp. or triple blind.mp. or triple blinded.mp. or exp prospective study/ or prospective study.mp.	3476781
70	exp clinical study/ or exp case control study/ or exp family study/ or exp longitudinal study/ or exp retrospective study/ or exp cohort analysis/ or (cohort adj1 stud*).mp. or (case control adj1 stud*).mp. or	2982544

#	Searches	Results
	(exp prospective study/ not randomi?ed controlled trials.mp.) or (follow up adj1 stud*).mp. or (observational adj1 stud*).mp. or (epidemiologic* adj1 stud*).mp. or (cross sectional adj1 stud*).mp.	
71	"case report"/	1887103
72	(editorial or letter or comment or historical article).pt.	1969551
73	71 or 72	3656632
74	(animals/ or nonhuman/) not humans/	4443785
75	(67 and 68) not (72 or 74)	29
76	(67 and 69) not (73 or 74 or 75)	316
77	(67 and 70) not (73 or 74 or 75 or 76)	190

A1.3 Evidence-Based Medicine Reviews

EBM Reviews combines several resources into a single database and includes the following:

- ACP Journal Club 1991 to June 2018,
- Cochrane Database of Systematic Reviews 2005 to July 18, 2018,
- Database of Abstracts of Reviews of Effects 1st Quarter 2016,
- Cochrane Clinical Answers June 2018,
- Cochrane Central Register of Controlled Trials June 2018,
- Cochrane Methodology Register 3rd Quarter 2012,
- Health Technology Assessment 4th Quarter 2016,
- NHS Economic Evaluation Database 1st Quarter 2016.

Table A.3 Search results Questions 1 to 4: EBM Reviews

Search via Ovid conducted 19 July 2018

#	Searches	Results
1	exp "obstetrics"/ or exp "obstetric care"/ or exp "pregnancy"/ or exp "pregnancy disorder"/ or exp "prenatal disorder"/	19993
2	(obstetric or obstetrics or pregnancy or maternal).ti,ab,kw.	37479
3	(prepartum or pre partum or pre-partum or intrapartum or intra partum or intra-partum or perinatal or peri natal or peri-natal).ti,ab,kw.	4694
4	(antenatal or ante natal or ante-natal or prenatal or pre natal or pre-natal).ti,ab,kw.	5834
5	(postnatal or post natal or post-natal or postpartum or post partum or post-partum).ti,ab,kw.	8587
6	1 or 2 or 3 or 4 or 5	50733
7	exp "fetus"/	1614
8	(fetu* or fetal* or f?etu* or f?etal*).ti,ab,kw.	8812
9	7 or 8	9664
10	exp alloimmunization/	0
11	exp Rh Isoimmunization/	30
12	(Rh* Isoimmuni?ation or Rh* D Isoimmuni?ation).ti,ab.	13
13	(Rh* alloimmuni?ation or Rh* D alloimmuni?ation).ti,ab.	6
14	(Rh* incompatibility or Rh* D incompatibility or blood group incompatibility).ti,ab.	25
15	(((Rh* adj3 incompatib*) or Rh* D) adj3 incompatibl*).ti,ab.	2
16	((Rh or RhD or rhesus) adj5 sensiti*).ti,ab.	23
17	((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) adj2 immuni?ation).ti,ab.	0
18	((rh or RhD or rhesus) adj2 (immuni?ation or autoimmuni?ation)).ti,ab.	30
19	10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18	116

#	Searches	Results
20	exp rhesus D antigen/	0
21	rhesus D antigen.ti,ab.	0
22	rh* D antigen.ti,ab.	0
23	(RhD or rhesus D or Rh?D or Rh-?D or Rh D).ti,ab.	139
24	(Rh-negative or Rh-positive).ti,ab.	23
25	(Rhesus negative or Rhesus positive).ti,ab.	17
26	((rh or rhesus) adj2 (factor or factors or antigen$ or system or group)).ti,ab.	117
27	20 or 21 or 22 or 23 or 24 or 25 or 26	283
28	(Macaca mulatta or Simian Immunodeficiency Virus or zika or macaque?).ti,ab.	151
29	27 not 28	283
30	(isoimmuni?ation or alloimmuni?ation).ti,ab,kw.	175
31	(isoimmuni* or iso-immuni* or isoimmune or iso-immune).ti,ab,kw.	46
32	(alloimmuni* or allo-immuni* or alloimmune or allo-immune).ti,ab,kw.	266
33	(unsensiti?ed or un-sensiti?ed or non-sensiti?ed).ti,ab,kw.	49
34	(sensiti?ation* or sensiti?ed).ti,ab,kw.	2899
35	30 or 31 or 32 or 33 or 34	3200
36	exp Erythroblastosis, Fetal/	70
37	((erythroblastoses or erythroblastosis) adj2 (fetal* or f?etal*)).ti,ab,kw.	14
38	(h?emolytic disease* or h?emolytic disorder*).ti,ab,kw.	106
39	(HDFN or HDN).ti,ab,kw.	21
40	36 or 37 or 38 or 39	150
41	6 or 9 or 19 or 29 or 35 or 40	55540
42	exp Rh D immunoglobulin/	0
43	exp Rho D Immune Globulin/	169
44	exp "Rho(D) Immune Globulin"/	169
45	exp anti-D immunoglobulin/	169
46	Rh* D Immune Globulin.ti,ab.	9
47	(rh* immunoglobulin or rh* d immunoglobulin).ti,ab.	13
48	(rh* immuni?ation or rh* d immuni?ation).ti,ab.	28
49	42 or 43 or 44 or 45 or 46 or 47 or 48	208
50	exp rhesus D antibody/	0
51	rhesus D antibody.ti,ab.	0
52	(rh* D antibody or rh*D antibody).ti,ab.	5
53	(anti-D or anti D or anti?D).ti,ab.	145
54	50 or 51 or 52 or 53	150
55	exp rhogam/	169
56	rhogam.ti,ab.	2
57	exp winrho/	0
58	winrho.ti,ab.	5
59	exp rhophylac/	169
60	rhophylac.ti,ab.	5
61	exp MICRhoGam/	169
62	exp BayRHo-D/	0
63	exp rhesonativ/	0
64	'RhD immunoglobulin vf'.ti,ab.	0
65	55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64	176
66	49 or 54 or 65	310
67	41 and 66	102

A1.4 PubMed

The PubMed search is restricted to records that are not indexed for MEDLINE (i.e. in-process citations and citations from journals (or parts of journals) that are not currently MEDLINE-indexed) and to records added to PubMed since January 2006.

The search comprises free-text terms only and replicates the free-text sets in the Embase search (converted from the Ovid syntax).

Table A.4 Search results Questions 1 to 4: Pubmed (not MEDLINE)

Search conducted 20 July 2018

#	Searches	Results
#48	(#47 AND pubmednotmedline[sb])	200
#47	(#32 AND #46)	4737
#46	(#36 OR #45)	8156
#45	(#38 OR #39 OR #40 OR #41 OR #42 OR #43 OR #44)	53
#44	RhD immunoglobulin vf[tiab]	0
#43	rhesonativ[tiab]	2
#42	BayRHo-D[tiab]	0
#41	MICRhoGam[tiab]	2
#40	RhD immunoglobulin-vf[tiab]	0
#39	rhophylac[tiab]	8
#38	winrho[tiab]	42
#37	rhogam[tiab]	33
#36	(#33 OR #34 OR #35)	8146
#35	(anti-d[tiab] OR anti d[tiab])	2811
#34	((rhesus[tiab] OR rh[tiab] OR rho[tiab]) AND antibody[tiab])	4989
#33	((rhesus[tiab] OR rh[tiab] OR rho[tiab] OR RHD[tiab]) AND (immunoglobulin[tiab] OR immune globulin[tiab]))	1576
#32	(#5 OR #6 OR #14 OR #22 OR #27 OR #31)	1018102
#31	(#28 OR #29 OR #30)	20920
#30	(hdfn[tiab] OR hdn[tiab])	553
#29	((hemolytic OR haemolytic) AND (disorder[tiab] OR disorders[tiab] OR disease[tiab] OR diseases[tiab]))	18492
#28	((erythroblastoses[tiab] OR erythroblastosis[tiab]) AND (fetal[tiab] OR foetal[tiab] OR fetalis[tiab] OR foetalis[tiab]))	3161
#27	(#23 OR #24 OR #25 OR #26)	98817
#26	(sensitisation*[tiab] OR sensitization*[tiab] OR sensitised[tiab] OR sensitized[tiab])	90526
#25	((rh[tiab] OR rho[tiab] OR rhesus[tiab]) AND (sensitising[tiab] OR sensitizing[tiab] OR sensitisation[tiab] OR sensitization[tiab] OR sensitised[tiab] OR sensitized[tiab]))	1599
#24	(alloimmuni*[tiab] OR allo-immuni*[tiab] OR alloimmune[tiab] OR allo-immune[tiab])	6637
#23	(isoimmuni*[tiab] OR iso-immuni*[tiab] OR isoimmune[tiab] OR iso-immune[tiab])	2006
#22	(#20 NOT #21)	23409
#21	(Macaca mulatta[tiab] OR Simian Immunodeficiency Virus[tiab] OR zika[tiab] OR macaque[tiab] OR macaques[tiab])	33079
#20	(#15 OR #16 OR #17 OR #18 OR #19)	27458
#19	(rh[tiab] OR rhesus[tiab]) AND (factor[tiab] OR factors[tiab] OR antigen*[tiab] OR antigens[tiab] OR system[tiab] OR group[tiab])	24249
#18	(rhesus negative[tiab] OR rhesus positive[tiab])	240
#17	(rh-negative[tiab] OR rh-positive[tiab] OR rh negative[tiab] OR rh positive[tiab])	949
#16	(RhD[tiab] OR rhesus d[tiab] OR Rh D[tiab])	3887

#	Searches	Results
#15	((rh[tiab] OR rhd[tiab] OR rhesus[tiab]) AND antigen[tiab])	3429
#14	(#7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13)	11065
#13	((rh[tiab] OR RhD[tiab] OR rhesus[tiab]) AND (immunisation[tiab] OR immunization[tiab] OR autoimmunisation[tiab] OR autoimmunization[tiab]))	2055
#12	((fetomaternal[tiab] OR feto-maternal[tiab] OR foetomaternal[tiab] OR foeto-maternal[tiab]) AND (immunisation[tiab] OR immunization[tiab]))	166
#11	((Rh[tiab] OR RhD[tiab] OR rhesus[tiab]) AND (sensiti*[tiab]))	4933
#10	((rh[tiab] OR rhd[tiab] OR rhesus[tiab]) AND (incompatib*[tiab]))	1307
#9	((rh[tiab] OR rhd[tiab] OR rhesus[tiab]) AND (incompatibility[tiab]) OR (blood group incompatibility[tiab]))	1636
#8	((rh[tiab] OR rhd[tiab] rhesus[tiab]) AND (isoimmunization[tiab] OR isoimmunisation[tiab]))	63
#7	(alloimmunization[tiab] OR alloimmunisation[tiab])	2590
#6	(fetus[tiab] OR foetus[tiab] OR fetu*[tiab] OR foetu*[tiab] OR fetal*[tiab] OR foetal*[tiab])	299655
#5	(#1 OR #2 OR #3 OR #4)	738834
#4	(postnatal[tiab] OR post natal[tiab] OR post-natal[tiab] OR postpartum[tiab] OR post partum[tiab] OR post-partum[tiab])	153623
#3	(antenatal[tiab] OR ante natal[tiab] OR ante-natal[tiab] OR prenatal[tiab] OR pre natal[tiab] OR pre-natal[tiab])	115113
#2	(prepartum[tiab] OR pre partum[tiab] OR pre-partum[tiab] OR intrapartum[tiab] OR intra partum[tiab] OR intra-partum[tiab] OR perinatal[tiab] OR peri natal[tiab] OR peri-natal[tiab])	73169
#1	(Obstetric[tiab] OR obstetrics[tiab] OR pregnancy[tiab] OR maternal[tiab])	554296

A1.5 CINAHL

Table A.5 Search results Questions 1 to 4: CINAHL

Searched conducted 20 July 2018

#	Query	Results
S1	(MH "Obstetrics") or (MH "Obstetric Care+") or (MH "Pregnancy+") or “pregnancy disorder” or “prenatal disorder”	128,235
S2	TI (obstetric or obstetrics or pregnancy or maternal) OR AB (obstetric or obstetrics or pregnancy or maternal) or (“obstetric” or “obstetrics” or “pregnancy” or “maternal”)	157,109
S3	TI (obstetric or obstetrics or pregnancy or maternal) OR AB (obstetric or obstetrics or pregnancy or maternal) or (“obstetric” or “obstetrics” or “pregnancy” or “maternal”)	157,109
S4	TI (antenatal or ante natal or ante-natal or prenatal or pre natal or pre-natal) OR AB (antenatal or ante natal or ante-natal or prenatal or pre natal or pre-natal) OR ("antenatal" or "ante natal" or "ante-natal" or "prenatal" or "pre natal" or "pre-natal")	29,500
S5	TI (postnatal or post natal or post-natal or postpartum or post partum or post-partum) OR AB (postnatal or post natal or post-natal or postpartum or post partum or post-partum) OR ("postnatal" or "post natal" or "post-natal" or "postpartum" or "post partum" or "post-partum")	23,198
S6	S1 OR S2 OR S3 OR S4 OR S5	172,767
S7	(MH "Fetus+")	17,301
S8	TI (fetu* or fetal* or f#etu* or f#etal*) OR AB (fetu* or fetal* or f#etu* or f#etal*) OR ("fetu*" or "fetal*" or "f#etu*" or "f#etal*")	2,598,213
S9	S7 OR S8	2,598,237
S10	"alloimmuni?ation"	343
S11	(MH "RH Isoimmunization")	275
S12	TI (Rh* Isoimmuni?ation or Rh* D Isoimmuni?ation) OR AB (Rh* Isoimmuni?ation or Rh* D Isoimmuni?ation)	29
S13	TI (Rh* alloimmuni?ation or Rh* D alloimmuni?ation) OR AB (Rh* alloimmuni?ation or Rh* D alloimmuni?ation)v	25

S14	TI (Rh* incompatibility or Rh* D incompatibility or blood group incompatibility) OR AB (Rh* incompatibility or Rh* D incompatibility or blood group incompatibility)	49
S15	TI ((Rh* N3 incompatib*) OR (Rh* D N3 incompatibl*)) OR AB ((Rh* N3 incompatib*) OR (Rh* D N3 incompatibl*))	37
S16	TI ((Rh or RhD or rhesus) N5 sensiti*) OR AB ((Rh or RhD or rhesus) N5 sensiti*)	60
S17	TI (fetomaternal or feto-maternal or foetomaternal or foeto-maternal) N2 immuni?ation) OR AB (fetomaternal or feto-maternal or foetomaternal or foeto-maternal) N2 immuni?ation)	2
S18	TI (((rh or RhD or rhesus) N2 (immuni?ation or autoimmuni?ation))) OR AB (((rh or rhesus) N2 (immuni?ation or autoimmuni?ation)))	10
S19	S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18	656
S20	"rhesus D antigen"	2
S21	TI rhesus D antigen OR AB rhesus D antigen	3
S22	TI rh* D antigen OR AB rh* D antigen	36
S23	TI (RhD or rhesus D or Rh D or Rh-D) OR AB (RhD or rhesus D or Rh D or Rh-D)	528
S24	TI (Rh negative OR Rh positive) OR AB (Rh negative OR Rh positive))	88
S25	TI (Rhesus negative or Rhesus positive) OR AB (Rhesus negative or Rhesus positive)	32
S26	TI (rh or rhesus) N2 (factor or factors or antigen* or system or group)) OR AB (rh or rhesus) N2 (factor or factors or antigen* or system or group))	156
S27	S20 OR S21 OR S22 OR S23 OR S24 OR S25 OR S26	688
S28	TI (Macaca mulatta or Simian Immunodeficiency Virus or zika or macaque#) OR AB (Macaca mulatta or Simian Immunodeficiency Virus or zika or macaque#)	1,516
S29	S27 NOT S28	683
S30	TI (isoimmuni?ation or alloimmuni?ation) OR AB (isoimmuni?ation or alloimmuni?ation) OR ("isoimmuni?ation" or "alloimmuni?ation")	579
S31	TI (isoimmuni* or iso-immuni* or isoimmune or iso-immune) OR AB (isoimmuni* or iso-immuni* or isoimmune or iso-immune) OR ("isoimmuni*" or "iso-immuni*" or "isoimmune" or "iso-immune")	310
S32	TI (alloimmuni* or allo-immuni* or alloimmune or allo-immune) OR AB (alloimmuni* or allo-immuni* or alloimmune or allo-immune) OR ("alloimmuni*" or "allo-immuni*" or "alloimmune" or "allo-immune")	607
S33	TI (unsensiti?ed or un-sensiti?ed or non-sensiti?ed) OR AB (unsensiti?ed or un-sensiti?ed or non-sensiti?ed) OR ("unsensiti?ed" or "un-sensiti?ed" or "non-sensiti?ed")	20
S34	TI (sensiti?ation* or sensiti?ed) OR AB (sensiti?ation* or sensiti?ed) OR ("sensiti?ation*" or "sensiti?ed")	3,426
S35	S30 OR S31 OR S32 OR S33 OR S34	4,227
S36	(MH "Erythroblastosis, Fetal+")	616
S37	TI (((erythroblastoses or erythroblastosis) N2 (fetal* or f#etal*))) OR AB (((erythroblastoses or erythroblastosis) N2 (fetal* or f#etal*))) OR ((("erythroblastoses" or "erythroblastosis") N2 ("fetal*" or "f#etal*")))	240
S38	TI ((h#emolytic disease* or h#emolytic disorder*)) OR AB ((h#emolytic disease* or h#emolytic disorder*)) OR (("h#emolytic disease*" or "h#emolytic disorder*"))	376
S39	TI (HDFN or HDN) OR AB (HDFN or HDN) OR ("HDFN" or "HDN")	57
S40	S36 OR S37 OR S38 OR S39	873
S41	S6 OR S9 OR S19 OR S35 OR S40	2,610,641
S42	"Rh D immunoglobulin"	1
S43	"Rho D Immune Globulin"	222
S44	(MH "Rho(D) Immune Globulin")	219
S45	"anti-D immunoglobulin"	34
S46	TI Rh* D Immune Globulin OR AB Rh* D Immune Globulin	27
S47	TI (rh* immunoglobulin or rh* d immunoglobulin) OR AB (rh* immunoglobulin or rh* d immunoglobulin)	56
S48	TI (rh* immuni?ation or rh* d immuni?ation) OR AB (rh* immuni?ation or rh* d immuni?ation)	24
S49	S42 OR S43 OR S44 OR S45 OR S46 OR S47 OR S48	298
S50	"rhesus D antibody"	0

S51	TI rhesus D antibody OR AB rhesus D antibody	4
S52	TI (rh* D antibody or rh*D antibody) OR AB (rh* D antibody or rh*D antibody)	409
S53	TI (anti-D or anti D or anti?D) OR AB (anti-D or anti D or anti?D)	466
S54	S50 OR S51 OR S52 OR S53	849
S55	“rhogam”	7
S56	TI rhogam OR AB rhogam	7
S57	"winrho"	6
S58	TI winrho OR AB winrho	6
S59	TI rhophylac OR AB rhophylac OR "rhophylac"	2
S60	TI RhD immunoglobulin vf OR AB RhD immunoglobulin vf' OR "RhD immunoglobulin vf"	0
S61	TI MICRhoGam OR AB MICRhoGam OR "MICRhoGam"	0
S62	TI BayRHo-D OR AB BayRHo-D OR "BayRHo-D"	0
S63	TI rhesonativ OR AB rhesonativ OR "rhesonativ"	0
S64	TI RhD immunoglobulin vf OR AB RhD immunoglobulin vf	0
S65	S55 OR S56 OR S57 OR S58 OR S59 OR S60 OR S61 OR S62 OR S63 OR S64	15
S66	S49 OR S54 OR S65	1,019
S67	S41 AND S66	973
S68	PT (Editorial or letter or comment or historical article)	364,194
S69	S67 NOT S68	920

A2 Subquestion 3

A2.1 Embase

Table A.6 Search results subquestion 3: Embase <1974 to 2018 July 17>

Search via Ovid for Level I, Level II and Level III studies conducted 19 July 2018

#	Searches	Results
1	exp Prenatal Diagnosis/	100703
2	Maternal Serum Screening Tests/	232
3	Hematologic Tests/	12148
4	((prenatal or pre-natal or antenatal or ante-natal) adj3 (test* or screen* or diagnos* or determin* or detect*)).ti,ab.	47618
5	(f?etal adj3 (test* or screen* or diagnos* or determin* or detect*)).ti,ab.	23936
6	((non-invasive adj7 screening) or (non?invasive adj7 screening)).ti,ab.	4412
7	(NIPD or NIPT or NIPS or NIPA).ti,ab.	2110
8	or/1-7	144660
9	Cell-Free Nucleic Acids/	0
10	(cffCDNA or cell-free f?etal DNA).ti,ab.	1056
11	((cell free dna or cfDNA) adj3 (obstetric or obstetrics or pregnancy or maternal)).ti,ab.	443
12	((cell free dna or cfDNA) adj3 (fetu* or fetal* or f?etu* or f?etal*)).ab,ti.	267
13	Genotyping Techniques/	5856
14	((genotype* or genotyping) adj3 (obstetric or obstetrics or pregnancy or maternal)).ti,ab.	1424
15	((genotype* or genotyping) adj3 (fetu* or fetal* or f?etu* or f?etal*)).ti,ab.	1113
16	(RHD adj3 gene).ti,ab.	667
17	or/9-16	9881
18	8 or 17	152700
19	exp "obstetrics"/ or exp "obstetric care"/ or exp "pregnancy"/ or exp "pregnancy disorder"/ or exp "prenatal disorder"/	1138381

#	Searches	Results
20	(obstetric or obstetrics or pregnancy or maternal).kw,ab,ti.	688764
21	(prepartum or pre partum or pre-partum or intrapartum or intra partum or intra-partum or perinatal or peri natal or peri-natal).kw,ab,ti.	98043
22	(antenatal or ante natal or ante-natal or prenatal or pre natal or pre-natal).kw,ab,ti.	151208
23	(postnatal or post natal or post-natal or postpartum or post partum or post-partum).kw,ab,ti.	194268
24	or/19-23	1433924
25	exp "fetus"/	189793
26	(fetu* or fetal* or f?etu* or f?etal*).kw,ab,ti.	375396
27	or/25-26	428926
28	exp alloimmunization/	4372
29	exp Rh Isoimmunization/	1604
30	(Rh* Isoimmuni?ation or Rh* D Isoimmuni?ation).ti,ab.	719
31	(Rh* alloimmuni?ation or Rh* D alloimmuni?ation).ti,ab.	381
32	(Rh* incompatibility or Rh* D incompatibility or blood group incompatibility).ti,ab.	1102
33	(((Rh* adj3 incompatib*) or Rh* D) adj3 incompatibl*).ti,ab.	203
34	((Rh or RhD or rhesus) adj5 sensiti*).ti,ab.	1325
35	((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) adj2 immuni?ation).ti,ab.	81
36	((rh or rhesus) adj2 (immuni?ation or autoimmuni?ation)).ti,ab.	770
37	or/28-36	9210
38	exp rhesus D antigen/	1158
39	rhesus D antigen.ti,ab.	55
40	rh* D antigen.ti,ab.	234
41	(RhD or rhesus D or Rh?D or Rh-?D or Rh D).ti,ab.	7551
42	(Rh-negative or Rh-positive).ti,ab.	1311
43	(Rhesus negative or Rhesus positive).ti,ab.	362
44	((rh or rhesus) adj2 (factor or factors or antigen* or system or group)).ti,ab.	4806
45	or/38-44	12533
46	(Macaca mulatta or Simian Immunodeficiency Virus or zika or macaque?).ti,ab.	38059
47	45 not 46	12358
48	(isoimmuni?ation or alloimmuni?ation).ti,ab,kw.	5920
49	(isoimmuni* or iso-immuni* or isoimmune or iso-immune).ti,ab,kw.	2122
50	(alloimmuni* or allo-immuni* or alloimmune or allo-immune).ti,ab,kw.	11071
51	(unsensiti?ed or un-sensiti?ed or non-sensiti?ed).ti,ab,kw.	2409
52	(sensiti?ation* or sensiti?ed).ti,ab,kw.	119495
53	or/48-52	132300
54	exp Erythroblastosis, Fetal/	11404
55	((erythroblastoses or erythroblastosis) adj2 (fetal* or f?etal*)).kw,ab,ti.	1103
56	(h?emolytic disease* or h?emolytic disorder*).ti,ab,kw.	5204
57	(HDFN or HDN).ti,ab,kw.	1169
58	or/54-57	14942
59	24 or 27	1568681
60	37 or 47 or 53 or 58	156987
61	59 and 60	23151
62	18 and 61	5024
63	(diagnos*.mp. and (exp performance/ or yield.mp.)) or accura*.mp. or exp accuracy/ or exp diagnostic accuracy/ or sensitivity.mp. or specificity.mp. or exp "sensitivity and specificity"/ or exp "specificity and sensitivity"/ or exp precision/ or exp positive predictive value/ or exp negative predictive value/ or positive likelihood ratio.mp. or exp negative predictive value/ or positive likelihood ratio.mp. or negative likelihood ratio.mp. or receiver operating.mp. or diagnostic odds.mp. or ppv.mp. or npv.mp. or plr.mp. or nlr.mp. or roc.mp. or exp sroc/ or dor.mp. or exp reliability/ or repeatability.mp. or exp reproducibility/ or reference	5845182

#	Searches	Results
	standard.mp. or index test.mp. or reference test.mp. or exp gold standard/ or exp false positive result/ or exp false negative result/ or true positive.mp. or true negative.mp. or false positive.mp. or false negative.mp. or concord*.mp. or agreement.mp. or correlat*.mp. or accord*.mp. or (predictive adj4 value).mp.	
64	62 and 63	1442
65	(editorial or letter or comment or historical article).pt.	1600105
66	64 not 65	1415
67	(animals/ or nonhuman/) not humans/	6124874
68	66 not 67	1402

A2.2 MEDLINE

Table A.7 Search results subquestion 3: Medline <1946 to May 30, 2018>

Ovid MEDLINE(R) Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE and Versions(R)

Search via Ovid for Level I, Level II and Level III studies conducted 19 July 2018

#	Searches	Results
1	exp Prenatal Diagnosis/	68829
2	Maternal Serum Screening Tests/	330
3	Hematologic Tests/	8696
4	((prenatal or pre-natal or antenatal or ante-natal) adj3 (test* or screen* or diagnos* or determin* or detect*)).ti,ab.	36975
5	(f?etal adj3 (test* or screen* or diagnos* or determin* or detect*)).ti,ab.	17863
6	((non-invasive adj7 screening) or (non?invasive adj7 screening)).ti,ab.	2893
7	(NIPD or NIPT or NIPS or NIPA).ti,ab.	1344
8	or/1-7	104364
9	Cell-Free Nucleic Acids/	198
10	(cffCDNA or cell-free f?etal DNA).ti,ab.	666
11	((cell free dna or cfDNA) adj3 (obstetric or obstetrics or pregnancy or maternal)).ti,ab.	273
12	((cell free dna or cfDNA) adj3 (fetu* or fetal* or f?etu* or f?etal*)).ab,ti.	157
13	Genotyping Techniques/	5403
14	((genotype* or genotyping) adj3 (obstetric or obstetrics or pregnancy or maternal)).ti,ab.	1115
15	((genotype* or genotyping) adj3 (fetu* or fetal* or f?etu* or f?etal*)).ti,ab.	779
16	(RHD adj3 gene).ti,ab.	323
17	or/9-16	8282
18	8 or 17	111426
19	exp "obstetrics"/ or exp "obstetric care"/ or exp "pregnancy"/ or exp "pregnancy disorder"/ or exp "prenatal disorder"/	846400
20	(obstetric or obstetrics or pregnancy or maternal).kw,ab,ti.	542881
21	(prepartum or pre partum or pre-partum or intrapartum or intra partum or intra-partum or perinatal or peri natal or peri-natal).kw,ab,ti.	72582
22	(antenatal or ante natal or ante-natal or prenatal or pre natal or pre-natal).kw,ab,ti.	113470
23	(postnatal or post natal or post-natal or postpartum or post partum or post-partum).kw,ab,ti.	152751
24	or/19-23	1127977
25	exp "fetus"/	151416
26	(fetu* or fetal* or f?etu* or f?etal*).kw,ab,ti.	296787
27	or/25-26	368773

#	Searches	Results
28	exp alloimmunization/	0
29	exp Rh Isoimmunization/	1672
30	(Rh* Isoimmuni?ation or Rh* D Isoimmuni?ation).ti,ab.	602
31	(Rh* alloimmuni?ation or Rh* D alloimmuni?ation).ti,ab.	215
32	(Rh* incompatibility or Rh* D incompatibility or blood group incompatibility).ti,ab.	909
33	(((Rh* adj3 incompatib*) or Rh* D) adj3 incompatibl*).ti,ab.	155
34	((Rh or RhD or rhesus) adj5 sensiti*).ti,ab.	1195
35	((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) adj2 immuni?ation).ti,ab.	78
36	((rh or rhesus) adj2 (immuni?ation or autoimmuni?ation)).ti,ab.	713
37	or/28-36	4718
38	exp rhesus D antigen/	0
39	rhesus D antigen.ti,ab.	37
40	rh* D antigen.ti,ab.	183
41	(RhD or rhesus D or Rh?D or Rh-?D or Rh D).ti,ab.	4499
42	(Rh-negative or Rh-positive).ti,ab.	951
43	(Rhesus negative or Rhesus positive).ti,ab.	238
44	((rh or rhesus) adj2 (factor or factors or antigen* or system or group)).ti,ab.	3881
45	or/38-44	8683
46	(Macaca mulatta or Simian Immunodeficiency Virus or zika or macaque?).ti,ab.	32790
47	45 not 46	8526
48	(isoimmuni?ation or alloimmuni?ation).ti,ab,kw.	3791
49	(isoimmuni* or iso-immuni* or isoimmune or iso-immune).ti,ab,kw.	2000
50	(alloimmuni* or allo-immuni* or alloimmune or allo-immune).ti,ab,kw.	6616
51	(unsensiti?ed or un-sensiti?ed or non-sensiti?ed).ti,ab,kw.	1629
52	(sensiti?ation* or sensiti?ed).ti,ab,kw.	90214
53	or/48-52	98682
54	exp Erythroblastosis, Fetal/	11580
55	((erythroblastoses or erythroblastosis) adj2 (fetal* or f?etal*)).kw,ab,ti.	858
56	(h?emolytic disease* or h?emolytic disorder*).ti,ab,kw.	4554
57	(HDFN or HDN).ti,ab,kw.	552
58	or/54-57	13562
59	24 or 27	1260402
60	37 or 47 or 53 or 58	118578
61	59 and 60	17363
62	18 and 61	2898
63	(diagnos*.mp. and (exp performance/ or yield.mp.)) or accura*.mp. or exp accuracy/ or exp diagnostic accuracy/ or sensitivity.mp. or specificity.mp. or exp "sensitivity and specificity"/ or exp "specificity and sensitivity"/ or exp precision/ or exp positive predictive value/ or exp negative predictive value/ or positive likelihood ratio.mp. or exp negative predictive value/ or positive likelihood ratio.mp. or negative likelihood ratio.mp. or receiver operating.mp. or diagnostic odds.mp. or ppv.mp. or npv.mp. or plr.mp. or nlr.mp. or roc.mp. or exp sroc/ or dor.mp. or exp reliability/ or repeatability.mp. or exp reproducibility/ or reference standard.mp. or index test.mp. or reference test.mp. or exp gold standard/ or exp false positive result/ or exp false negative result/ or true positive.mp. or true negative.mp. or false positive.mp. or false negative.mp. or concord*.mp. or agreement.mp. or correlat*.mp. or accord*.mp. or (predictive adj4 value).mp.	4531794
64	62 and 63	716
65	(editorial or letter or comment or historical article).pt.	1970016
66	64 not 65	702
67	(animals/ or nonhuman/) not humans/	4441716
68	66 not 67	699

A2.3 Evidence-Based Medicine Reviews

EBM Reviews combines several resources into a single database and includes the following:

- ACP Journal Club 1991 to June 2018,
- Cochrane Database of Systematic Reviews 2005 to July 11, 2018,
- Database of Abstracts of Reviews of Effects 1st Quarter 2016,
- Cochrane Clinical Answers June 2018,
- Cochrane Central Register of Controlled Trials June 2018,
- Cochrane Methodology Register 3rd Quarter 2012,
- Health Technology Assessment 4th Quarter 2016,
- NHS Economic Evaluation Database 1st Quarter 2016.

Table A.8 Search results subquestion 3: EBM Reviews

Search conducted 19 July 2018

#	Searches	Results
1	exp Prenatal Diagnosis/	939
2	Maternal Serum Screening Tests/	8
3	Hematologic Tests/	204
4	((prenatal or pre-natal or antenatal or ante-natal) adj3 (test* or screen* or diagnos* or determin* or detect*)).ti,ab.	852
5	(f?etal adj3 (test* or screen* or diagnos* or determin* or detect*)).ti,ab.	692
6	((non-invasive adj7 screening) or (non?invasive adj7 screening)).ti,ab.	159
7	(NIPD or NIPT or NIPS or NIPA).ti,ab.	137
8	or/1-7	2540
9	Cell-Free Nucleic Acids/	3
10	(cffCDNA or cell-free f?etal DNA).ti,ab.	14
11	((cell free dna or cfDNA) adj3 (obstetric or obstetrics or pregnancy or maternal)).ti,ab.	11
12	((cell free dna or cfDNA) adj3 (fetu* or fetal* or f?etu* or f?etal*)).ab,ti.	11
13	Genotyping Techniques/	60
14	((genotype* or genotyping) adj3 (obstetric or obstetrics or pregnancy or maternal)).ti,ab.	31
15	((genotype* or genotyping) adj3 (fetu* or fetal* or f?etu* or f?etal*)).ti,ab.	13
16	(RHD adj3 gene).ti,ab.	3
17	or/9-16	122
18	8 or 17	2636
19	exp "obstetrics"/ or exp "obstetric care"/ or exp "pregnancy"/ or exp "pregnancy disorder"/ or exp "prenatal disorder"/	19993
20	(obstetric or obstetrics or pregnancy or maternal).kw,ab,ti.	37479
21	(prepartum or pre partum or pre-partum or intrapartum or intra partum or intra-partum or perinatal or peri natal or peri-natal).kw,ab,ti.	4694
22	(antenatal or ante natal or ante-natal or prenatal or pre natal or pre-natal).kw,ab,ti.	5834
23	(postnatal or post natal or post-natal or postpartum or post partum or post-partum).kw,ab,ti.	8586
24	or/19-23	50733
25	exp "fetus"/	1614
26	(fetu* or fetal* or f?etu* or f?etal*).kw,ab,ti.	8812
27	or/25-26	9664
28	exp alloimmunization/	0
29	exp Rh Isoimmunization/	30
30	(Rh* Isoimmuni?ation or Rh* D Isoimmuni?ation).ti,ab.	13

#	Searches	Results
31	(Rh* alloimmuni?ation or Rh* D alloimmuni?ation).ti,ab.	6
32	(Rh* incompatibility or Rh* D incompatibility or blood group incompatibility).ti,ab.	25
33	(((Rh* adj3 incompatib*) or Rh* D) adj3 incompatibl*).ti,ab.	2
34	((Rh or RhD or rhesus) adj5 sensiti*).ti,ab.	23
35	((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) adj2 immuni?ation).ti,ab.	0
36	((rh or rhesus) adj2 (immuni?ation or autoimmuni?ation)).ti,ab.	27
37	or/28-36	113
38	exp rhesus D antigen/	0
39	rhesus D antigen.ti,ab.	0
40	rh* D antigen.ti,ab.	0
41	(RhD or rhesus D or Rh?D or Rh-?D or Rh D).ti,ab.	139
42	(Rh-negative or Rh-positive).ti,ab.	23
43	(Rhesus negative or Rhesus positive).ti,ab.	17
44	((rh or rhesus) adj2 (factor or factors or antigen* or system or group)).ti,ab.	117
45	or/38-44	283
46	(Macaca mulatta or Simian Immunodeficiency Virus or zika or macaque?).ti,ab.	151
47	45 not 46	283
48	(isoimmuni?ation or alloimmuni?ation).ti,ab,kw.	175
49	(isoimmuni* or iso-immuni* or isoimmune or iso-immune).ti,ab,kw.	46
50	(alloimmuni* or allo-immuni* or alloimmune or allo-immune).ti,ab,kw.	266
51	(unsensiti?ed or un-sensiti?ed or non-sensiti?ed).ti,ab,kw.	49
52	(sensiti?ation* or sensiti?ed).ti,ab,kw.	2899
53	or/48-52	3200
54	exp Erythroblastosis, Fetal/	70
55	((erythroblastoses or erythroblastosis) adj2 (fetal* or f?etal*)).kw,ab,ti.	14
56	(h?emolytic disease* or h?emolytic disorder*).ti,ab,kw.	106
57	(HDFN or HDN).ti,ab,kw.	21
58	or/54-57	150
59	24 or 27	52330
60	37 or 47 or 53 or 58	3577
61	59 and 60	367
62	18 and 61	38
63	(diagnos*.mp. and (exp performance/ or yield.mp.)) or accura*.mp. or exp accuracy/ or exp diagnostic accuracy/ or sensitivity.mp. or specificity.mp. or exp "sensitivity and specificity"/ or exp "specificity and sensitivity"/ or exp precision/ or exp positive predictive value/ or exp negative predictive value/ or positive likelihood ratio.mp. or exp negative predictive value/ or positive likelihood ratio.mp. or negative likelihood ratio.mp. or receiver operating.mp. or diagnostic odds.mp. or ppv.mp. or npv.mp. or plr.mp. or nlr.mp. or roc.mp. or exp sroc/ or dor.mp. or exp reliability/ or repeatability.mp. or exp reproducibility/ or reference standard.mp. or index test.mp. or reference test.mp. or exp gold standard/ or exp false positive result/ or exp false negative result/ or true positive.mp. or true negative.mp. or false positive.mp. or false negative.mp. or concord*.mp. or agreement.mp. or correlat*.mp. or accord*.mp. or (predictive adj4 value).mp.	233823
64	62 and 63	25
65	(editorial or letter or comment or historical article).pt.	7477
66	64 not 65	25
67	(animals/ or nonhuman/) not humans/	25
68	66 not 67	25

A2.4 PubMed

The PubMed search is restricted to records that are not indexed for MEDLINE (i.e. in-process citations and citations from journals (or parts of journals) that are not currently MEDLINE-indexed) and to records added to PubMed since January 2006.

The search comprises free-text terms only and replicates the free-text sets in the Embase search (converted from the Ovid syntax).

Table A.9 Search results subquestion 3: Pubmed (not MEDLINE)

Searched conducted 20 July 2018

#	Search terms	Results
#1	(Maternal[tiab] OR obstetric[tiab] OR obstetrics[tiab] OR pregnant[tiab] OR pregancy[tiab] OR prenatal[tiab] OR pre-natal[tiab]) AND (serum[tiab] OR sera[tiab]) AND (test[tiab] OR tests[tiab] OR testing[tiab] OR screen*[tiab] OR diagnos*[tiab] OR determin*[tiab] OR detect*[tiab])	21171
#2	(Blood[tiab] OR serum[tiab] OR sera[tiab] OR haematologic*[tiab] OR hematologic*[tiab]) AND (test[tiab] OR tests[tiab] OR testing[tiab])	344458
#3	(prenatal[tiab] OR pre-natal[tiab] OR antenatal[tiab] OR ante-natal[tiab]) AND (test[tiab] OR tests[tiab] OR testing[tiab] OR screen*[tiab] OR diagnos*[tiab] OR determin*[tiab] OR detect*[tiab])	70051
#4	(foetal[tiab] OR fetal[tiab]) AND (test[tiab] OR tests[tiab] OR testing[tiab] OR screen*[tiab] OR diagnos*[tiab] OR determin*[tiab] OR detect*[tiab])	103239
#5	(noninvasive[tiab] OR non-invasive[tiab]) AND (screening[tiab])	8460
#6	NIPD[tiab] OR NIPT[tiab] OR NIPS[tiab] OR NIPA[tiab]	1368
#7	#1 OR #2 OR #3 OR #4 OR #5 OR #6	502555
#8	cffCDNA[tiab] OR cell free fetal DNA[tiab] OR cell free foetal DNA[tiab]	706
#9	(cell free dna[tiab] OR cfDNA[tiab]) AND (obstetric[tiab] OR obstetrics[tiab] OR pregnancy[tiab] OR maternal[tiab])	576
#10	(cell free dna[tiab] OR cfDNA[tiab]) AND (fetu*[tiab] OR fetal*[tiab] OR foetu*[tiab] OR foetal*[tiab])	593
#11	(genotype[tiab] OR genotyping[tiab] OR allele[tiab] OR alleles[tiab]) AND (test[tiab] OR tests[tiab] OR testing[tiab] OR screen*[tiab] OR diagnos*[tiab] OR determin*[tiab] OR detect*[tiab])	180704
#12	(genotype*[tiab] OR genotyping[tiab]) AND (obstetric[tiab] OR obstetrics[tiab] OR pregnancy[tiab] OR maternal[tiab])	7324
#13	(genotype*[tiab] OR genotyping[tiab]) AND (fetu*[tiab] OR fetal*[tiab] OR foetu*[tiab] OR foetal*[tiab])	3628
#14	(RHD[tiab] AND gene[tiab])	607
#15	#8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14	186624
#16	#7 OR #15	675038
#17	Obstetric[tiab] OR obstetrics[tiab] OR pregnancy[tiab] OR maternal[tiab]	554296
#18	prepartum[tiab] OR pre partum[tiab] OR pre-partum[tiab] OR intrapartum[tiab] OR intra partum[tiab] OR intra-partum[tiab] OR perinatal[tiab] OR peri natal[tiab] OR peri-natal[tiab]	73169
#19	antenatal[tiab] OR ante natal[tiab] OR ante-natal[tiab] OR prenatal[tiab] OR pre natal[tiab] OR pre-natal[tiab]	115113
20#	postnatal[tiab] OR post natal[tiab] OR post-natal[tiab] OR postpartum[tiab] OR post partum[tiab] OR post-partum[tiab]	153623
#21	#17 OR #18 OR #19 OR #20	738834
#22	fetus[tiab] OR foetus[tiab] OR fetu*[tiab] OR foetu*[tiab] OR fetal*[tiab] OR foetal*[tiab]	299655
#23	alloimmunization[tiab] OR alloimmunisation[tiab]	2590
#24	(rh[tiab] OR rhd[tiab] rhesus[tiab]) AND (isoimmunization[tiab] OR isoimmunisation[tiab])	63

#	Search terms	Results
#25	(rh[tiab] OR rhd[tiab] OR rhesus[tiab]) AND (incompatibility[tiab]) OR (blood group incompatibility[tiab])	1636
#26	(rh[tiab] OR rhd[tiab] OR rhesus[tiab]) AND (incompatib*[tiab])	1307
#27	(Rh[tiab] OR RhD[tiab] OR rhesus[tiab]) AND (sensiti*[tiab])	4933
#28	(fetomaternal[tiab] OR feto-maternal[tiab] OR foetomaternal[tiab] OR foeto-maternal[tiab]) AND (immunisation[tiab] OR immunization[tiab])	166
#29	(rh[tiab] OR RhD[tiab] OR rhesus[tiab]) AND (immunisation[tiab] OR immunization[tiab] OR autoimmunisation[tiab] OR autoimmunization[tiab])	2055
#30	#23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29	11065
#31	(rh[tiab] OR rhd[tiab] OR rhesus[tiab]) AND antigen[tiab]	3429
#32	RhD[tiab] OR rhesus d[tiab] OR rh-d[tiab] OR Rh D[tiab]	3887
#33	rh-negative[tiab] OR rh-positive[tiab] OR rh negative[tiab] OR rh positive[tiab]	949
#34	rhesus negative[tiab] OR rhesus positive[tiab]	240
#35	(rh[tiab] OR rhesus[tiab]) AND (factor[tiab] OR factors[tiab] OR antigen*[tiab] OR antigens[tiab] OR system[tiab] OR group[tiab])	24249
#36	#31 OR #32 OR #33 OR #34 OR #35	27458
#37	Macaca mulatta[tiab] OR Simian Immunodeficiency Virus[tiab] OR zika[tiab] OR macaque[tiab] OR macaques[tiab]	33079
#38	#36 NOT #37	23409
#39	isoimmuni*[tiab] OR iso-immuni*[tiab] OR isoimmune[tiab] OR iso-immune[tiab]	2006
#40	alloimmuni*[tiab] OR allo-immuni*[tiab] OR alloimmune[tiab] OR allo-immune[tiab]	6637
#41	(rh[tiab] OR rho[tiab] OR rhesus[tiab]) AND (sensitising[tiab] OR sensitizing[tiab] OR sensitisation[tiab] OR sensitization[tiab] OR sensitised[tiab] OR sensitized[tiab])	1599
#42	sensitisation*[tiab] OR sensitization*[tiab] OR sensitised[tiab] OR sensitized[tiab]	90526
#43	#39 OR #40 OR #41 OR #42	98817
#44	(erythroblastoses[tiab] OR erythroblastosis[tiab]) AND (fetal[tiab] OR foetal[tiab] OR fetalis[tiab] OR foetalis[tiab])	3161
#45	(hemolytic OR haemolytic) AND (disorder[tiab] OR disorders[tiab] OR disease[tiab] OR diseases[tiab])	18492
#46	hdfn[tiab] OR hdn[tiab]	553
#47	#44 OR #45 OR #46	20920
#48	#21 OR #22	887315
#49	#30 OR #38 OR #43 OR #47	143560
#50	#48 AND #49	12773
#51	#16 AND #50	3926
	Diagnos*[tiab] AND (performance[tiab] or yield[tiab]) OR accura*[tiab] OR diagnostic accuracy[tiab] OR sensitivity[tiab] OR specificity [tiab] OR precision[tiab] OR positive predictive value [tiab] OR negative predictive value[tiab] OR positive likelihood ratio[tiab] OR negative likelihood ratio[tiab] OR receiver operating[tiab] OR diagnostic odds[tiab] OR ppv[tiab] OR npv[tiab] OR plr[tiab] OR nlr[tiab] OR ROC[tiab] OR sroc[tiab] OR dor[tiab] OR reliability[tiab] OR repeatability[tiab] OR reproducibility[tiab] OR reference standard[tiab] OR index test[tiab] OR reference test[tiab] OR gold standard[tiab] OR false positive[tiab] OR false negative[tiab] OR true positive[tiab] OR true negative[tiab] OR concord*[tiab] OR agreement[tiab] OR correlate*[tiab] OR accord*[tiab] OR (predictive[tiab] AND value[tiab])	3585762
#53	#51 AND #52	981
#54	#53 AND pubmednotmedline[sb]	40

A2.5 CINAHL

Table A.10 Search results subquestion 3: CINAHL

Searched conducted 19 July 2018

#	Query	Results
S1	(MH "Prenatal Diagnosis+")	8232
S2	"Maternal Serum Screening Tests"	0
S3	(MH "Hematologic Tests+")	22714
S4	TI (((prenatal or pre-natal or antenatal or ante-natal) N3 (test* or screen* or diagnos* or determin* or detect*))) OR AB (((prenatal or pre-natal or antenatal or ante-natal) N3 (test* or screen* or diagnos* or determin* or detect*)))	4126
S5	TI ((f#etal N3 (test* or screen* or diagnos* or determin* or detect*))) OR AB ((f#etal N3 (test* or screen* or diagnos* or determin* or detect*)))	2104
S6	TI (((non-invasive N7 screening) or (non#invasive N7 screening))) OR AB (((non-invasive N7 screening) or (non#invasive N7 screening)))	333
S7	TI (NIPD or NIPT or NIPA or NIPS) OR AB (NIPD or NIPT or NIPA or NIPS)	222
S8	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7	34153
S9	"Cell Free Nucleic Acids" OR "Cell Free dna"	247
S10	TI ((cffCDNA or cell free f#etal DNA)) OR AB ((cffCDNA or cell free f#etal DNA))	134
S11	TI (((cell free dna or cfDNA) N3 (obstetric or obstetrics or pregnancy or maternal))) OR AB (((cell free dna or cfDNA) N3 (obstetric or obstetrics or pregnancy or maternal)))	96
S12	TI (((cell free dna or cfDNA) N3 (fetu* or fetal* or f#etu* or f#etal*))) OR AB (((cell free dna or cfDNA) N3 (fetu* or fetal* or f#etu* or f#etal*)))	378
S13	(MH "Molecular Diagnostic Techniques")	729
S14	TI (((genotype* or genotyping) N3 (obstetric or obstetrics or pregnancy or maternal))) OR AB (((genotype* or genotyping) N3 (obstetric or obstetrics or pregnancy or maternal)))	128
S15	TI (((genotype* or genotyping) N3 (fetu* or fetal* or f#etu* or f#etal*))) OR AB (((genotype* or genotyping) N3 (fetu* or fetal* or f#etu* or f#etal*)))	3291
S16	TI RHD N3 gene OR AB RHD N3 gene	49
S17	S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16	4459
S18	S8 OR S17	38260
S19	(MH "Obstetrics") or (MH "Obstetric Care+") or (MH "Pregnancy+") or “pregnancy disorder” or “prenatal disorder”	128201
S20	TI (obstetric or obstetrics or pregnancy or maternal) OR AB (obstetric or obstetrics or pregnancy or maternal) or (“obstetric” or “obstetrics” or “pregnancy” or “maternal”)	157074
S21	TI (prepartum or pre partum or pre-partum or intrapartum or intra partum or intra-partum or perinatal or peri natal or peri-natal) OR AB (prepartum or pre partum or pre-partum or intrapartum or intra partum or intra-partum or perinatal or peri natal or peri-natal) OR ("prepartum" or "pre partum" or "pre-partum" or "intrapartum" or "intra partum" or "intra-partum" or "perinatal" or "peri natal" or "peri-natal")	20549
S22	TI (antenatal or ante natal or ante-natal or prenatal or pre natal or pre-natal) OR AB (antenatal or ante natal or ante-natal or prenatal or pre natal or pre-natal) OR ("antenatal" or "ante natal" or "ante-natal" or "prenatal" or "pre natal" or "pre-natal")	29496
S23	TI (postnatal or post natal or post-natal or postpartum or post partum or post-partum) OR AB (postnatal or post natal or post-natal or postpartum or post partum or post-partum) OR ("postnatal" or "post natal" or "post-natal" or "postpartum" or "post partum" or "post-partum")	23191
S24	S19 OR S20 OR S21 OR S22 OR S23	176163
S25	(MH "Fetus+")	17287
S26	TI (fetu* or fetal* or f#etu* or f#etal*) OR AB (fetu* or fetal* or f#etu* or f#etal*) OR ("fetu*" or "fetal*" or "f#etu*" or "f#etal*")	2597789
S27	S25 OR 26	40390
S28	"alloimmuni?ation" TI alloimmuni?ation OR AB alloimmuni?ation OR "alloimmuni?ation"	343

S29	(MH "RH Isoimmunization")	275
S30	TI (Rh* Isoimmuni?ation or Rh* D Isoimmuni?ation) OR AB (Rh* Isoimmuni?ation or Rh* D Isoimmuni?ation)	29
S31	TI (Rh* alloimmuni?ation or Rh* D alloimmuni?ation) OR AB (Rh* alloimmuni?ation or Rh* D alloimmuni?ation)	55
S32	TI (Rh* incompatibility or Rh* D incompatibility or blood group incompatibility) OR AB (Rh* incompatibility or Rh* D incompatibility or blood group incompatibility)	49
S33	TI ((Rh* N3 incompatib*) OR (Rh* D N3 incompatibl*)) OR AB ((Rh* N3 incompatib*) OR (Rh* D N3 incompatibl*))	37
S34	TI ((Rh or RhD or rhesus) N5 sensiti*) OR AB ((Rh or RhD or rhesus) N5 sensiti*)	60
S35	TI (fetomaternal or feto-maternal or foetomaternal or foeto-maternal) N2 immuni?ation) OR AB (fetomaternal or feto-maternal or foetomaternal or foeto-maternal) N2 immuni?ation)	2
S36	TI (((rh or RhD or rhesus) N2 (immuni?ation or autoimmuni?ation))) OR AB (((rh or rhesus) N2 (immuni?ation or autoimmuni?ation)))	10
S37	S28 OR S29 OR S30 OR S31 OR S32 OR S33 OR S34 OR S35 OR S36	656
S38	"rhesus D antigen"	2
S39	TI rhesus D antigen OR AB rhesus D antigen	3
S40	TI rh* D antigen OR AB rh* D antigen	36
S41	TI (RhD or rhesus D or Rh D or Rh-D) OR AB (RhD or rhesus D or Rh D or Rh-D)	528
S42	TI (Rh negative OR Rh positive) OR AB (Rh negative OR Rh positive))	88
S43	TI (Rhesus negative or Rhesus positive) OR AB (Rhesus negative or Rhesus positive)	32
S44	TI (rh or rhesus) N2 (factor or factors or antigen* or system or group)) OR AB (rh or rhesus) N2 (factor or factors or antigen* or system or group))	156
S45	S38 OR S39 OR S40 OR S41 OR S42 OR S43 OR S44	688
S46	TI (Macaca mulatta or Simian Immunodeficiency Virus or zika or macaque#) OR AB (Macaca mulatta or Simian Immunodeficiency Virus or zika or macaque#)	1514
S47	S45 NOT S46	683
S48	TI (isoimmuni?ation or alloimmuni?ation) OR AB (isoimmuni?ation or alloimmuni?ation) OR ("isoimmuni?ation" or "alloimmuni?ation")	579
S49	TI (isoimmuni* or iso-immuni* or isoimmune or iso-immune) OR AB (isoimmuni* or iso-immuni* or isoimmune or iso-immune) OR ("isoimmuni*" or "iso-immuni*" or "isoimmune" or "iso-immune")	310
S50	TI (alloimmuni* or allo-immuni* or alloimmune or allo-immune) OR AB (alloimmuni* or allo-immuni* or alloimmune or allo-immune) OR ("alloimmuni*" or "allo-immuni*" or "alloimmune" or "allo-immune")	607
S51	TI (unsensiti?ed or un-sensiti?ed or non-sensiti?ed) OR AB (unsensiti?ed or un-sensiti?ed or non-sensiti?ed) OR ("unsensiti?ed" or "un-sensiti?ed" or "non-sensiti?ed")	20
S52	TI (sensiti?ation* or sensiti?ed) OR AB (sensiti?ation* or sensiti?ed) OR ("sensiti?ation*" or "sensiti?ed")	3421
S53	S48 OR S49 OR S50 OR S51 OR S52	4222
S54	(MH "Erythroblastosis, Fetal+")	616
S55	TI (((erythroblastoses or erythroblastosis) N2 (fetal* or f#etal*))) OR AB (((erythroblastoses or erythroblastosis) N2 (fetal* or f#etal*))) OR ((("erythroblastoses" or "erythroblastosis") N2 ("fetal*" or "f#etal*")))	240
S56	TI ((h#emolytic disease* or h#emolytic disorder*)) OR AB ((h#emolytic disease* or h#emolytic disorder*)) OR (("h#emolytic disease*" or "h#emolytic disorder*"))	376
S57	TI (HDFN or HDN) OR AB (HDFN or HDN) OR ("HDFN" or "HDN")	57
S58	S54 OR S55 OR S56 OR S57	873
S59	S24 OR S27	199864
S60	S37 OR S47 OR S53 OR S58	5450
S61	S59 AND S60	1142
S62	S18 AND S61	361
S63	(diagnos* and (performance or yield)) or (accura* or “diagnostic accuracy”) or “sensitivity” or “specificity” or (MH "Sensitivity and Specificity") or (MH "Precision") or (MH "Predictive Value of Tests") or “positive predictive value” or “negative predictive value” or “positive likelihood ratio” or	374650

	“negative likelihood ratio” or (MH "ROC Curve") or "receiver operating" or “diagnostic odds” or ppv or npv or plr or nlr or roc or sroc or dor or reliability or repeatability or reproducibility or “reference standard” or “index test” or “reference test” or “gold standard” or “false positive result” or (MH "False Positive Results") or “false negative result” or (MH “False Negative Results”) or “true positive” or “true negative” or “false positive” or “false negative” or concord* or agreement or correlate* or accord* or (predictive N4 value) or (MH "Predictive Validity")	
S64	S62 and S63	96
S65	PT (Editorial or letter or comment or historical article)	364150
S66	S64 NOT S65	94

Ovid syntax

Exp explodes controlled vocabulary term (i.e. includes all narrower terms in the hierarchy)

* denotes a term that has been searched as a major subject heading

/ denotes controlled vocabulary terms (EMTREE)

$ truncation character (unlimited truncation)

$n truncation limited to specified number (n) of characters (e.g. time$1 identifies time, timed, timer, times but not timetable)

* truncation character (unlimited truncation)

? substitutes any letter (e.g. oxidi?ed identifies oxidised and oxidized)

adjn search terms within a specified number (n) of words from each other in any order

.ti. limit to title field

.ti,ab. limit to title and abstract fields

.kw,ti,ab. limit to keyword, title and abstract field

.pt limit to publication type

PubMed syntax

* truncation character (unlimited truncation)

[TI] limit to title field

[TIAB] limit to title and abstract fields

[EDAT] date citation added to PubMed

[SB] PubMed subset

CINHAL syntax

* truncation character (unlimited truncation)

wildcard character will replace 1 or 0 characters (e.g. f#etus will retrieve fetus and foetus)

? wildcard character will replace one character (e.g. wom?n will retrieve women and woman)

MH - Search the exact CINAHL® subject heading; searches both major and minor headings

MH”heading”+ Search an exploded subheading

TI search title fields

AB search abstract fields

Nn – Proximity “near” operator will find a result if the terms are within a certain number (n) words of each other, regardless of the order in which they appear. (e.g. eating N5 disorders for results that contain eating disorders, as well as mental disorders and eating pathology.)

PT limit to publication type

Appendix B Excluded studies

This appendix documents studies that met the prespecified inclusion criteria for a systematic review on the prophylactic use of Rh D Immunoglobulin (Anti-D) in pregnant women but were later excluded. These studies, and their reasons for exclusion, are listed below.

B1 Studies excluded from Questions 1-4

Not able to be retrieved (2)

Chilcott, J., Lloyd Jones, M., Wight, J., Forman, K., Wray, J., Beverley, C., & Tappenden, P. (2016). A review of the clinical effectiveness and cost-effectiveness of routine anti-D prophylaxis for pregnant women who are rhesus-negative (Structured abstract). *Health Technology Assessment Database* (Issue 4).

Pilgrim, H., Lloyd Jones, M., & Rees, A. (2016). Routine antenatal anti-D prophylaxis for RhD negative women: a systematic review and economic evaluation (Structured abstract). *Health Technology Assessment Database* (Issue 4).

Publication not available in English (6)

Bader W., Behrens O., Holle W. and Maas D.H.A. (1993). The effect of antenatal rhesus prophylaxis on the antibodies. *Archives of Gynecology and Obstetrics*. 254: 1421-1423.

Branger B. and Winer N. (2006). Epidemiology of anti-D allo-immunization during pregnancy. *//Journal de gynecologie, obstetrique et biologie de la reproduction //*. 35: 1S87-81S92.

Corosu R. and Tillo R. (2008). The role of pre-partum anti-D immunoprophylaxis: Scientific evidences. *Giornale Italiano di Ostetricia e Ginecologia*. 30: 295-299.

Holle W., Behrens O., Bader W. and Maas D.H.A. (1993). Progression of anti-D antibodytitres after antenatal rhesusprophylaxis: Evaluation of four testmethods. *Laboratoriums Medizin*. 17: 62-64.

Ksibi, I., Achour, R., Bel Haj Ammar, W., Cheour, M., Ben Amara, M., Neji, K., & Kacem, S. (2017). Anti-D prophylaxis in fetal-maternal erythrocyte incompatibility in Tunisia. *Archives de Pediatrie, 24*(10), 942-949.

Parant, O. (2006). Comparison of the efficacy of different methods for the prevention of anti-D allo-immunization during pregnancy: targeted strategy limited to risk situations or associated with systematic prevention in the 3rd trimester. *Journal de gynecologie, obstetrique et biologie de la reproduction, 35*(1 Suppl), 1S93-91S103.

Level II study already included in Level I (8)

Clausen, F. B., Christiansen, M., Steffensen, R., Jorgensen, S., Nielsen, C., Jakobsen, M. A., . . . Dziegiel, M. H. (2012). Report of the first nationally implemented clinical routine screening for fetal RHD in D-pregnant women to ascertain the requirement for antenatal RhD prophylaxis. *Transfusion, 52*(4), 752-758. doi:http://dx.doi.org/10.1111/j.1537-2995.2011.03362.x

Damkjaer, M. B., Perslev, A., Clausen, F. B., Dziegiel, M. H., & Jorgensen, F. S. (2012). Study of compliance with a new, targeted antenatal D immunization prevention programme in Denmark. *Vox Sanguinis, 103*(2), 145-149. doi:https://dx.doi.org/10.1111/j.1423-0410.2012.01602.x

Hensleigh PA, L. W., Dixon E, Hall E, Kitay DZ, Jackson JE. (1977). Reduced dose of Rho(D) immune globulin following induced first-trimester abortion (Vol. 129, pp. 413).

Huchet J, D. S., Huchet C, Brossard Y, Larsen M, Parnet-Mathieu F. (1987). Ante-partum administration of preventive treatment of Rh-D immunization in rhesus-negative women. Parallel evaluation of transplacental passage of fetal blood cells. Results of a multicenter study carried out in the Paris region *Application ante-partum du traitement preventif d'immunisation rhesus D chez les femmes rhesus negatif. Evaluation parallele des passages transplacentaires d'hematies foetales. Resultats d'une etude multicentrique menee en region parisienne* 16(1), 101.

Katz, J., & Marcus, R. G. (1973). Incidence of Rh immunization following abortion: possible detection of lymphocyte priming to Rh antigen. *American Journal of Obstetrics and Gynecology, 117*(2), 261-267.

MacKenzie, I. Z., Bowell, P., Gregory, H., Pratt, G., Guest, C., & Entwistle, C. C. (1999). Routine antenatal Rhesus D immunoglobulin prophylaxis: the results of a prospective 10 year study. *British Journal of Obstetrics & Gynaecology, 106*(5), 492-497.

Tiblad, E., Taune Wikman, A., Ajne, G., Blanck, A., Jansson, Y., Karlsson, A., . . . Westgren, M. (2013). Targeted Routine Antenatal Anti-D Prophylaxis in the Prevention of RhD Immunisation - Outcome of a New Antenatal Screening and Prevention Program. *PLoS ONE, 8*(8), e70984. doi:http://dx.doi.org/10.1371/journal.pone.0070984

Tovey, L. A., Townley, A., Stevenson, B. J., & Taverner, J. (1983). The Yorkshire antenatal anti-D immunoglobulin trial in primigravidae. *Lancet (London, England), 2*(8344), 244-246.

No usable data (33)

Controlled trial of various anti-D dosages in suppression of Rh sensitization following pregnancy. Report to the Medical Research Council by the working party on the use of anti-D-immunoglobulin for the prevention of isoimmunization of Rh negative women during pregnancy. (1974). *2*(5910), 75.

U.S. Preventive Services Task Force Screening for Rh(D) incompatibility: recommendation statement. (2005). *American Family Physician, 72*(6), 1087-1997.

Practice Bulletin No. 181 Summary: Prevention of Rh D Alloimmunization. (2017). *Obstetrics & Gynecology, 130*(2), 481-483. doi:10.1097/AOG.0000000000002226

Alrahmani, L., Adekola, H., & Awonuga, A. (2016). Trauma in multi-fetal gestations. *Reproductive Sciences, 1)*, 111A. doi:http://dx.doi.org/10.1177/1933719116641257

Amirthanayagam, A., & Regan, F. (2013). Why is anti-D sensitisation still happening in women of child-bearing age? *Transfusion Medicine, 2)*, 60-61. doi:http://dx.doi.org/10.1111/tme.12070

Bennardello, F., Coluzzi, S., Curciarello, G., Todros, T., & Villa, S. (2015). Recommendations for the prevention and treatment of haemolytic disease of the foetus and newborn. *Blood Transfusion, 13*(1), 109-134. doi:http://dx.doi.org/10.2450/2014.0119-14

Bolton-Maggs, P., Davies, T., Poles, D., Cohen, H., & Bolton-Maggs, P. H. B. (2013). Errors in anti-D immunoglobulin administration: retrospective analysis of 15 years of reports to the UK confidential haemovigilance scheme. *BJOG: An International Journal of Obstetrics & Gynaecology, 120*(7), 873-878. doi:10.1111/1471-0528.12175

Crowther, C. A., & Middleton, P. (2000). Anti-D administration after childbirth for preventing Rhesus alloimmunisation. *Cochrane Database of Systematic Reviews*, N.PAG-N.PAG.

Dajak, S., Roje, D., Haspl, Z. H., & Maglic, P. E. (2014). The importance of antenatal prevention of RhD immunisation in the first pregnancy. *Blood transfusion = Trasfusione del sangue, 12*(3), 410-415. doi:https://dx.doi.org/10.2450/2014.0167-13

Davis, V. J. (2006). Induced Abortion Guidelines. *Journal of Obstetrics and Gynaecology Canada, 28*(11), 1014-1027. doi:http://dx.doi.org/10.1016/S1701-2163%2816%2932296-4

Engelfriet, C. P., Reesink, H. W., Judd, W. J., Ulander, V. M., Kuosmanen, M., Koskinen, S., . . . Mayr, W. R. (2003). Current status of immunoprophylaxis with anti-D immunoglobin. *Vox Sanguinis, 85*(4), 328-337.

Fung, K. F. K., & Eason, E. (2018). No. 133-Prevention of Rh Alloimmunization. *Journal of Obstetrics and Gynaecology Canada, 40*(1), e1-e10. doi:http://dx.doi.org/10.1016/j.jogc.2017.11.007

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Moussa, H., Tsochandaridis, M., Jemni-Yacoub, S., Hmida, S., Khairi, H., Gabert, J., & Levy-Mozziconacci, A. (2012). Fetal RhD genotyping by real-time quantitative PCR in maternal plasma of RhD negative pregnant women from the Sahel of Tunisia. *Annales de Biologie Clinique, 70*(6), 683-688. doi:http://dx.doi.org/10.1684/abc.2012.0769

Plesinac, S., Plecas, D., & Babovic, I. (2018). The Determination of Fetal RhD Status from Maternal Blood in Serbia. *Indian Journal of Hematology and Blood Transfusion*((Plesinac, Plecas, Babovic) Clinic for Obstetrics and Gynecology, Clinical Center of Serbia, Medical School University Belgrade, Majke Jevrosime 8, Belgrade 11000, Serbia), 1-5. doi:http://dx.doi.org/10.1007/s12288-018-0919-z

Sedrak, M., Hashad, D., Adel, H., Azzam, A., & Elbeltagy, N. (2011). Use of free fetal DNA in prenatal noninvasive detection of fetal RhD status and fetal gender by molecular analysis of maternal plasma. *Genetic testing and molecular biomarkers, 15*(9), 627-631.

Sillence, K., Roberts, L. A., Hollands, H. J., Thompson, H. P., Kiernan, M., Madgett, T. E., . . . Avent, N. D. (2015). Fetal sex and RHD genotyping with digital PCR demonstrates greater sensitivity than real-time PCR. *61*(11), 1399.

Tounta, G., Christina, V., Kolialexi, A., Papantoniou, N., Destouni, A., Tsangaris, G. T., . . . Mavrou, A. (2011). A multiplex PCR for non-invasive fetal RHD genotyping using cell-free fetal DNA. *In Vivo, 25*(3), 411-417.

Xiong, Y., Jeronis, S., Hoffman, B., Liebermann, D. A., & Geifman-Holtzman, O. (2017). First trimester noninvasive fetal RHD genotyping using maternal dried blood spots. *Prenatal Diagnosis, 37*(4), 311-317. doi:http://dx.doi.org/10.1002/pd.5006

Yenilmez, E. D., Kokbas, U., Kartlasmis, K., Kayrin, L., & Tuli, A. (2018). A new biosensor for noninvasive determination of fetal RHD status in maternal blood of RhD negative pregnant women. *PLoS ONE, 13*(6), e0197855. doi:http://dx.doi.org/10.1371/journal.pone.0197855

Not comparable to the Australian context (2)

Boggione, C. T., Lujan Brajovich, M. E., Mattaloni, S. M., Di Monaco, R. A., Garcia Borras, S. E., Biondi, C. S., & Cotorruelo, C. M. (2017). Genotyping approach for non-invasive foetal RHD detection in an admixed population. *Blood Transfusion, 15*(1), 66-73. doi:http://dx.doi.org/10.2450/2016.0228-15

Vivanti, A., Benachi, A., Huchet, F. X., Ville, Y., Cohen, H., & Costa, J. M. (2016). Diagnostic accuracy of fetal rhesus D genotyping using cell-free fetal DNA during the first trimester of pregnancy. *215*(5), 606.

Appendix C Literature screening results

This appendix documents the literature search screening results for a systematic review on the prophylactic use of Rh D Immunoglobulin (Anti-D) in pregnant women.

A PRIMSA flow illustrating the screening results is provided in Volume 1 of the technical report.

Table C.1 Literature search and title/abstract screening results

Number of citations identified	Questions 1-4			Q3
	Level I [a]	Level II (not Level I)	Level III (not Level II)	Diagnostic accuracy
Database				
Embase 1974 to 18 July 2018	69	470	990	1402
MEDLINE 1946 to 18 July 2018	29	316	190	699
Cochrane 18 July 2018	102	--		25
PubMed	200	--		40
CINAHL	920	--		94
TOTAL	**1320**	**786**	**1180**	**2261**
Date limit [b]	NA	NA	712	NA
Duplicates removed by Covidence [c]	51	257	97	584
TITLE/ABSTRACT SCREENING				
Number of citations screened in Covidence	**1269**	**529**	**371**	**1677**
Published prior to 2002 (Q1 only)	41	10	0	NA
Additional duplicates identified	4	5	3	2
Nonhuman	87	31	0	6
Population out of scope	844	265	293	1135
Intervention out of scope	70	96	23	180
Comparator out of scope	1	0	0	9
Outcome out of scope	2	0	1	5
Publication type out of scope. Not a systematic review.	18	13	0	63
Publication type out of scope. Opinion piece.	13	2	0	6
Publication type out of scope. Editorial.	24	2	0	0
Publication type out of scope. Other.	15	0	0	14
Study type out of scope. Level IV or below.	18	0	0	27
TOTAL irrelevant	**1137**	**424**	**320**	**1447**
Additional studies identified in grey literature	0	0	0	1
Cost-effectiveness studies (not included)	6	0	0	14

a. NHMRC evidence level filters were applied in the Ovid interface. Studies identified in the Cochrane Collection and those retrieved via PubMed and CINAHL did not have filters applied but were screened in the first pass. (see Technical report, volume one)

b. A date limit was applied to studies relevant to Question 1 in the search strategy. If appropriate, additional date limits were applied for each question after screening of the higher level evidence (see Technical report, volume one).

c. https://www.covidence.org/home

Table C.2 Full text screening results

Number of citations identified	Questions 1-4			Q3
	Level I [a]	Level II (not Level I)	Level III (not Level II)	Diagnostic accuracy
FULL TEXT REVIEW				
Number of citations screened in Covidence [c]	**132**	**105**	**51**	**230**
Published prior to 2002 (Q1 only) [b]	10			--
Duplicate citation	66			0
Not available in English	6			14
Population out of scope	22			21
Intervention out of scope	17			9
Comparator out of scope	5			1
Outcome out of scope	33			20
Publication type out of scope. Not a systematic review.	23			3
Publication type out of scope. Opinion piece.	16			2
Publication type out of scope. Editorial.	3			2
Level II or III study already included in Level I	8			49
Wrong study type (Level IV or below)	17			2
No usable data	33			46
Superseded	5			6
Duplicate data (published elsewhere)	10			16
Not able to be retrieved	2			0
Small sample size	0			23
Not comparable to the Australian context	0			2
TOTAL EXCLUDED	**276**			**216**
Not yet retrieved	0			0
Still to be screened	0			0
TOTAL INCLUDED	**12**			**14**

a. NHMRC evidence level filters were applied in the Ovid interface. Studies identified in the Cochrane Collection and those retrieved via PubMed and CINAHL did not have filters applied but were screened in the first pass. (see Technical report, volume one)

b. A date limit was applied to studies relevant to Question 1 in the search strategy. If appropriate, additional date limits were applied for each question after screening of the higher level evidence (see Technical report, volume one).

c. https://www.covidence.org/home

Appendix D Critical appraisal

D1 Question 1

D1.1 Level I – Systematic review of RCTs

Study ID	McBain 2015	
Question	**Judgement**	**Comments**
1. Was an 'a priori' design provided?	Yes	The authors refer to a published protocol with predetermined research objectives and report deviations.
2. Was there duplicate study selection and data extraction?	Yes	Two authors independently assessed all studies for inclusion and data extraction (p 7)
3. Was a comprehensive literature search performed?	Yes	The literature search is outlined and included six different sources including Embase, MEDLINE, CINHAL and Cochrane (p 7)
4. Was the status of publication (i.e. grey literature) used as an inclusion criterion?	Yes	All published, unpublished and ongoing randomised, quasirandomised and cluster randomised trials. Abstracts were also included (p.7)
5. Was a list of studies (included and excluded) provided?	Yes	Studies that were included (p 18) and excluded (p 21) were listed, as well as ongoing studies
6. Were the characteristics of the included studies provided?	Yes	All included studies were described in detail: PICO and risk of bias provided (p 18)
7. Was the scientific quality of the included studies assessed and documented?	Yes	Risk of bias was assessed (p8) and reported (p11) for all identified studies
8. Was the scientific quality of the included studies used appropriately in formulating conclusions?	Yes	Moderate to high risk of bias was noted however, due to paucity of data, studies were still used
9. Were the methods used to combine the findings of studies appropriate?	Yes	Heterogeneity was assessed using Chi-squared and I2, and used during analysis (p13, p24), where heterogeneity was high (Analysis 1.3 and 3) a random effects model was used, and the heterogeneity was highlighted.
10. Was the likelihood of publication bias assessed?	Yes	Publication bias was assessed via GRADE however, due to low number of studies, a funnel plot was not constructed (p9)
11. Was the conflict of interest stated?	Yes	Authors stated conflict of interest and declared funding source for the systematic review (p30) there was no mention of conflict of interests of included studies.
Total score	***11***	
Overall risk of bias of the review	***Low***	***The systematic review provides an accurate and comprehensive summary of the results of the available studies that address the question of interest.***

Source: Shea et al. 2007. BMC Medical Research Methodology 7:10 doi:10.1186/1471-2288-7-10

D1.2 Level I – Systematic review of observational and cohort studies

Study ID	Turner 2012	
Question	**Judgement**	**Comments**
1. Did the research questions and inclusion criteria for the review include the components of the PICO?	Yes	PICO and study inclusion criteria are clearly defined (p2)
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	No	No reference is made to a protocol, a priori design or prespecified methods
3. Did the review authors explain their selection of the study designs for inclusion in the review?	Yes	The authors only included published studies identified by Pilgrim 2009 and Chilcott 2003
4. Did the review authors use a comprehensive literature search strategy?	Yes	Both Pilgrim 2009 and Chilcott 2003 undertook comprehensive literature reviews therefore the search us current up to the search dates of Pilgrim 2009.
5. Did the review authors perform study selection in duplicate?	No	Study selection in Pilgrim was conducted by one reviewer.
6. Did the review authors perform data extraction in duplicate?	No	Data were abstracted by one researcher (see Pilgrim 2009).
7. Did the review authors provide a list of excluded studies and justify the exclusions?	Partial yes	List of excluded studies can be found in Pilgrim 2009.
8. Did the review authors describe the included studies in adequate detail?	Yes	Summary of included studies (Table 1, p 4)
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	Yes	A checklist was completed by four assessors for each study. Four categories of external bias were considered : population bias, intervention bias, control bias, and outcome bias., as well as other bias (p 3). Biases identified in Table 2 (p 5)
10. Did the review authors report on the sources of funding for the studies included in the review?	Partial yes	There was no mention of conflict of interests of included studies. It is stated in the original texts but is not clear if this was considered in the review.
11. If meta-analysis was performed, did the review authors use appropriate methods for statistical combination of results?	Yes	Methods were previously published meta-analytic method that allows for adjustment of methodological limitations and differences in study design. (p2)
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	Yes	Meta-analysis was performed, adjusting for bias determined by assessors (p 7)
13. Did the review authors account for RoB in individual studies when interpreting/discussing the results of the review?	Yes	Meta-analysis was performed, adjusting for bias determined by assessors (p 7)
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Yes	Statistical heterogeneity was assessed using the I2 statistic and a random effects meta-analysis was used to combine the bias-adjusted results. (p 3)
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	No	No reference to assessment of publication bias.
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Yes	Competing interests and funding sources stated (p1)
Overall risk of bias of the review	***Moderate***	***More than one non-critical weakness – the systematic review has more than one weakness but no critical flaws. It may provide an accurate summary of the results of the available studies that were included in the review.***

Source: Shea et al. 2017. BMJ 358: j4008 doi:10.1136 (https://doi.org/10.1136/bmj.j4008)

Study ID	Pilgrim 2009	
Question	**Judgement**	**Comments**
1. Did the research questions and inclusion criteria for the review include the components of the PICO?	Yes	PICO and study inclusion criteria are clearly defined p 23
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	Yes	"The protocol was agreed in August 2007. The assessment report began editorial review in November 2007 and was accepted for publication in June 2008."
3. Did the review authors explain their selection of the study designs for inclusion in the review?	Yes	Study design for study selection: any of systematic reviews, RCTs (p 23) or non-RCTs. Only one quasi-RCT and one true RCT was identified. because of the shortage of RCTs, all relevant non randomised studies were retained (p 29)
4. Did the review authors use a comprehensive literature search strategy?	Yes	The literature search is outlined and included six different sources and was supplemented by reviewing reference lists. (Databases p 25, Literature search terms Appendix 1), including unpublished studies and grey literature
5. Did the review authors perform study selection in duplicate?	No	Study selection was undertaken by one researcher, and any studies that gave rise to uncertainty were reviewed by a second researcher (p25)
6. Did the review authors perform data extraction in duplicate?	No	Data were abstracted by one researcher using a standardised data extraction form (p25)
7. Did the review authors provide a list of excluded studies and justify the exclusions?	Yes	List of excluded studies with rationale was provided (p93 Appendix 3)
8. Did the review authors describe the included studies in adequate detail?	Yes	All included studies were described in detail: Characteristics of included studies, including quality assessment (Appendix 4, p95)
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	Partial yes	Critical appraisal strategy (p27) The quality of RCTs was assessed using those proposed by the NHS centre for reviews and dissemination (appendix 3), and nonrandomised studies was judged on comparability of the intervention and control groups and the use of intention to treat analysis
10. Did the review authors report on the sources of funding for the studies included in the review?	Yes	The source of funding was declared where possible (Table 11, p 30)
11. If meta-analysis was performed, did the review authors use appropriate methods for statistical combination of results?	Yes	p-value for heterogeneity was included (Table 16, p40). The meta-analyses were conducted using binary logistic regression with a fixed effects model (p26) which is appropriate for studies with low heterogeneity.
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	Yes	Moderate to high risk of bias was noted however studies were still used (probably due to paucity of studies, p75).
13. Did the review authors account for RoB in individual studies when interpreting/discussing the results of the review?	Partial yes	External validity of studies is considered in discussion. p75-76
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Yes	p-value for heterogeneity was included (Table 16, p40). No further discussion is included
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	No	Publication bias was not investigated (p25)
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Yes	Authors stated conflicts of interests and funding sources (title page).
Overall risk of bias of the review	***Moderate risk***	***More than one non-critical weakness – the systematic review has more than one weakness but no critical flaws. It may provide an accurate summary of the results of the available studies that were included in the review.***

Source: Shea et al. 2017. BMJ 358: j4008 doi:10.1136 (https://doi.org/10.1136/bmj.j4008)

Study ID	Chilcott 2003	
Question	**Judgement**	**Comments**
1. Did the research questions and inclusion criteria for the review include the components of the PICO?	Yes	PICO and study inclusion criteria is clearly defined p 9
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	No	No reference is made to a protocol, a priori design or prespecified methods
3. Did the review authors explain their selection of the study designs for inclusion in the review?	Yes	Study design for study selection: any of systematic reviews, RCTs (p 9). Only one quasi-RCT was identified, therefore non-randomised studies were retained (p 10)
4. Did the review authors use a comprehensive literature search strategy?	Yes	The literature search is outlined (p9) and included 12 different databases. Search strategy is provided in Appendix 1, including unpublished studies and grey literature.
5. Did the review authors perform study selection in duplicate?	No	The method for study selection was not outlined.
6. Did the review authors perform data extraction in duplicate?	No	Data were extracted by one researcher, and checked by another (p9)
7. Did the review authors provide a list of excluded studies and justify the exclusions?	Partial yes	No studies were excluded from this review at full text. (p11)
8. Did the review authors describe the included studies in adequate detail?	Yes	All included studies were described in detail: Characteristics of included studies, including quality assessment (Appendix 2, p57)
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	Yes	Quality assessment (p9) NHS CRD.41 This proved to be a poor means of discriminating between the studies relevant to this review, which used several different study designs. Only the question 'were the groups similar at baseline in terms of prognostic factors?' appeared to discriminate meaningfully between studies, and they have therefore been awarded a quality score of good, fair or poor based on this criterion alone
10. Did the review authors report on the sources of funding for the studies included in the review?	Yes	The source of funding was declared where possible (Table 3, p12)
11. If meta-analysis was performed, did the review authors use appropriate methods for statistical combination of results?	Yes	p-value for heterogeneity was included (Table 10, p22). The meta-analyses were conducted using binary logistic regression with a fixed effects model (p22) which is appropriate for studies with low heterogeneity.
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	Yes	Moderate to high risk of bias was noted however studies were still used (probably due to paucity of studies.)
13. Did the review authors account for RoB in individual studies when interpreting/discussing the results of the review?	Yes	Size and contribution of studies with regards to their study design is discussed (p 25)
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Yes	p-value for heterogeneity was included (Table 10, p22). No further discussion is included
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	No	No reference to assessment of publication bias.
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Yes	Authors stated conflicts of interests and funding sources (title page).
Overall risk of bias of the review	***Moderate risk***	***More than one non-critical weakness – the systematic review has more than one weakness but no critical flaws. It may provide an accurate summary of the results of the available studies that were included in the review.***

Source: Shea et al. 2017. BMJ 358: j4008 doi:10.1136 (https://doi.org/10.1136/bmj.j4008)

D1.3 Level II - Randomised controlled trials

Source: Chapter 8 of the Cochrane Handbook for Systematic Reviews of Interventions, version 5.1.0

No studies identified

D1.4 Level III – Comparative observational studies

Study ID	Pennell 2017		
Domain	**Judgement**	**Description**	**Source**
Random sequence generation (selection bias)	Low risk	Permuted Block Randomisation used	ANZCTR
Allocation concealment (selection bias)	Low risk	Sealed Opaque Envelopes	ANZCTR
Blinding of participants and personnel (performance bias)	Low risk	No blinding used in study, however, unlikely to affect outcome due to quantitative outcomes (correct dosing timing, anti-D level, safety)	ANZCTR
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, nature of outcomes means that detection bias is unlikely to affect outcomes (correct dosing timing, sensitisations, safety)	Abstract
Incomplete outcome data addressed (attrition bias)	Low risk	No attritions reported, analyses performed on ITT and treatment received basis	Abstract
Selective reporting (reporting bias)	Unclear risk	Protocol on ANZCTR appears to match primary outcomes stated however insufficient data is reported and some outcomes are missing	Abstract
Other sources of bias*	Unclear risk	Method of detecting residual anti-D not reported in abstract or protocol, insufficient information in the protocol to evaluate other potential sources of bias.	Abstract
Overall risk of bias of the review	***High risk***	***Plausible bias that seriously weakens confidence in the results.***	

Source: Table 5.5 GRADE handbook http://gdt.guidelinedevelopment.org/app/handbook/handbook.html#h.m9385o5z3li7

Study ID	Koelewijn 2008	
Domain	**Judgement**	**Description**
Bias due to failure to develop and apply appropriate eligibility criteria	Moderate risk	Historical controls used; however, they are relatively contemporary and overlap, therefore differences in medical care or population characteristics over time are not considered substantial. The two groups are otherwise the same (Dutch, RH negative Parae-1 women) and the prevalence of anti-D immunisations detected at weeks 30 did not differ between groups. No other comparisons were reported. Due to the large number of patients (All women who participated in Dutch anti-D program) patient selection bias is generally avoided.
Bias due to flawed measurement of both exposure and outcome	Low risk	No mention of outcome blinding, however, outcomes are objective (alloimmunisation, HDFN) or unlikely to be significantly affected by bias.
Bias due to failure to adequately control confounding	Low risk	The prevalence of anti-D immunisations detected at weeks 30 did not differ between groups.
Bias due to incomplete or inadequately short follow-up	Moderate risk	Women were only those who were undergoing a second pregnancy following delivery of first Rh+ child, therefore more sensitisations may have occurred who did not give birth in the relevant period or have a second child.
Overall risk of bias	***Moderate risk***	***The study appears to provide sound evidence for a non-randomised study but cannot be considered comparable to a well-performed randomised trial.***

Source: Table 5.5 GRADE handbook http://gdt.guidelinedevelopment.org/app/handbook/handbook.html#h.m9385o5z3li7

D2 Question 2

D2.1 Level I – Systematic review of RCTs

Study ID	**Karanth 2013**	
Question	**Judgement**	**Comments**
1. Was an 'a priori' design provided?	Yes	Published protocol, predetermined outcomes stated, and deviations from protocol stated p 18
2. Was there duplicate study selection and data extraction?	Yes	Two authors independently assessed for inclusion all the potential studies (p 4)
3. Was a comprehensive literature search performed?	Yes	The literature search is outlined and included five different sources including Embase, MEDLINE, CINHAL and Cochrane (p 4)
4. Was the status of publication (i.e. grey literature) used as an inclusion criterion?	No	No mention of attempts to include unpublished literature
5. Was a list of studies (included and excluded) provided?	Yes	Lists of Included (p13) and excluded (p14) were provided
6. Were the characteristics of the included studies provided?	Yes	Characteristics of studies, including quality assessment was provided (p13)
7. Was the scientific quality of the included studies assessed and documented?	Yes	Risk of bias was independently assessed by two reviewers using the Cochrane Handbook for Systematic Reviews of Interventions (p 4)
8. Was the scientific quality of the included studies used appropriately in formulating conclusions?	Yes	Only one study was identified. The authors acknowledge the poor quality of available evidence.
9. Were the methods used to combine the findings of studies appropriate?	Not applicable	Only one study was identified. Heterogeneity would have been assessed had more studies been identified (p6)
10. Was the likelihood of publication bias assessed?	Yes	The authors note that if 10 or more studies are identified in future updates, assessment of publication bias will be conducted. (p6)
11. Was the conflict of interest stated?	Yes	Declarations of interest were stated (p17)
Total score	***9***	
Overall risk of bias of the review	***Low***	***The systematic review provides an accurate and comprehensive summary of the results of the available studies that address the question of interest.***

Source: Shea et al. 2017. BMJ 358: j4008 doi:10.1136 (https://doi.org/10.1136/bmj.j4008)

D2.2 Level I-III – Systematic review of observational and cohort studies

Study ID	NICE 2014	
Question	**Judgement**	**Comments**
1. Did the research questions and inclusion criteria for the review include the components of the PICO?	Yes	PICO and inclusion criteria outlined in Protocol in Appendix D
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	Yes	Protocol outlined for each question (Appendix D)
3. Did the review authors explain their selection of the study designs for inclusion in the review?	Yes	Evidence statements and outlining of comparable data (Table 9.2, p216-217)
4. Did the review authors use a comprehensive literature search strategy?	Yes	Six electronic databases were searched (Appendix E)
5. Did the review authors perform study selection in duplicate?	No	Not stated in original guidelines, "automated and manual sifts were conducted to produce a list of the most relevant references" stated in update (p3)
6. Did the review authors perform data extraction in duplicate?	No	Not stated
7. Did the review authors provide a list of excluded studies and justify the exclusions?	Yes	Excluded studies with reason (Table G.23, Appendix G)
8. Did the review authors describe the included studies in adequate detail?	Yes	Summary of included studies Appendix F (Figure F.25)
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	Yes	GRADE Method (Section 3.3.) and tables are reported (Table I.9.2)
10. Did the review authors report on the sources of funding for the studies included in the review?	Yes	Funding where available was stated in Appendix p 605
11. If meta-analysis was performed, did the review authors use appropriate methods for statistical combination of results?	Partial yes	Not applicable - methods p 31
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	Partial yes	Not applicable - methods p 31
13. Did the review authors account for RoB in individual studies when interpreting/discussing the results of the review?	Yes	Quality of evidence was considered when interpreting and discussing the results of the review (p219)
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Partial yes	Heterogeneity was not assessed but where statistically significant heterogeneity was identified random effects models would be used. P31
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	Partial yes	Quantitative synthesis was not performed (p31) however, publication bias was considered
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	No	No conflicts of interest stated
Overall risk of bias of the review	***Moderate***	***More than one non-critical weakness – the systematic review has more than one weakness but no critical flaws. It may provide an accurate summary of the results of the available studies that were included in the review.***

Source: Shea et al. 2017. BMJ 358: j4008 doi:10.1136 (https://doi.org/10.1136/bmj.j4008)

D2.3 Level II – Randomised controlled trials

Source: Chapter 8 of the Cochrane Handbook for Systematic Reviews of Interventions, version 5.1.0

No studies identified

D2.4 Level III – Comparative observational studies

Source: Table 5.5 GRADE handbook http://gdt.guidelinedevelopment.org/app/handbook/handbook.html#h.m9385o5z3li7

No studies identified

D3 Question 3

D3.1 Level I – Systematic review of diagnostic accuracy studies

Study ID	Geifman-Holtzman 2006	
Question	**Judgement**	**Comments**
1. Did the research questions and inclusion criteria for the review include the components of the PICO?	Yes	PICO is outlined p. 1164
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	No	Protocol not stated.
3. Did the review authors explain their selection of the study designs for inclusion in the review?	Yes	Study selection is outlined. p. 114
4. Did the review authors use a comprehensive literature search strategy?	Yes	Multiple databases were searched (p. 1164) Literature search strings were not outlined.
5. Did the review authors perform study selection in duplicate?	No	Study selection is not outlined.
6. Did the review authors perform data extraction in duplicate?	No	Data extraction was not performed in duplicate.
7. Did the review authors provide a list of excluded studies and justify the exclusions?	No	Excluded studies are not provided
8. Did the review authors describe the included studies in adequate detail?	Yes	Included studies are summarised in table I (p. 1165) and in the reference list
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	No	Risk of bias was not assessed.
10. Did the review authors report on the sources of funding for the studies included in the review?	No	No reporting of funding was outlined.
11. If meta-analysis was performed, did the review authors use appropriate methods for statistical combination of results?	Yes	Statistical methods are outlined p. 1164-1165
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	No	No assessment of the potential impact of RoB in individual studies.
13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?	No	Critical appraisal of studies was not included in the discussion.
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	No	Heterogeneity was not assessed or discussed.
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	No	Publication bias was not assessed or discussed. Studies with less than 10 samples and studies with more than one sample per woman were excluded.
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	No	Funding and conflicts of interest are not reported.
Overall risk of bias of the review	***Serious risk***	***More than one critical flaw with or without non-critical weaknesses – the review has more than one critical flaw and should not be relied on to provide an accurate and comprehensive summary of the available studies.***

Source: Shea et al. 2017. BMJ 358: j4008 doi:10.1136 (https://doi.org/10.1136/bmj.j4008)

Study ID	**Mackie 2016**	
Question	**Judgement**	**Comments**
1. Did the research questions and inclusion criteria for the review include the components of the PICO?	Yes	PICO is outlined p. 33
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	Yes	This review was performed according to recommended methods, and used a protocol that was designed and registered a priori (PROSPERO CRD42014007174), p.33
3. Did the review authors explain their selection of the study designs for inclusion in the review?	Yes	Study design selection is outlined p. 33
4. Did the review authors use a comprehensive literature search strategy?	Yes	Literature search is outlined p.33. Five databases were searched, and authors attempted to source grey literature.
5. Did the review authors perform study selection in duplicate?	Yes	Study selection was performed in duplicate, and disagreements were resolved by a third reviewer (p. 33)
6. Did the review authors perform data extraction in duplicate?	Yes	Data was extracted in duplicate. Data was also extracted on factors that may affect test accuracy and test characteristics. (p. 33)
7. Did the review authors provide a list of excluded studies and justify the exclusions?	No	No list provided, reasons for exclusions and PRISMA flow chart are supplied in Supp. Fig. 1
8. Did the review authors describe the included studies in adequate detail?	Yes	Included studies are listed in Figure 2 and in the reference list
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	Yes	QUADAS-2 was used to assess risk of bias p. 34. Appendix S2. sFig 4.
10. Did the review authors report on the sources of funding for the studies included in the review?	No	No reporting of funding was outlined
11. If meta-analysis was performed, did the review authors use appropriate methods for statistical combination of results?	Yes	Statistical methods are outlined p. 34 and Appendix S2
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	Yes	Sensitivity analysis was performed on different test methods and other *a priori* specified subgroups. The authors noted overall risk of bias in most studies was low, thus subgroup analysis or meta-regression based on quality assessment was not possible. Appendix S3.
13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?	Yes	The authors noted concerns regarding poor reporting of methodology and a lack of transparency particularly regarding patient flow. This was noted as a limitation of the review.
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Yes	The authors explored potential factors of heterogeneity including analysis platform and reference standard. Heterogeneity was tested for (p. 34) with forest plots and ROC curves reported.
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	No	Publication bias was not discussed. Small case series were not included
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Yes	Conflicts of interest and funding is reported p. 41
Overall risk of bias of the review	***Moderate risk***	***More than one non-critical weakness – the systematic review has more than one weakness but no critical flaws. It may provide an accurate summary of the results of the available studies that were included in the review.***

Source: Shea et al. 2017. BMJ 358: j4008 doi:10.1136 (https://doi.org/10.1136/bmj.j4008)

Study ID	Saramago 2018	
Question	**Judgement**	**Comments**
1. Did the research questions and inclusion criteria for the review include the components of the PICO?	Yes	PICO is clearly outlined p. 8
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	Yes	The protocol was agreed in January 2016. The assessment report began editorial review in June 2016 and was accepted for publication in February 2017
3. Did the review authors explain their selection of the study designs for inclusion in the review?	Yes	Study design selection is outlined p. 8
4. Did the review authors use a comprehensive literature search strategy?	Yes	Literature search is outlined p7. Multiple databases were searched including an attempt to source grey literature. Full study strings are provided in the appendix.
5. Did the review authors perform study selection in duplicate?	Yes	At least two reviewers independently screened the titles and abstracts (if available) of all reports identified by the search strategy. Full text studies were independently assessed for inclusion. Disagreements were resolved by a third reviewer, p. 9
6. Did the review authors perform data extraction in duplicate?	Partial yes	The data was extracted by one reviewer, and another reviewer checked the data extraction. Disagreements were resolved by consensus or by recourse to a third reviewer, p.9
7. Did the review authors provide a list of excluded studies and justify the exclusions?	Yes	Excluded studies are listed in appendix 3 with reasons, p. 137
8. Did the review authors describe the included studies in adequate detail?	Yes	Included studies are listed in appendix 2 p. 133
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	Yes	QUADAS-2 was used to assess risk of bias, p. 10. The results of the critical appraisal are reported in detail in appendix 5
10. Did the review authors report on the sources of funding for the studies included in the review?	No	No reporting of funding was outlined
11. If meta-analysis was performed, did the review authors use appropriate methods for statistical combination of results?	Yes	Data synthesis methods are described p. 10. Heterogeneity was assessed, bivariate meta-analysis was carried out, random effects models were used and sensitivity analysis was undertaken.
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	Yes	Sensitivity analyses based on test location and whether inconclusive results were reported was included, p. 11 and p. 25
13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?	Yes	Critical appraisal was discussed with regards to the included studies p. 13.
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Yes	Heterogeneity was found and discussed (p. 19-20, p. 97)
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	No	Due to inclusion of only high throughput studies, most studies were of considerable size. The authors did not discuss the possible impacts of publication bias.
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Yes	Study funding and author affiliations are reported (p. iii-iv)
Overall risk of bias of the review	***Moderate risk***	***More than one non-critical weakness – the systematic review has more than one weakness but no critical flaws. It may provide an accurate summary of the results of the available studies that were included in the review.***

Source: Shea et al. 2017. BMJ 358: j4008 doi:10.1136 (https://doi.org/10.1136/bmj.j4008)

Study ID	**Zhu 2014**	
Question	**Judgement**	**Comments**
1. Did the research questions and inclusion criteria for the review include the components of the PICO?	Yes	PICO is outlined p.1840
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	No	Protocol not stated.
3. Did the review authors explain their selection of the study designs for inclusion in the review?	Yes	Study selection is outlined p. 1840
4. Did the review authors use a comprehensive literature search strategy?	No	Only the PMC Highwire database was listed a search.
5. Did the review authors perform study selection in duplicate?	No	Selection method was not outlined
6. Did the review authors perform data extraction in duplicate?	No	Data extraction was not outlined
7. Did the review authors provide a list of excluded studies and justify the exclusions?	No	Excluded studies are not provided
8. Did the review authors describe the included studies in adequate detail?	No	Included studies are not referenced
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	No	Risk of bias was not assessed
10. Did the review authors report on the sources of funding for the studies included in the review?	No	No reporting of funding was outlined
11. If meta-analysis was performed, did the review authors use appropriate methods for statistical combination of results?	Yes	Statistical methods outlined p 1840
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	No	No assessment of the potential impact of RoB in individual studies
13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?	No	Critical appraisal of studies was not included in the discussion.
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Partial yes	Heterogeneity was shown in a ROC curve (Figure 1, p 1841), but was not discussed
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	No	Publication bias was not discussed
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Yes	Conflicts of interest was declared p. 1844
Overall risk of bias of the review	***Serious risk***	***More than one critical flaw with or without non-critical weaknesses – the review has more than one critical flaw and should not be relied on to provide an accurate and comprehensive summary of the available studies.***

Source: Shea et al. 2017. BMJ 358: j4008 doi:10.1136 (https://doi.org/10.1136/bmj.j4008)

D3.2 Level II – Consecutive patients with a valid reference standard

Study ID	De Haas 2016	
Domain	**Risk of bias**	**Applicability**
Patient selection	Consecutive patients were enrolled. All RhD negative pregnant women were offered fetal RHD testing and repeated RBC antibody screening in GW 27. Only women who were at risk of sensitisation were included (p.2) 62 women were pregnant twice during the study period (p. 4) therefore have increased risk of sensitisation. Women with anti-RBC antibodies and those with multiple pregnancies were excluded. The exclusion of multiple pregnancies may favour diagnostic accuracy of the index test. No. of women excluded not reported. Presence of weak D type 1, 2, or 3 were regarded as RhD positive and excluded from the analysis. 18 samples showed weak (≤2+) reactivity. Exclusion of these women favours the apparent accuracy of the index test, however, the number is small so the effect is considered negligible.	The study was conducted Jul 2011 – Oct 2012 in a centralised setting in the Netherlands. The applicability to the Australian population is unclear in terms of prevalence of RHD genotype/ Rh D phenotype and exclusion of multiple pregnancies. Centralised setting is probably not comparable to diagnostic screening in Australia.
Index test	Duplex RT-PCR analysis for RHD exon 5 & 7 (p. 2-3) using maternal plasma as the source of cffDNA. DNA was automatically extracted (High throughput, p. 2). No internal control was used for the PCR. The study implemented a non-human sequence into the assay as an internal control for DNA isolation to further reduce the false negative rate. (p. 3, p. 6) The index test was carried out without knowledge of the reference standard. (p. 3) Not all women received the same index test: during the study period, the authors changed the predefined prediction algorithm of computer software once, after about 7700 samples when the protocol changed from a fast to slow PCR protocol. (p. 3) 112 women did not get the index test. (p. 2)	Uncertain what platform, genes or algorithms will be used in the Australian setting.
Reference standard	Neonate birth serology determined by cord blood testing, which was performed and interpreted without knowledge of the fetal RHD test results (p. 3) Cord blood serology was performed with anti-D reagents (LHM 59/20 [LDM3]+175-2 and ESD-1M+175-2) that detect RhD category VI (DVI) phenotype. Of the 225 false positives, 100 of the samples had a variant gene in the mother and newborn (n=55) or only the newborn (n=45). For 25 of these 45 newborns, follow-up serology or molecular testing showed the presence of a variant RHD gene that produced RHD positivity of the RBCs (i.e. false negative blood serology, p. 5).	Birth serology is applicable to the research question.
Patient flow	The interval between the index test and reference standard was appropriate. All patients received the same reference standard. Not all patients included in the analysis. A high proportion of patients enrolled in the study (20%) did not have cord blood serology available. The reason for this is unclear (p. 5). The authors indicate that in all women with the most commonly occurring RHD variants RHD*Ψ and RHD*DVI, a positive test result can be issued, and no additional cord blood serology is needed. It is not clear if this accounts for the missing cord blood serology.	

Source: QUADAS-2 (Whiting et al., 2011)

Study ID	Haimila 2017	
Domain	**Risk of bias**	**Applicability**
Patient selection	Consecutive patients. All RhD negative women (12% of the population) participating in the national screening program for HDFN (p. 1229). NIPT was offered to non-alloimmunised women at GW 24–26 (p. 1229). The authors estimated they captured 83.2% of the expected number of RhD negative, non-immunised women in Finland. Women with multiple pregnancies were included (p. 1232)	Study conducted in Finland in 2014 and 2015. The applicability to the Australian population is unclear in terms of prevalence of RHD genotype/ Rh D phenotype. Centralised setting is probably not similar to diagnostic screening in Australia.
Index test	RT-PCR method targeting RHD exons 5 and 7 (p. 1230). Maternal plasma was the source of the cffDNA. DNA was automatically extracted (High throughput) (p. 1230). No internal control was used for the PCR. (p. 1232) The index test was carried out without knowledge of the reference standard. (as antenatal prophylaxis was administered, p.1230) The algorithm used to interpret the results was not outlined. The authors discuss changing the algorithm further to the reduce the number of inconclusive results. (p. 1231)	Uncertain what platform, genes or algorithms will be used in the Australian setting.
Reference standard	Unclear if cord serology was interpreted without knowledge of the test however unlikely to affect test results.	Birth serology is applicable to the research question.
Patient flow	Inconclusive results were reported (86/10814). The majority of which (60/86, 69%) were due to mothers' RHD null variants. One birth sample was missing for inconclusive results. (p. 1231) Compliance was 69.7% at the beginning of the study and 98.3% at the end of the study. (p. 1231)	

Source: QUADAS-2 (Whiting et al., 2011)

Study ID	Hyland 2017	
Domain	**Risk of bias**	**Applicability**
Patient selection	Patient enrolment was prospective after informed consent (p. 2). Not stated if patients were consecutive, but authors contacted and confirmed consecutive women invited to participate. It was not stated if women with multiple pregnancies were eligible for inclusion. It was not stated whether women who were sensitised were eligible for inclusion in this study.	Applicability to the Australian population is high and the setting is similar to a diagnostic screening in Australia.
Index test	NIPT was provided at median 19.29 weeks (range 9–37.1) (p. 2). Maternal plasma was the source of the cffDNA DNA was automatically extracted, there was no manual sample handling (High throughput). (p. 2) qPCR of RHD exons 5 and 10. CCR5 was used as an internal control to verify that cfDNA (maternal and fetal) was extracted from each sample and to compare background cfDNA levels as a measure of maternal white cell lysis. (p. 2) Not stated, but likely the index test was carried out without knowledge of the reference standard as cord blood testing occurs postnatally.	Uncertain what platform, genes or algorithms will be used in the Australian setting.
Reference standard	Unclear if cord serology was interpreted without knowledge of the test, however, unlikely to affect test results.	Birth serology (taken at delivery) is applicable to the research question.
Patient flow	15 women were excluded from the analysis as they were determined to carry maternal variants (p. 3). Exclusion of these women would increase the apparent accuracy of the index test. Inconclusive results were reported, all were attributed to inherited paternal variants. Variants were sent for massively parallel sequencing for identification. Cord serology was missing for 48/647 (7.4%) women including fetal death (n=8), and n=40 (6.2%) lost to follow-up (p. 4).	

Source: QUADAS-2 (Whiting et al., 2011)

Study ID	Macher 2012	
Domain	**Risk of bias**	**Applicability**
Patient selection	Consecutive enrolment: all RhD negative women at GW 10–28 included (p. 491). Multiple pregnancies were not excluded (p. 492). Exclusions not reported. It was not stated whether women with RHD variants or Rh alloimmunised women were eligible for inclusion.	Single institution in Spain. The applicability to the Australian population is unclear in terms of RHD genotype and phenotype prevalence The setting is probably similar to diagnostic screening in Australia.
Index test	Maternal plasma was the source of the cffDNA. DNA was automatically extracted (High throughput). (p. 491) Single/Multiplex RT-PCR on exons 5 and exon 7. The primers and probe designed for exons 5 and 7 do not permit amplification of the majority of non-functional rearranged RHD/RHCD genes seen in Exon 10. The SRY gene served as an internal control marker to confirm the presence of fetal DNA. The index test was carried out without knowledge of the reference standard (before birth).	Uncertain what platform, genes or algorithms will be used in the Australian setting. Repeat testing and request for second sample may not be feasible in the target population.
Reference standard	Description of reference standard not provided but unlikely its conduct/interpretation would have introduced bias. Reference standard is not perfect, but further interrogation of FP/FN samples not reported. Not stated if cord serology results interpreted without knowledge of the results of the index test.	Birth serology is applicable to the research question
Patient flow	Index test conducted at GW10–28. Reference standard conducted at birth in all patients. This is appropriate. Not all patients included in the analysis (missing 981 samples due to pregnancy ongoing).	

Source: QUADAS-2 (Whiting et al., 2011)

Study ID	Manfroi 2018	
Domain	**Risk of bias**	**Applicability**
Patient selection	Consecutive Rh D negative pregnant women presenting for screening or invasive diagnostic procedures (p. 2) Only mothers with known positive or unknown paternal phenotypes screened. There were no exclusions (p. 4). Multiple pregnancies were permitted, the population included nulliparous and multiparous women, and women with positive antenatal screening were included. The study states that RHD genotyping is performed in Italy for women who are alloimmunised.	Study conducted in Italy. Unclear applicability in terms of prevalence of RHD genotypes and Rh D phenotype. The likelihood of a positive fetus in this patient population (known Rh D positive partner) would be higher than in all pregnant RhD women. This may affect the reported accuracy of the test.
Index test	Maternal plasma was the source of the cffDNA (p. 4). RT-qPCR on exons 5, 7 and 10. Thresholds were predefined and outlined in Table I (p. 3) Internal controls for fetal DNA maize DNA, with positive and negative plasma controls (not described) used for PCR (p. 3) Unclear if results were interpreted without knowledge of reference standard but likely due to timing of tests performed.	Uncertain what platform, genes or algorithms will be used in the Australian setting.
Reference standard	Unclear if cord serology was interpreted without knowledge of the test, the authors state that the serology was performed locally following validated procedures (p. 3) , however, unlikely to affect test results.	Birth serology is applicable to the research question.
Patient flow	NIPT was offered at GW 11–30 however the analysis was restricted analysis to GW 24–28^{+6}. The exclusion of samples prior to GW 23 favours the index text. Inconclusive results were reported (p. 3, p. 4).	

Source: QUADAS-2 (Whiting et al., 2011)

Study ID	Moise 2016	
Domain	**Risk of bias**	**Applicability**
Patient selection	Consecutive RhD negative women in their first trimester with no evidence of Rh alloimmunisation were eligible to participate (p. 1341). Multiple pregnancies were permitted (p. 1342) and no inappropriate exclusions.	Applicability to the Australian population is unclear in terms of RHD genotype and phenotype prevalence. The setting is similar to diagnostic screening in Australia.
Index test	cffDNA was removed from whole blood and extraction automated using magnetic bead-based separation (p. 1341) SensiGENE Fetal RHD Genotyping test used to interrogate alleles for exons 4, 5, and 7 of the RHD gene as well as the RHD pseudogene. TGIF served as a control. (p. 1341). MALDI-TOF mass spectrometry platform used to detect the control and fetal genetic sequences. (p. 1341) Samples found to contain less than 104 fetal copies were excluded from the final analysis as 'quantity not sufficient'. Samples were from frozen samples and tested in batches (not prospectively as in clinical practice). Final results were released to the principal investigator at the end of the study (p. 1341)	Uncertain what platform, genes or algorithms will be used in the Australian setting. Unlikely MALDI-TOF to be used as means of detecting RHD alleles.
Reference standard	Neonatal serology for typing after birth was used, and was conducted without knowledge of the index test (p. 1340)	Birth serology is applicable to the research question.
Patient flow	There was some patients lost to follow-up at each trimester (p. 1341). Of 520 patients initially enrolled, results for 425 were available at the third trimester. Many missing samples did not have available cord serology (p. 1343). Others were not tested as a result of improper labelling, receipt at the laboratory after the stability period, or inability of the sample to be located (Fig. 1). Not clear if this missing data is likely to affect the interpretation of test results but exclusion of inconclusive results in the analysis favours the index test.	

Source: QUADAS-2 (Whiting et al., 2011)

Study ID	Picchassi 2014	
Domain	**Risk of bias**	**Applicability**
Patient selection	RhD negative women consecutively enrolled (p. 23). NIPT was offered in GW 10–14 . Unclear if women were sensitised, or if multiple pregnancies were permitted. Not clear if difficult to diagnose were excluded (weak Rh D etc.)	Study conducted in a single centre in Italy. Applicability to the Australian population is unclear in terms of RHD genotype and phenotype prevalence. The setting is probably similar to a diagnostic screening in Australia, but samples collected prior to GW 12 may not be applicable to the Australian context.
Index test	RT-qPCR, exon 5 and exon 7 were used (p. 23) TERT gene internal control for total DNA (p. 23). Two RT-qPCRs were performed, in the first test 216 were analysed, in the 2nd test a total of 108 samples were reanalysed. (p.23) It is unclear if the threshold was predetermined. (p. 23) DNA was extracted using a kit (p. 23; not high throughput) Unclear if results were interpreted without knowledge of reference standard.	Uncertain what platform, genes or algorithms will be used in the Australian setting.
Reference standard	Unclear if cord serology was interpreted without knowledge of the test , however, unlikely to affect test results. There was insufficient information about cord serology to define bias.	Birth serology is applicable to the research question.
Patient flow	Serology was obtained in 193/216 participants. Inconclusive data was not reported (p. 23) and not clear if there were any. Exclusion of difficult to diagnose may favour the index test.	

Source: QUADAS-2 (Whiting et al., 2011)

D3.3 Level III-1 – Non-consecutive patients with valid reference standard

Study ID	Jakobsen 2018	
Domain	**Risk of bias**	**Applicability**
Patient selection	Data retrieved from electronic medical records of Rh D negative women in a region south of Denmark (p. 2). Multiple pregnancies were permitted (p. 2) and 30 women delivered twice during the study period (p. 2). BMIs were recorded at week 12 but is not available at time of NIPT. (p. 2). It is not stated whether women who were Rh D alloimmunised were excluded. Of 4500 eligible pregnancies, data were available for 1649 neonates (1618 pregnancies) (not clear or explained why)	Applicability to the Australian population is unclear in terms of RHD genotype and phenotype prevalence, however, the setting is similar to a diagnostic screening in Australia.
Index test	Maternal plasma was the source of the cffDNA (p. 2) Automated DNA extraction was used (High throughput, p. 2) PCR on RHD exon 5 and exon 10 (p. 2). CCR5 was used as an internal control (p. 2) Results were interpreted without the knowledge of the reference standard (p. 3). Unclear if algorithms or thresholds were predetermined from text.	Uncertain what platform, genes or algorithms will be used in the Australian setting.
Reference standard	Unclear if cord serology was interpreted without knowledge of the test , however, unlikely to affect test results. There was insufficient information about cord serology to define bias.	Birth serology is applicable to the research question.
Patient flow	NIPT was offered in week 25 (p.2). Serology tested at birth. Inconclusive results were reported (Table 1, p. 3)	

Source: QUADAS-2 (Whiting et al., 2011)

Study ID	Orzińska 2015	
Domain	**Risk of bias**	**Applicability**
Patient selection	Unclear if consecutive or random sample (p. 362) Women are those referred due to suspected alloimmunisation (p. 363) including Rh D negative pregnant women Unclear if multiple pregnancies were permitted. 6 women were excluded as they had a variant RHD gene, and NIPT was impossible. 1 woman had an RHD*R variant and was included. Other exclusions not clearly reported.	Study conducted in Poland. Applicability to Australian population is unclear in terms of RHD genotype and phenotype prevalence, May not be applicable as includes women with suspected alloimmunisation
Index test	The source of the cffDNA was maternal plasma (p. 362) DNA isolation was conducted automatically (p. 362) RT-qPCR exon 5 and exon 7 (p. 362) from 2012. Prior to this was exon 7, 10 and intron 4. CCR5 was used as an internal control, to confirm the presence of fetal DNA, SRY or another marker inherited from the father (from a panel of 21 biallelic polymorphisms)(p. 362) Uncertain if a threshold or algorithm was prespecified.	Uncertain what platform, genes or algorithms will be used in the Australian setting.
Reference standard	Birth serology from cord blood or material from swabs	Birth serology is applicable to the research question.
Patient flow	NIPT was offered from week 5–39 of pregnancy but week 15 recommended as a minimum age. Insufficient information about timing of postnatal serology, but not likely to influence test results. Serious concerns regarding reporting of missing data and inconclusive results.	

Source: QUADAS-2 (Whiting et al., 2011)

Study ID	Papasavva 2016	
Domain	**Risk of bias**	**Applicability**
Patient selection	Rh D negative pregnant women with RhD positive partners at risk of HDN were referred by their obstetrician (p. 3) It is unclear if a random or consecutive sample was enrolled. It is unclear if there were any inappropriate exclusions. It is unclear if women with multiple pregnancies were excluded.	Study conducted in Cyprus. Applicability to Australian population is low. Prevalence of Rh D negative phenotype is <10% and the population is narrower (as only those with Rh D positive fathers included). The likelihood of a positive fetus in this patient population is higher than in all pregnant RhD negative women.
Index test	Maternal plasma was the source of the cffDNA. (p. 3) DNA isolation was extracted using a kit (p. 3; not high throughput) RT-PCR was used to amplify exons 4, 5 and 10 (p. 3). SRY gene was used as the internal control (p. 3) Results were interpreted without knowledge of reference standard (p. 5) MPLA was performed on two inconclusive results (p. 4) Unclear if threshold was predetermined.	Uncertain what platform, genes or algorithms will be used in the Australian setting.
Reference standard	Cord serology was interpreted without knowledge of index test (p. 5)	Birth serology is applicable to the research question.
Patient flow	NIPT was offered after GW 16. Inconclusive results were reported for two women but cord serology of the newborns missing. Cord serology was obtained for all women. No report if any women were lost to follow-up.	

Source: QUADAS-2 (Whiting et al., 2011)

Study ID	Sørenson 2018	
Domain	**Risk of bias**	**Applicability**
Patient selection	Patient recruitment was not consecutive - 283 samples collected fresh, 90 samples from frozen. (p. 2) Women were not alloimmunised (p. 5) It is unclear if multiple pregnancies were allowed.	Study conducted in Norway. Applicability to the Australian population is unclear in terms of RHD genotype and phenotype prevalence. The setting is probably similar to a diagnostic screening in Australia, unclear applicability regarding the use of frozen samples.
Index test	Source of cffDNA is maternal plasma (p. 2) DNA was automatically extracted (p. 2) RT-qPCR targeting exon 7 and exon 10. GAPDH was used as an internal control (housekeeping gene) (p. 2) It is unclear if the algorithm (Table 1) was prespecified. It is unclear if test was performed without knowledge of the reference standard, but due to timing this is unlikely.	Uncertain what platform, genes or algorithms will be used in the Australian setting.
Reference standard	Unknown if reference standard was carried out without knowledge of index test. Discrepancies between birth serology and RHD testing were investigated using molecular techniques (p. 2)	Birth serology is applicable to the research question.
Patient flow	Samples were collected GW 24 (range 16–36) and serology collected at birth. Inconclusive results from nine patients had maternal variants that hampered the determination of the RHD type of the fetus. These data are sufficiently reported.	

Source: QUADAS-2 (Whiting et al., 2011)

D4 Question 4

D4.1 Level I – Systematic review of RCTs

Source: Shea et al. 2007. BMC Medical Research Methodology 7:10 doi:10.1186/1471-2288-7-10 http://amstar.ca/Amstar_checklist.php

No studies identified

D4.2 Level I – Systematic review of observational and cohort studies

Source: Shea et al. 2017. BMJ 358: j4008 doi:10.1136 (https://doi.org/10.1136/bmj.j4008)

No studies identified

D4.3 Level II – Prospective cohort studies

Source: Chapter 8 of the Cochrane Handbook for Systematic Reviews of Interventions, version 5.1.0

Study ID	**MacKenzie 2006**	
Domain	**Judgement**	**Description**
Bias due to failure to develop and apply appropriate eligibility criteria	Low risk	45 unsensitised Rh D negative pregnant women. The women were not selected based on maternal body weight.
Bias due to flawed measurement of both exposure and outcome	Low risk	Surrogate outcome: peak serum anti-D levels may not directly correlate with protection against alloimmunisation. Assessors were not blinded however, peak serum level is quantitative and unlikely to be affected by assessors knowledge.
Bias due to failure to adequately control confounding	Serious risk	Possible confounding due to most patients BSA being normally distributed, with few patients at the higher end. No potential attempt to correct for confounders.
Bias due to incomplete or inadequately short follow-up	Serious risk	Follow-up was 84 days after first injection. Follow-up is sufficient for peak serum levels and demonstrated persistence of antibodies but follow-up was insufficient and cohort too small to detect new sensitisations. Data relating to persistence of anti-D not adequately reported.
Overall risk of bias	***Serious risk***	***The study has important problems and cannot be considered comparable to a well-performed randomised trial.***

Source: Table 5.5 GRADE handbook http://gdt.guidelinedevelopment.org/app/handbook/handbook.html#h.m9385o5z3li7

Study ID	**Woelfer 2004**	
Domain	**Judgement**	
Bias due to failure to develop and apply appropriate eligibility criteria	Low risk	26 Rh D negative women who gave birth to positive child were consecutively enrolled. There was no attempt to specifically enrol women with high and low BMI. The study does not report range, median, mean or any statistics to illustrate weight characteristics of participants. No mention of dropouts or loss to follow-up
Bias due to flawed measurement of both exposure and outcome	Moderate risk	Surrogate outcome: peak serum anti-D levels may not be directly correlated with protection against alloimmunisation. No mention of blinding of authors to outcome assessment however, peak serum levels are a quantitative measure and unlikely to be affected by blinding. Low patient numbers. Critical risk for outcome of alloimmunisation.
Bias due to failure to adequately control confounding	Moderate risk	Multiple and univariate linear regression analyses were applied using each measurement as a case and a general linear model was constructed. Caesarean section considered a potential confounder, but other factors not considered or discussed.
Bias due to incomplete or inadequately short follow-up	Low risk	Follow-up for only 14 days. Follow-up was sufficient for pharmacokinetic outcomes but not sufficient to see if women were sensitised. The evidence is insufficient to know if lower peak serum levels of IgG translates to a higher sensitisation rate
Overall risk of bias	***Moderate risk***	***The study appears to provide sound evidence for a non-randomised study but cannot be considered comparable to a well-performed randomised trial for the outcome of anti-D levels.***

Source: Table 5.5 GRADE handbook http://gdt.guidelinedevelopment.org/app/handbook/handbook.html#h.m9385o5z3li7

D4.4 Level III – All or none, retrospective cohort studies

Study ID	Bichler 2003	
Domain	**Judgement**	
Bias due to failure to develop and apply appropriate eligibility criteria	Serious risk	Rh D negative pregnant women were enrolled if they were less than 28 weeks gestation. Population was not specifically recruited to compare high BMI vs low BMI. Only two women weighed over 80kg, both in the i.m. group. Block randomisation, but allocation concealment not attempted (i.e. patients and clinicians aware of treatment group).
Bias due to flawed measurement of both exposure and outcome	Serious risk	Surrogate outcome: peak serum anti-D levels may not directly correlate with protection against alloimmunisation. No recording of when weight and height were measured. The study was not sufficiently powered to detect differences in weight and peak serum concentrations.
Bias due to failure to adequately control confounding	Critical	Only two patients who had a high BMI, and both patients who were >80 kg were in the i.m. group. No statistical tests were performed, and study was insufficiently powered.
Bias due to incomplete or inadequately short follow-up	Moderate risk	Both groups were followed for 6 months after birth to test for sensitisations using indirect antiglobulin test. One woman who tested positive was followed for an additional two weeks with the enzyme test; however, women were not followed to next pregnancy. Follow-up was appropriate for pharmacokinetic outcomes. The evidence is insufficient to know if lower peak serum levels of IgG translates to a higher sensitisation rate.
Overall risk of bias	***Critical risk***	***The study is too problematic to provide any useful evidence on the outcome of interest.***

Source: Table 5.5 GRADE handbook http://gdt.guidelinedevelopment.org/app/handbook/handbook.html#h.m9385o5z3li7

Study ID	Koelewijn 2009	
Domain	**Judgement**	**Description**
Bias due to failure to develop and apply appropriate eligibility criteria	Moderate risk	Cases were Rh D negative pregnant women who were parae-1 who had sensitisation detected in their 1st trimester. Controls were Rh D negative and positive, randomly selected and matched based on GW and case worker. No baseline characteristics reported. All patients from same population but over-representation of women from primary setting in the control group.
Bias due to flawed measurement of both exposure and outcome	Moderate risk	Retrospective collection of potential risk factors from medical records and interview with obstetric care worker or pregnant woman (controls). Involves recall bias. Due to time constraints, an over-representation of women from primary setting (midwives, GPs) vs obstetric setting (3:1) in the control group necessitated weighting in the final analysis to avoid over (or under)-estimation of exposure to potential risk factors.
Bias due to failure to adequately control confounding	Low risk	The authors take sufficient measure to assess potential confounding variables in the analysis.
Bias due to incomplete or inadequately short follow-up	Moderate risk	Cases were identified over a 5-year period (1999–2004) whereas controls were selected over a 10 month period (Sept 2002–Jun 2003). The short exposure period and low case numbers suggest possible bias in the control group
Overall risk of bias	***Moderate risk***	***The study appears to provide sound evidence for a non-randomised study but cannot be considered comparable to a well-performed randomised trial.***

Source: Table 5.5 GRADE handbook http://gdt.guidelinedevelopment.org/app/handbook/handbook.html#h.m9385o5z3li7

Appendix E Data extraction forms

E1 Question 1

E1.1 Level I – Systematic review of RCTs

STUDY DETAILS: SR/MA			
Citation			
McBain 2015 McBain, R. D., Crowther, C. A., & Middleton, P. (2015). Anti-D administration in pregnancy for preventing Rhesus alloimmunisation. *Cochrane Database Syst Rev*(9), CD000020.			
Affiliation/Source of funds			
Cochrane Pregnancy and Childbirth Group			
Study design	**Level of evidence**	**Location**	**Setting**
SR and MA of Level II studies (RCTs)	Level I	Various Lee 1995 (UK); Huchet 1987 (France)	Obstetrics and maternal care
Intervention		**Comparator**	
Anti-D immunoglobulin at 28 weeks or more of gestation (regardless of timing, dose and route of administration) Huchet 1987 administered 100 µg (500 IU) Rh D IgG at 28 and 34weeks' gestation (total dose of 200 µg). Lee 1995 administered 50 µg (250 IU) Rh D IgG at 28 and 34 weeks' gestation (total dose of 100 µg).		No treatment; or a placebo; or comparisons of different anti-D regimens.	
Population characteristics			
Rh negative women without anti-D antibodies at 28 weeks gestation			
Length of follow-up		**Outcomes measured**	
Not applicable		- Incidence of Rh D alloimmunisation (during pregnancy, postpartum and in subsequent pregnancies) - Incidence of positive Kleihauer test (a test that detects fetal cells in the maternal blood) - Neonatal morbidity (e.g. neonatal jaundice, anaemia and kernicterus) in current or subsequent pregnancies - Adverse events attributed to anti-D treatment	
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			
Rating: Low Description: Cochrane review. The systematic review provides an accurate and comprehensive summary of the results of the available studies that address the question of interest. Data from Huchet 1987 was obtained from a translation of the paper. Included studies: Overall risk of bias was judged to be high for Huchet 1987 and unclear for Lee 1995. The Huchet 1987 trial was quasirandomised, and thus at high risk of selection bias; the Lee 1995 trial did not clearly detail its selection methods. Neither trial used a placebo, and both trials had high rates of attrition.			
RESULTS:			

Outcome **No. patients (No. trials)**	**RAADP** **n/N (%)**	**No therapy** **n/N (%)**	**Risk estimate** **RR (95% CI)**	***Statistical significance*** ***p-value*** **Heterogeneity [a]** **I^2 (*p*-value)**
RAADP (two-dose) versus no therapy				

Incidence of Rh D alloimmunisation (during pregnancy) N=3902 (2 trials)	5/1879 (0.27)	13/2023 (0.64)	RR 0.42 (0.15, 1.17)	*No significant difference* *p = 0.096* No significant heterogeneity I^2 = 13% (*p* = 0.28)
Incidence of Rh D alloimmunisation (at birth of Rh positive infant) N=2297 (2 trials)	5/1112 (0.45)	13/1185 (1.10)	RR 0.42 (0.15, 1.17)	*No significant difference* *p = 0.096* No significant heterogeneity I^2 = 22% (*p* = 0.26)
Incidence of Rh D alloimmunisation (at birth of Rh positive infant and at up to 12 months follow-up) N=2048 (2 trials)	6/985 (0.61)	16/1063 (1.51)	RR 0.39 (0.10, 1.62)	*No significant difference* *p = 0.20* Moderate heterogeneity I^2 = 39% (*p* = 0.20)
Incidence of Rh D alloimmunisation (after birth of Rh positive infant and at 2–12 months follow-up, primigravidae) N=722 (1 trial)	0/362 (0)	4/360 (1.11)	RR 0.11 (0.01, 2.04)	*No significant difference* *p = 0.14*
Incidence of Rh D alloimmunisation (subsequent pregnancy) No studies identified	No data	No data	No data	No data
Incidence of a positive Kleihauer test (32 to 35 weeks gestation) N=1884 (1 trial)	39/927 (4.21)	67/957 (7.00)	RR 0.60 (0.41, 0.88)	*Favours RAADP* *p = 0.0094*
Incidence of a positive Kleihauer test (at birth of Rh positive infant) N=1189 (1 trial)	73/599 (12.19)	119/590 (20.17)	RR 0.60 (0.46, 0.79)	*Favours RAADP* *p = 0.00023*
Incidence of positive Kleihauer test (Kleihauer > 1/10,000, Rh positive infant) N=1189 (1 trial)	31/599 (5.18)	32/590	RR 0.95 (0.59, 1.54)	*No significant difference* *p = 0.85*
Neonatal morbidity (jaundice) N=1882 (1 trial)	1/927 (0.11)	4/955 (0.42)	RR 0.26 (0.03, 2.30)	*No significant difference* *p = 0.22*
Neonatal morbidity (other) No studies identified	No data	No data	No data	No data
Maternal adverse events No studies identified	No data	No data	No data	No data
RAADP (one-dose) versus placebo				
No studies identified	No data	No data	No data	No data
RAADP (one-dose) versus RAADP (two-dose)				
No studies identified	No data	No data	No data	No data

EXTERNAL VALIDITY
Generalisability (relevance of the study population to the Guidelines target population)
The evidence is directly generalisable to the target population with some caveats. The meta-analysis included two studies of Rh negative pregnant women, one of which (Lee 1995) was restricted to primigravidae women. The other study (Huchet 1987) included both primigravidae and multigravidae women. No studies included Indigenous Australians or Torres Strait Islander people.
Applicability (relevance of the evidence to the Australian health care system)
The evidence is directly applicable the Australian health care context. Both studies were carried out in health care systems comparable with Australia: Lee 1995 (UK) 250 IU at 28 and 34 weeks, Huchet 1987 (France) 500 IU at 28 and 34 weeks
Additional comments
Authors conclusions Existing studies do not provide conclusive evidence that the use of anti-D during pregnancy benefits either mother or baby in terms of incidence of Rhesus D alloimmunisation during the pregnancy or postpartum, or the incidence of neonatal morbidity (jaundice) (low to very low quality evidence). However, women receiving anti-D may be less likely to register a positive Kleihauer test in pregnancy and at the birth of a Rh positive infant (low quality evidence). Fewer women who receive anti-D during pregnancy may have Rhesus D antibodies in a subsequent pregnancy, with benefits for the baby however this needs to be tested in studies of robust design.

CI, confidence interval; IU international units; MA, meta-analysis; µg, microgram; RAADP, routine antenatal anti-D prophylaxis; RCT, randomised controlled trial; RR, relative risk; SD, standard deviation; SR, systematic review; UK, United Kingdom

a. Heterogeneity defined as follows: (i) no significant heterogeneity if $P_{het} > 0.1$ and $I^2 < 25\%$; (ii) mild heterogeneity if $I^2 < 25\%$; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity $I^2 > 50\%$

E1.2 Level I - Systematic review of observational and cohort studies

STUDY DETAILS: SR/MA			
Citation			
Turner 2012 Turner, R. M., Lloyd Jones, M., Anumba, D. O. C., Smith, G. C. S., Spiegelhalter, D. J., Squires, H., Stevens, J. W., Sweeting, M. J., Urbaniak, S. J., Webster, R., & Thompson, S. G. (2012). Routine antenatal anti-D prophylaxis in women who are Rh(D) negative: Meta-analyses adjusted for differences in study design and quality. *PLoS ONE*, 7(2), e30711. doi:http://dx.doi.org/10.1371/journal.pone.0030711.			
Affiliation/Source of funds			
RT, DS, MS and ST were supported by the United Kingdom Medical Research Council grants U.1052.00.001, U.1052.00.011 and U.1052.00.005. MLJ and HS were supported by grants awarded by the National Institute on Health Research (NIHR) Health Technology Assessment Programme. GS was supported by the NIHR Cambridge Comprehensive Biomedical Research Centre. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript			
Study design	**Level of evidence**	**Location**	**Setting**
Meta-analysis of Level II and Level III studies	Level I-III	Various Studies conducted in Canada, Denmark, UK, Sweden, France,	Obstetrics and Maternity
Intervention		**Comparator**	
Dose of 500 IU anti-D immunoglobulin offered i.m. at 28 and 34 weeks in addition to comparator antenatal care		No treatment or placebo, plus postpartum anti-D or during sensitising events according to UK policy	
Population characteristics			
Non-sensitised Rh D negative pregnant women. Some included studies selected primigravidae women only			
Length of follow-up		**Outcomes measured**	
Not specified (various)		Rh D alloimmunisation (during pregnancy, at delivery, or at postpartum follow-up)	
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			
Rating: Moderate Description: No critical flaws. SR *may* provide an accurate summary of the results of the available studies that were included in the review. Included studies: Doses and timing of RAADP differed between studies and there was also substantial variation in timing of follow-up for sensitisation to Rh D. Study populations varied in terms of geography & baseline characteristics (e.g. primigravidae, multigravidae) such that a simple meta-analysis is inappropriate. Selection bias is of critical concern in seven studies, due to use of historical controls who likely differed from the intervention group.			
RESULTS:			

Outcome **No. patients (No. trials)**	**RAADP** **n/N (%)**	**no RAADP** **n/N (%)**	**Risk estimate (95% CI)**	***Statistical significance*** ***p-value*** **Heterogeneity [a]** **I^2 (*p*-value)**
RAADP (one- or two-dose) versus routine care and no RAADP				
Incidence of Rh D alloimmunisation (bias-adjusted)[b] N=NR (10 studies)	NR	NR	OR 0.31 (0.17, 0.56)	*Favours intervention* *p = NR* No significant heterogeneity I^2 = 0% (*p* = NR)

Incidence of Rh D alloimmunisation (naive conventional random effects) N=NR (10 studies)	NR	NR	OR 0.25 (0.18, 0.36)	*Favours intervention* p = NR Mild heterogeneity I^2 = 19% (p = NR)
Incidence of Rh D alloimmunisation (adjustment for internal biases) N=NR (10 studies)	NR	NR	OR 0.28 (0.15, 0.53)	*Favours intervention* *p = NR* No significant heterogeneity I^2 = 0% (p = NR)
Incidence of Rh D alloimmunisation (excluding two studies by Bowman that share control group with Bowman 1978) N=NR (8 studies)	NR	NR	OR 0.31 (0.16, 0.61)	*Favours intervention* *p = NR* No significant heterogeneity I^2 = 0% (p = NR)
Two-dose RAADP (500 IU at 28 and 34 weeks) versus routine care and no RAADP				
Incidence of Rh D alloimmunisation			OR 0.31 (0.09, 0.65)* *estimated (meta-regression analysis)[c]	*Favours intervention* *p = NR* Heterogeneity not reported
Two-dose RAADP (1250 IU at 28 and 34 weeks) versus routine care and no RAADP				
Incidence of Rh D alloimmunisation			OR 0.18 (0.03, 0.53)* *estimated (meta-regression analysis)[c]	*Favours intervention* *p = NR* Heterogeneity not reported
Single dose RAADP (1500 IU at 28-30 weeks) versus routine care and no RAADP				
Incidence of Rh D alloimmunisation			OR 0.42 (0.17, 0.73)* *estimated (meta-regression analysis)[c]	*Favours intervention* *p = NR* Heterogeneity not reported
Individual studies				
Bowman 1978	NR	NR	OR 0.02 (0.001,0.33)	NR
Bowman & Pollock 1978	NR	NR	OR 0.34 (0.18, 0.65)	NR
Bowman 1987	NR	NR	OR 0.18 (0.12, 0.65)	NR
Hermann 1984	NR	NR	OR 0.24 (0.05, 1.10)	NR
Huchet 1987	NR	NR	OR 0.14 (0.02, 1.14)	NR
Lee 1995	NR	NR	OR 0.56 (0.14, 2.24)	NR
MacKenzie 1999	NR	NR	OR 0.44 (0.22, 0.86)	NR
Mayne 1997	NR	NR	OR 0.25 (0.08, 0.74)	NR
Tovey 1983	NR	NR	OR 0.16 (0.04, 0.67)	NR
Trolle 1989	NR	NR	OR 0.08 (0.005, 1.49)	NR
EXTERNAL VALIDITY				
Generalisability (relevance of the study population to the Guidelines target population)				
The evidence is directly applicable to the Australian Guidelines target population with few caveats. No studies included Indigenous Australians or Torres Strait Islander people. Five of the nine studies identified included only primigravidae or primiparae women, which may not be reflective of the Guidelines target population. Three allowed for primigravidae or unsensitised multigravidae, which is reflective of the Guidelines target population. One study was completely unselected (28% primiparae). The studies are two decades old and therefore the population may not be reflective of modern factors such as comorbidities. Some of the participants in the historical control arms were from rural populations which may differ in variables such as rates of invasive procedures.				

Applicability (relevance of the evidence to the Australian health care system)
The evidence is directly applicable to the Australian health care system. The studies were conducted in Canada, Denmark, France, US and UK. The studies are quite old, and used different doses and different preparations of Anti-D. Some did not state the route of administration.
Additional comments
Authors conclusion: Available evidence supports the policy of RAADP (one or two-dose). The probability that a dose of 1250 IU at 28 and 34 weeks is most effective is 83%, while the probability that a dose of 500 IU at 28 and 34 weeks is most effective is 15%, The probability that a single dose of 1500 IU is least effective among the three regimens is 76%. MacKenzie, Mayne and Hutchet were considered higher quality studies.

CI, confidence interval; i.m., intramuscular; IU, international units; MD, mean difference; OR, odds ratio; RAADP, routine antenatal anti-D prophylaxis; RCT, randomised controlled trial; RR, relative risk; SD, standard deviation; UK, United Kingdom; US, United States

a. Heterogeneity defined as follows: (i) no significant heterogeneity if $P_{het} > 0.1$ and $I^2 < 25\%$; (ii) mild heterogeneity if $I^2 < 25\%$; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity I^2 >50%

b. The authors adjusted each study for internal and external biases as rated by four assessors after consideration of a bias checklist. Internal biases related to study design (e.g., patient selection, performance, attrition, outcome) and external biases related to anti-D prophylaxis.

c. Subgroup analysis of different dosing regimes were not conclusive, therefore the authors elicited opinion on the relative effectiveness of all RAADP treatment regimens and performed a meta-regression model to estimate the association between the relative and observed effectiveness for different treatment regimes.

STUDY DETAILS: SR/MA			
Citation			
Pilgrim 2009 Pilgrim, H., Lloyd Jones, M., & Rees, A. (2009). Routine antenatal anti-D prophylaxis for RhD negative women: a systematic review and economic evaluation. *Health Technology Assessment*, 13(37), 1-126.			
Affiliation/Source of funds			
National Institute for Health and Clinical Excellence			
Study design	**Level of evidence**	**Location**	**Setting**
Systematic review of Level II and Level III studies	Level I-III	No restriction Studies identified from Canada, Denmark, France, US and UK	Obstetrics and maternal care
Intervention		**Comparator**	
Routine antenatal anti-D prophylaxis (RAADP) using either two doses of at least 500 IU at 28 and 34 weeks gestation or a single dose of at least 1500 IU at 28 weeks gestation, in either case followed by a further dose of anti-D given at or within 72 hrs of delivery of a positive infant		RAADP using different dosing regimens and/or methods of administration and no RAADP	
Population characteristics			
Pregnant women who are RhD negative			
Length of follow-up		**Outcomes measured**	
None specified		- Rh D alloimmunisation rates (overall and in subsequent pregnancies), - incidence of HDFN - infant mortality - Infant disability - health-related quality of life - adverse effects of treatment	
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			

Rating: Moderate

Description: No critical flaws. SR *may* provide an accurate summary of the results of the available studies that were included in the review.

Included studies: Many were poorly designed. Lack of blinding is considered relatively unimportant due to the incidence of alloimmunisation (presence or absence of anti-D) is objective. Six of the included studies use historical controls which introduces serious bias. The baseline demographics of treatment and controls may not be comparable and could underestimate the incidence of sensitisation (e.g. earlier tests for anti-D were less sensitive). For example, a decrease in Rh D alloimmunisation is seen in the control group of MacKenzie 1999 over time, although not to the same extent as the intervention group. This change is likely due to changes in obstetric practice, possibly including more comprehensive use of anti-D following sensitising events. Changes or differences in obstetric practice over time (e.g. rates of caesarean section, induction before GW40) may equally influence the incidence of sensitisation among either group, suggesting studies that use historic controls may overestimate rather than underestimate the degree of protection when compared to current practice.

Timing of outcome measure is also of concern and could possibly underestimate the incidence of sensitisation with few studies reporting number of women sensitised in a subsequent pregnancy. Poor reporting of intent to treat numbers (number of women eligible for the intervention vs number of women who received the intervention) also introduces bias. Exclusion of failures over estimates the clinical effectiveness of RAADP.

RESULTS:

Outcome **No. patients (No. trials)**	**RAADP** **n/N (%)** **% (95% CI)**	**No treatment** **n/N (%)** **% (95% CI)**	**Risk estimate (95% CI)**	***Statistical significance*** ***p-value*** **Heterogeneity [a]** **I^2 (*p*-value)**
RAADP (one or two-dose) versus no therapy or placebo				
Overall number of women sensitised *Individual study data (authors' figures, Table 13, p35-36)*				
MacKenzie 2004	0/248 (0)	Not applicable	Not calculable	
MacKenzie 1999	12/3320 (0.4)	26/3146 (0.8)	RR 0.44 (0.22, 0.86)	
Mayne 1997	4/1425 (0.3)	16/1426 (1.1)	RR 0.25 (0.08, 0.74)	
Trolle 1989	0/346 (0)	6/354 (1.7)	RR 0.08 (0.00, 1.38)	
Bowman 1987	25/9303 (0.3)	62/3533 (1.8)	RR 0.15 (0.10, 0.24)	
Huchet 1987	1/599 (0.2)	7/590 (1.2)	RR 0.14 (0.02, 1.14)	
Tovey 1983	6/2037 (0.3)	32/2721 (1.2)	RR 0.25 (0.10, 0.59)	
Bowman & Pollock 1978	5/1804 (0.3)	62/3533 (1.8)	RR 0.16 (0.06, 0.39)	
Bowman 1978	1/1357 (0.1)	45/2768 (1.6)	RR 0.04 (0.01, 0.32)	
Overall number of women sensitised N=NR (9 studies)	54/20439 (0.26)	NR/NR	NR	NR
Overall number of women sensitised [b] N=31961 (8 studies)* *Excluding MacKenzie 2004 *Bowman 1978, Bowman & Pollock 1978, Bowman 1987 combined to avoid triple counting controls	54/20191 (0.27)	149/11770 (1.27)	M-H Random OR 0.22 (0.13, 0.36) RR 0.22 (0.14, 0.37)	*Favours RAADP* *p < 0.00001* Moderate heterogeneity I^2 = 41% (*p* = 0.13)
Overall number of women sensitised [b] N=30598 (7 studies)* *Excluding Bowman 1978 and MacKenzie 2004 *Bowman & Pollock 1978 and Bowman 1987 combined to avoid double counting controls	53/18834 (0.28)	149/11770 (1.27)	M-H Random OR 0.23 (0.14, 0.36) RR 0.23 (0.15, 0.36)	*Favours RAADP* *p < 0.00001* Moderate heterogeneity I^2 = 31% (*p* = 0.20)

Overall number of women sensitised *Individual study data (including women excluded from published analysis for various reasons, Table 14, p38)*				
MacKenzie 2004	NR	Not applicable	Not calculable	
MacKenzie 1999	12/3320 (0.36) 0.4 (0.2, 0.6)	26/3146 (0.83) 0.8 (0.5, 1.1)	RR 0.44 (0.22, 0.87)	
Mayne 1997	4/1425 (0.28) 0.3 (0.0, 0.6)	16/1426 (1.12) 1.1 (0.6, 1.7)	RR 0.25 (0.08, 0.75)	
Trolle 1989	0/346 (0) 0.0 (0.0, 0.0)	6/354 (1.69) 1.7 (0.4, 3.0)	RR 0.08 (0.00, 1.39)	
Bowman 1987	30/9295 (0.32) 0.3 (0.2, 0.4)	62/3533 (1.75) 1.8 (1.3, 2.2)	RR 0.18 (0.12, 0.28)	
Huchet 1987	1/599 (0.17) 0.2 (-0.2, 0.5)	7/590 (1.19) 1.2 (0.3, 2.1)	RR 0.14 (0.02, 1.14)	
Tovey 1983	6/2037 (0.29) 0.3 (0.1, 0.5)	32/2721 (1.18) 1.2 (0.8, 1.6)	RR 0.25 (0.10, 0.60)	
Bowman & Pollock 1978	11/1806 (0.61) 0.6 (0.3, 1.0)	62/3533 (1.75) 1.8 (1.3, 2.2)	RR 0.34 (0.18, 0.65)	
Bowman 1978	1/1357 (0.07) 0.1 (–0.1, 0.3)	45/2768 (1.63) 1.6 (1.2, 2.1)	RR 0.05 (0.01, 0.33)	
Women found to be sensitised at a subsequent pregnancy *Individual study data (table 12, p34)*				
Bowman 1978	0/343 (0.0) (0.0, 0.0)	No data	not calculable	not calculable
Tovey 1983	2/325 (0.6)* 0.6 (–0.2, 1.5) *Not reported in Chilcott 2003	11/582 (1.9) 1.9 (0.8, 3.0)	not calculable	not calculable
Mayne 1997	4/1425 (0.3) 0.3 (0.0, 0.6)	16/1425 (1.1) 1.1 (0.6, 1.7)	NR	NR
MacKenzie 1999	12/3320 (0.4) 0.4 (0.2, 0.6)	26/3146 (0.8) 0.8 (0.5, 1.1)	NR	NR
Women sensitised during pregnancy or within 3 days of delivery *Individual study data (table 39, p102)*				
Bowman 1978	1/1357 (0.1) 0.1 (–0.1, 0.3)	45/2768 (1.6) (1.2, 2.1)	NR	NR
Bowman & Pollock 1978	5/1804 (0.3) 0.3 (0.0, 0.5)	62/3533 (1.8) (1.3, 2.2)	NR	NR
Tovey 1983	4/1563 (0.3) 0.3 (0.0, 0.6)	29/2582 (1.1) 1.1 (0.7, 1.5)	NR	NR
Bowman 1987	18/9303 (0.2) 0.2 (0.1, 0.3)	62/3533 (1.8) (1.3, 2.2)	NR	NR
Huchet 1987	1/599 (0.2) 0.2 (0.0, 0.5)	6/590 (1.0) 1.0 (0.2, 1.8)	NR	NR
Trolle 1989	No data/346	No data/354	not calculable	not calculable
Mayne 1997	No data/1425	No data/1426	not calculable	not calculable
MacKenzie 1999	No data/3320	No data/3146	not calculable	not calculable
Women sensitised at postnatal follow-up *Individual study data (table 40, p103)*				

Bowman 1978	1/1004 (0.1) 0.1 (–0.1, 0.3)	45/2768 (1.6) [c] 1.6 (1.2, 2.1)	NR	
Bowman & Pollock 1978	No data/807	50/3533 (1.4)[c] 1.4 (1.0, 1.8)	not calculable	not calculable
Tovey 1983	2/1059 (0.2)[d] 0.2 (–0.1, 0.5)	No data	not calculable	not calculable
Bowman 1987	25/9303 (0.3) [c] 0.3 (0.2, 0.4)	50/3533 (1.4) [c] 1.4 (1.0, 1.8)	NR	NR
Huchet 1987	1/472 (0.2) 0.2 (–0.2, 0.6)	7/468 (1.5) 1.5 (0.4, 2.6)	NR	NR
Trolle 1989	0/291 (0.0) 0.0 (0.0, 0.0)	6/322 (1.9) 1.9 (0.4, 3.3)	NR	NR
Mayne 1997	No data/1425	No data/1426	not calculable	not calculable
MacKenzie 1999	No data/3320	No data/3146	not calculable	not calculable
Overall number of women sensitised [b] N=31955 (8 studies)* *excluding MacKenzie 2004 * Bowman 1978, Bowman & Pollock 1978 and Bowman 1987 combined to avoid triple counting control	65/20185 (0.32)	149/11770 (1.27)	OR 0.23 (0.17, 0.32) RR 0.24 (0.18, 0.32)	*Favours RAADP* *p < 0.00001* No significant heterogeneity I^2 = 1% (*p* = 0.41)
Overall number of women sensitised [b] N=30598 (7 studies)* * Excluding Bowman 1978 * Bowman & Pollock 1978 and Bowman 1987 combined to avoid double counting controls	64/18828 (0.34)	149/11770 (1.27)	OR 0.24 (0.18, 0.33) RR 0.25 (0.18, 0.33)	*Favours RAADP* *p < 0.00001* No significant heterogeneity I^2 = 0% (*p* = 0.51)
RAADP (one-dose, 1500IU) versus no therapy or placebo				
Rh D alloimmunisation N=15334 (3 studies)* (Trolle 1989, Bowman & Pollock 1978, Bowman 1987) *unselected women (primigravidae and multigravidae)	41/11447 (0.36) 0.34% (0.28, 0.40%)	68/3887 (1.75) 1.60% (0.37, 2.83%)	OR 0.20 (0.13, 0.29) [e]	*Favours RAADP* *p = NR* No significant heterogeneity I^2 = NR (*p* = 0.940)
RAADP (two-dose, 500IU) versus no therapy or placebo				
Rh D alloimmunisation N=13523 (4 studies)* (Huchet 1987, Mayne 1997, Tovey 1983, MacKenzie 1999) *primigravidae only	20/6444 (0.31) 0.30% (0.22, 0.38)	65/7026 (0.93) 0.89% (0.21, 1.56)	OR 0.33 (0.20, 0.55)	*Favours RAADP* *p = NR* No significant heterogeneity I^2 = NR (*p* = 0.812)
RAADP (two-dose, 500IU) versus no therapy or placebo				
Rh D alloimmunisation N= 9317 (2 studies)* (MacKenzie 1999, Mayne 1997) *primigravidae only, UK-based studies	16/4745 (0.34) 0.35% (0.29, 0.40)	42/4572 (0.92) 0.95% (0.18, 1.71)	OR 0.37 (0.20, 0.65)	*Favours RAADP* *No statistics reported* No significant heterogeneity I^2 = NR (*p* = 0.976)
Adverse neonatal events				

Treatment related to HDFN in first pregnancy 1 study (Tovey 1983) [f]	NR	2/18 (11)	NR	
Treatment related to HDFN in subsequent pregnancy 1 study (Tovey 1983) [f]	NR	3/11 (27)	NR	
Treatment related to HDFN in subsequent pregnancy 1 study (Bowman 1987) [g]	NR	7/17 (41)	NR	
Adverse maternal events attributed to treatment				
mild pain, soreness and itching at injection site	A few cases reported by MacKenzie 2004			*No serious adverse events reported by any studies* (qualitative, p43)
marked flushing and mild chest discomfort that disappeared within 30 seconds without the use of medication	Two out of 3733 women given WinRho either antenatally or postpartum. Noted by Bowman 1982 that this lot contained an unacceptable level of moisture and aggregated IgG.			
RAADP (one-dose versus two-dose)				
No studies identified that directly compare the two regimens	Pooled data from studies that used two-doses (500 IU at 28 and 34 weeks) gave a point estimate for sensitisation of 0.30% (0.22, 0.38) compared with 0.34% in studies that used a single dose (1500 IU at 28 weeks).			
EXTERNAL VALIDITY				
Generalisability (relevance of the study population to the Guidelines target population)				
The evidence is directly applicable to the Australian Guidelines target population with few caveats. No studies included Indigenous Australians or Torres Strait Islander people. Five of the nine studies identified included only primigravidae or primiparae women, which may not be reflective of the Guidelines target population. Three allowed for primigravidae or unsensitised multigravidae, which is reflective of the Guidelines target population. One study was completely unselected (28% primiparae). The studies are two decades old and therefore the population may not be reflective of modern factors such as comorbidities. Some of the participants in the historical control arms were from rural populations which may differ in variables such as rates of invasive procedures.				
Applicability (relevance of the evidence to the Australian health care system)				
The evidence is directly applicable to the Australian health care system. The studies were conducted in Canada, Denmark, France, US and UK . The studies are quite old, and used different doses and different preparations of Anti-D. Some did not state the route of administration. Meta-analysis of differing groups showed that regardless of location and dose, there was consistency within results (p 37)				
Additional comments				
Five studies were included in the previous review by Chilcott 2003 (Huchet 1987, MacKenzie 1999, Mayne 1997, Tovey 1983, and Trolle 1989) Query amount of overlap between Bowman 1978, Bowman & Pollock 1978, and Bowman 1987. All three trials use the same overlapping historical controls (Pilgrim 2009, p 42) and it is not clear if there is double counting of patients who received Rh D IgG. MacKenzie 2004 was an RCT comparing i.v. vs i.m. however the study was not powered to detect differences between administration groups. *Authors conclusion* The evidence indicates that RAADP reduces the incidence of sensitisation and hence of HDFN.				

CI, confidence interval; HDFN, haemolytic disease of the fetus and newborn; IgG, immunoglobulin; IU, international units; M-H, Mantzel-Hentzel; NR, not reported; OR, odds ratio; RAADP, routine antenatal Anti-D prophylaxis; RCT, randomised controlled trial; RR, relative risk; SD, standard deviation; UK, United Kingdom; US, United States

a. Heterogeneity defined as follows: (i) no significant heterogeneity if $P_{het} > 0.1$ and $I^2 < 25\%$; (ii) mild heterogeneity if $I^2 < 25\%$; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity I^2 >50%

b. Calculated post-hoc using RevMan 5.3 with M-H Fixed effects unless otherwise indicated.

c. Not clear if all women were screened at postnatal follow-up.

d. Data reported for primigravidae only.

e. This meta-analysis appears to have double counted the control groups (Bowman & Pollock 1978 and Bowman 1987)

f. Among the 18 sensitised women in the control group with first pregnancy, 14 were mildly affected, 2 required exchange transfusion, one died due to reasons other than HDFN, and one was Rh D negative. Among the 11 further pregnancies, five were mildly affected, two were moderately affected, and one was severely affected requiring 6 exchange transfusions.

g. Among the 62 sensitised women in the control group information on 17 subsequent pregnancies was provided. Treatment included fetal and exchange transfusion s (2), exchange transfusion and early delivery (3), phototherapy (2), Direct Coombs test positive but treatment not required (5), Direct Coombs test negative (unaffected).

<table>
<tr><td colspan="4">STUDY DETAILS: SR/MA</td></tr>
<tr><td colspan="4">Citation</td></tr>
<tr><td colspan="4">Chilcott 2003
Chilcott, J., Lloyd Jones, M., Wight, J., et al. 2003. A review of the clinical effectiveness and cost-effectiveness of routine anti-D prophylaxis for pregnant women who are rhesus-negative. Health Technol Assess, 7, iii-62.</td></tr>
<tr><td colspan="4">Affiliation/Source of funds</td></tr>
<tr><td colspan="4">National Institute for Health and Clinical Excellence</td></tr>
<tr><td>Study design</td><td>Level of evidence</td><td>Location</td><td>Setting</td></tr>
<tr><td>Systematic review of Level II and Level III studies</td><td>I-III</td><td>Various
Studies conducted in Canada, Denmark, UK, Sweden, France,</td><td>Obstetrics and maternal care</td></tr>
<tr><td colspan="2">Intervention</td><td colspan="2">Comparator</td></tr>
<tr><td colspan="2">Routine antenatal anti-D administration (RAADP)</td><td colspan="2">No treatment</td></tr>
<tr><td colspan="4">Population characteristics</td></tr>
<tr><td colspan="4">Pregnant women who are Rh D negative</td></tr>
<tr><td colspan="2">Length of follow-up</td><td colspan="2">Outcomes measured</td></tr>
<tr><td colspan="2">None specified</td><td colspan="2">- sensitisation rates among women at risk (i.e. Rh D negative women delivered of Rh D positive infants)
- adverse effects
- cost

Included studies reported sensitisation rates at different time points:
- during pregnancy and/or within three days of delivery
- at postnatal follow-up (6–12 months following delivery)
- in a subsequent pregnancy</td></tr>
<tr><td colspan="4">INTERNAL VALIDITY</td></tr>
<tr><td colspan="4">Overall risk of bias (descriptive)</td></tr>
</table>

Rating: Moderate

Description: No critical flaws. SR *may* provide an accurate summary of the results of the available studies that were included in the review.

Included studies: Many were poorly designed. Lack of blinding is considered relatively unimportant due to the incidence of alloimmunisation (presence or absence of anti-D) is objective. The used of historical and/or geographical controls introduces serious bias. The baseline demographics of treatment and controls may not be comparable and could underestimate the incidence of sensitisation (e.g. earlier tests for anti-D were less sensitive). Changes or differences in obstetric practice over time and geography (e.g. rates of caesarean section) may equally influence the incidence of sensitisation among either group. Timing of outcome measure is also of concern and could possibly underestimate the incidence of sensitisation with few studies reporting number of women sensitised in a subsequent pregnancy. Poor reporting of intent to treat numbers (number of women eligible for the intervention vs number of women who received the intervention) also introduces bias. Exclusion of failures over estimates the clinical effectiveness of RAADP.

RESULTS:

Outcome **No. patients (No. trials)**	**RAADP** **n/N (%)** **% (95% CI)**	**No treatment** **n/N (%)** **% (95% CI)**	**Risk estimate (95% CI)**	***Statistical significance*** ***p-value*** **Heterogeneity [a]** **I^2 (*p*-value)**
RAADP (one or two-dose) versus no therapy or placebo				
Overall number of women sensitised (any time point) N=41441 (11 studies)	147/29288* (0.5) *not clear how authors calculated overall number of women who received or were eligible for RAADP	167/12153 (1.4) (10 studies)* *data not reported for control group in one study (Parsons 1998)	NR	NR
Overall number of women sensitised *Individual study data (authors' figures, Table 7, p18)*				
Bowman 1978	1/1357 (0.1)	45/2768 (1.6)	RR 0.05 (0.01, 0.33)	
Bowman & Pollock 1978	5/1804 (0.3)	62/3533 (1.8)	RR 0.16 (0.06, 0.39)	
Bowman 1987	25/9303 (0.3)	62/3533 (1.8)	RR 0.15 (0.10, 0.24)	
Tovey 1983	6/2037 (0.3)	32/2721 (1.2)	RR 0.25 (0.10, 0.60)	
Hermann 1984	2/568 (0.4)	12/645 (1.9)	RR 0.19 (0.04, 0.84)	
Huchet 1987	1/599 (0.2)	7/590 (1.2)	RR 0.14 (0.02, 1.14)	
Trolle 1989	0/346 (0)	6/354 (1.7)	RR 0.08 (0.00, 1.39)	
Lee 1995	5/513 (1.0)	9/595 (1.5)	RR 0.64 (0.22, 1.91)	
Mayne 1997	4/1425 (0.3)	16/1426 (1.1)	RR 0.25 (0.08, 0.75)	
Parsons 1998	72/9684 (0.7)	No data (0.8)	not calculable	
MacKenzie 1999	12/3320 (0.4)	26/3146 (0.8)	RR 0.44 (0.22, 0.87)	
Overall number of women sensitised [b] N=NC (11 studies)	133/30956 (0.04)	215/NC	not calculable	
Overall number of women sensitised [b] N=34282 (10 studies)** ** excluding Parsons 1998 and three studies combined (Bowman 1978, Bowman & Pollock 1978 and Bowman 1987) to avoid double counting of control group	61/21272 (0.29)	170/13010 (1.31)	M-H Random OR 0.25 (0.15, 0.40) RR 0.25 (0.16, 0.40)	*Favours RAADP* *p < 0.00001* Moderate heterogeneity I^2 = 44% (*p* = 0.08)
Overall number of women sensitised *Individual study data (including women excluded from published analysis for various reasons, Table 8, p19)*				

Bowman 1978	1/1357 (0.1)	45/2768 (1.6)	RR 0.05 (0.01, 0.33)	
Bowman & Pollock 1978	11/1806 (0.6)	62/3533 (1.8)	RR 0.35 (0.18, 0.66)	
Bowman 1987	30/9295 (0.3)	62/3533 (1.8)	RR 0.18 (0.12, 0.28)	
Tovey 1983	6/2037 (0.3)	32/2721 (1.2)	RR 0.25 (0.10, 0.60)	
Hermann 1984	5/568 (0.9)	12/645 (1.9)	RR 0.47 (0.17, 1.33)	
Huchet 1987	1/599 (0.2)	7/590 (1.2)	RR 0.14 (0.02, 1.14)	
Trolle 1989	0/346 (0)	6/354 (1.7)	RR 0.08 (0.00, 1.39)	
Lee 1995	5/513 (1.0)	9/595 (1.5)	RR 0.64 (0.22, 1.91)	
Mayne 1997	4/1425 (0.3)	16/1426 (1.1)	RR 0.25 (0.08, 0.75)	
Parsons 1998	72/9684 (0.7)	no data (0.8)	Not calculable	
MacKenzie 1999	12/3320 (0.4)	26/3146 (0.8)	RR 0.44 (0.22-0.87)	
Overall number of women sensitised [b] N= not calculable (11 studies)	147/30950 (0.47)	277/ not calculable	not calculable	NR
Overall number of women sensitised [b] N=34276 (10 studies)** ** excludes Parsons 1998 and three studies combined (Bowman 1978, Bowman & Pollock 1978 and Bowman 1987) to avoid double counting of control group	75/21266 (0.36)	170/13010 (1.31)	OR 0.26 (0.20-0.35) RR 0.27 (0.20-0.35)	*Favours RAADP* *p < 0.00001* Moderate heterogeneity I^2 = 27% (*p* = 0.22)
Women found to be sensitised at a subsequent pregnancy *Individual study data (table 4, p14)*				
Bowman 1978	0/343 (0.0) (0.0, 0.0)	No data	not calculable	not calculable
Hermann 1984	0/39 (0.00) (0.0, 0.0)	No data	not calculable	not calculable
Tovey 1983	No data/325	11/582 (1.9) (0.8, 3.0)	not calculable	not calculable
Mayne 1997	4/1425 (0.3) (0.0, 0.6)	16/1425 (1.1) (0.6, 1.7)	NR	NR
MacKenzie 1999	12/3320 (0.4) (0.2, 0.6)	26/3146 (0.8) (0.5, 1.1)	NR	NR
Women sensitised during pregnancy or within 3 days of delivery *Individual study data (table 5, p16)*				
Bowman 1978	1/1357 (0.1) (0.0, 0.3)	45/2768 (1.6) (1.2, 2.1)	NR	NR
Bowman & Pollock 1978	5/1804 (0.3) (0.0, 0.5)	62/3533 (1.8) (1.3, 2.2)	NR	NR
Tovey 1983	4/1563 (0.3) (0.0, 0.6)	29/2582 (1.1) (0.7, 1.5)	NR	NR
Hermann 1984	0/568 (0.0) (0.0, 0.0)	10/645 (1.6) (0.6, 2.5)	NR	NR
Bowman 1987	18/9303 (0.2) (0.1, 0.3)	62/3533 (1.8) (1.3, 2.2)	NR	NR
Huchet 1987	1/599 (0.2) (0.0, 0.5)	6/590 (1.0) (0.2, 1.8)	NR	NR
Trolle 1989	No data/346	No data/354	not calculable	not calculable

Lee 1995	4/513 (0.8) (0.0, 1.5)	7/595 (1.2) (0.3, 2.0)	NR	NR
Mayne 1997	No data	No data	not calculable	not calculable
Parsons 1998	No data/9684	No data	not calculable	not calculable
MacKenzie 1999	No data	No data	not calculable	not calculable
Women sensitised at postnatal follow-up *Individual study data (table 6, p17)*				
Bowman 1978	1/1004 (0.1) (0.0, 0.3)	45/2768 (1.6)[c] (1.2, 2.1)	NR	
Bowman & Pollock 1978	No data/807	50/3533 (1.4)[c] (1.0, 1.8)	not calculable	not calculable
Tovey 1983	2/1059 (0.2)[d]	No data	not calculable	not calculable
Hermann 1984	2/568 (0.4)[c] (0.0, 0.9)	10/645 (1.6)[c] (0.6, 2.5)	NR	NR
Bowman 1987	25/9303 (0.3)[c] (0.2, 0.4)	50/3533 (1.4)[c] (1.0, 1.8)	NR	NR
Huchet 1987	1/472 (0.2) (0.0, 0.6)	7/468 (1.5) (0.4, 2.6)	NR	NR
Trolle 1989	0/291 (0.0) (0.0, 0.0)	6/322 (1.9) (0.4, 3.3)	NR	NR
Lee 1995	3/361 (0.8) (0.0, 1.8)	7/405 (1.7) (0.5, 3.0)	NR	NR
Mayne 1997	No data	No data	not calculable	not calculable
Parsons 1998	No data/9684	No data	not calculable	not calculable
MacKenzie 1999	No data	No data	not calculable	not calculable
RAADP (one-dose, I500 IU at 28 weeks) versus no therapy or placebo				
Rh D alloimmunisation N=18867 (3 studies)** (Trolle 1989, Bowman & Pollock 1978, Bowman 1987) **unselected women (primigravidae and multigravidae)	0.34 (0.28, 0.40)	1.60 (0.37, 2.83)	OR 0.20 (0.13, 0.29)	NR
RAADP (two-dose, 500 IU at 28 weeks and 34 weeks) versus no therapy or placebo				
Rh D alloimmunisation N=13490 (4 studies)** (Huchet 1987, Mayne 1997, Tovey 1983, MacKenzie 1997) **primigravidae only	0.30 (0.22, 0.38)	0.89 (0.21, 1.56)	OR 0.33 (0.20, 0.55)	NR
Rh D alloimmunisation N=9317 (2 studies)** (Mayne 1997, MacKenzie 1997) **primigravidae only, UK-based studies	0.35 (0.29, 0.40)	0.95 (0.18, 1.71)	OR 0.37 (0.21, 0.65)	NR
EXTERNAL VALIDITY				
Generalisability (relevance of the study population to the Guidelines target population)				

The evidence is directly applicable to the Australian Guidelines target population with few caveats. No studies included Indigenous Australians or Torres Strait Islander people. Four studies included only primigravidae or primiparae women, which may not be reflective of the Guidelines target population. Six allowed for primigravidae or unsensitised multigravidae, which is reflective of the Guidelines target population. One study did not state the target population (Parsons 1998). The studies are two decades old and therefore the population may not be reflective of modern factors such as comorbidities. Six of the included studies use historical controls.
Applicability (relevance of the evidence to the Australian health care system)
The evidence is directly applicable the Australian health care system. Included studies were conducted in Canada, Denmark, France, Sweden and UK. The studies are quite old, and used different doses and different preparations of Anti-D. Some did not state the route of administration. Meta-analysis of differing groups showed that regardless of location and dose, there was consistency within results (p 37)
Additional comments
Post-hoc calculations do not match the figures stated on p40 for the control group. Query amount of overlap between Bowman 1978, Bowman & Pollock 1978, and Bowman 1987. All three trials use the same overlapping historical controls and it is not clear is there is double counting of patients who received Rh D IgG. Three studies were not included in the updated review by Pilgrim 2009: Herman 1984, Lee 1995, and Parsons 1998 Five studies were included in the updated review by Pilgrim 2009: Huchet 1987, MacKenzie 1999, Mayne 1997, Tovey 1983, Trolle 1989 Authors conclusion: The evidence suggests that RAADP is effective in reducing the number of RhD negative pregnant women who are sensitised during pregnancy. However, it cannot prevent all instances of sensitisation, some of which occur either despite or before appropriate administration of anti-D.

CI, confidence interval; NC, not calculable; NR, not reported; OR, odds ratio; M-H, Mantzel-Hentzel; RAADP, routine antenatal anti-D prophylaxis; RCT, randomised controlled trial; RR, relative risk; SD, standard deviation; UK, United Kingdom;

a. Heterogeneity defined as follows: (i) no significant heterogeneity if $P_{het} > 0.1$ and $I^2 < 25\%$; (ii) mild heterogeneity if $I^2 < 25\%$; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity $I^2 > 50\%$

b. Calculated post-hoc using RevMan 5.3 with M-H Fixed effects unless otherwise indicated.

c. Not clear if all women were screened at postnatal follow-up.

d. Data reported for primigravidae only.

E1.3 Level II – Randomised controlled trials

STUDY DETAILS: RCT			
Citation			
Pennell 2017 Pennell, C., Cheng, J., Veselinovic, B. P., Wang, C., Ingleby, B., Arnold, C., Barr, A., Staples, N., White, M., & White, S. (2017). Single dose Anti-D prophylaxis in pregnancy: is it time to change*? Journal of Paediatrics and Child Health*, 53(S2), 112-113. doi:10.1111/jpc.13494_332			
Affiliation/Source of funds			
King Edward Memorial Hospital, Women and Infants Research Foundation (WIRF)			
Study design	**Level of evidence**	**Location**	**Setting**
RCT	Level II	King Edward Memorial Hospital, WA, Australia	Obstetrics and maternity care
Intervention		**Comparator**	
1500 IU Rh(D) Immunoglobulin-VF at 28 weeks gestation		625 IU Rh(D) Immunoglobulin-VF at 28 and 34 weeks gestation	
Population characteristics			
280 Rh negative pregnant women who had a negative antibody screen, and no history of adverse reactions to anti-D injections			
Length of follow-up		**Outcomes measured**	
Following delivery of each participant enrolled in this study		- Detectability of anti-D at delivery via standard diagnostic practices employed at King Edward Memorial Hospital - Proportion of women receiving doses at correct gestation (compliance) - Risk factors for no detectable antibody at delivery via questionnaire and review of medical records - Complication rates (obstetric and neonatal) via review of medical records - Total amount of anti-D used per participant	
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			
Rating: Not assessed Description: Study is reported as conference abstract and is difficult to judge internal bias. Not all outcomes reported. Details on testing methodology is lacking.			
RESULTS			
Population analysed	**Intervention**	**Comparator**	
Randomised	NR	NR	
Efficacy analysis (ITT)	NR	NR	
Efficacy analysis (PP)	NR	NR	
Safety analysis	NR	NR	

Outcome	**1500 IU Rh D IgG at 28 weeks n/N (%)**	**625 IU Rh D Ig G at 28 and 34 weeks n/N (%)**	**Risk estimate (95% CI)**	***Statistical significance p*-value**
One-dose (1500 IU anti-D at 28 weeks) RAADP versus two-dose (625 IU anti-D at 28 and 34 weeks) RAADP				
Proportion with undetectable anti-D at delivery (ITT)	NR (45.2%)	NR (14.2%)	OR 5.0 (NR)	*Favours two-dose* $p < 0.001$
	Multivariate regression analysis suggested time elapsed since last dose and third -trimester weight were significant predictors for undetectable anti-D at delivery. Anti-D regimen group was not a significant predictor.			

Compliance	NR (77.1%)	NR (60.7%)	NR	*Favours single dose* $p < 0.001$
Complication rates (obstetric and neonatal) via review of medical records	NR	NR	NR	
The total amount of Rh D IgG used per participant	NR	NR	NR	
EXTERNAL VALIDITY				
Generalisability (relevance of the study population to the Guidelines target population)				
The evidence is directly generalisable to the Guideline target population.				
Applicability (relevance of the evidence to the Australian health care system)				
The evidence is directly applicable to the Australian health care system.				
Additional comments				
Information provided in the conference abstract and on ANZCTR is sparse and likely incomplete. Study authors were contacted – and advised a manuscript is under final review with MJA. Upon review of the submitted abstract for the paper, it became apparent that the final analysis reported in the manuscript is different to that reported above. Here, the proportion of women with undetectable anti-D at delivery was higher in women randomised to the single dose (84% vs 56%, $p < 0.001$). Compliance was also slightly higher in the single dose group (61% vs 50%, $p = 0.056$). Compliance was defined as receiving a dose within the correct two-week interval (28–30 weeks or 34–36 weeks). Women who did not receive a dose at all, received a routine dose outside of this interval, or received the incorrect dose were deemed non-compliant. Women randomised to the two-dose group who delivered prior to 36 weeks were deemed compliant if the first dose had been administered at the correct gestation.				

ANZCTR, Australian New Zealand Clinical Trials Registry; CI, confidence interval; IgG, immunoglobulin; ITT, intent to treat; IU, international units; NR, not reported; OR, odds ratio; PP, per-protocol; RCT, randomised controlled trial; WA, Western Australia

E1.4 Level III – Comparative observational studies

STUDY DETAILS: Cohort / Case-control			
Citation			
Koelewijn 2008 Koelewijn, J. M., de Haas, M., Vrijkotte, T. G., Bonsel, G. J., & van der Schoot, C. E. (2008). One single dose of 200 [mu]g of antenatal RhIG halves the risk of anti-D immunization and hemolytic disease of the fetus and newborn in the next pregnancy. *Transfusion,* 48(8), 1721-1729.			
Affiliation/Source of funds			
OPZI (detection and prevention of pregnancy immunisation)-project: the nationwide evaluation of pregnancy screening for RBC antibodies in the Netherlands. Financed by the Council of Health Insurances.			
Study design	**Level of evidence**	**Location**	**Setting**
Historic control cohort study	Level III-3	The Netherlands	Obstetrics and maternity
Intervention		**Comparator**	
Single dose Rh D IgG (1000 IU) administered antenatal (Week 30) and postnatal (following birth of RhD positive newborn) Years: 2002 and 2004		Single dose Rh D IgG (1000 IU) administered postnatal (following birth of RhD positive newborn) Years: 1999 and 2002	
Population characteristics			
Rh D negative pregnant primiparae women who gave birth to their first child shortly before (controls) or after (intervention) the introduction of RAADP. The control group included women who had previously given birth to D+ child after the 30th week of pregnancy with anti-D immunisation detected after Week 30 in the previous pregnancy or not later than Week 30 in the current pregnancy. The intervention group included women who had RAADP, additional to postnatal Rh D IgG administered for a previous pregnancy.			
Length of follow-up		**Outcomes measured**	
Not determined		Rh D alloimmunisation Severe HDFN (perinatal mortality, the need for IUT, and/or the need for exchange transfusion attributed to anti-D immunisation)	
Method of analysis			
Primary data about all new Rh D sensitisations were retrieved from the two reference laboratories. Women with a previous pregnancy outside the Netherlands were excluded from the analysis, as were pregnancies delivered at or before Week 30, and women who did not receive postnatal Rh D IgG in their previous pregnancy because they were erroneously typed as D+ or their child was typed as D– The denominators for the calculation of the prevalence of new sensitisations were calculated from data from the Office of Vital Statistics (in Dutch: CBS). The estimated D genotype frequencies in the Netherlands used were 38.5% DD, 46.2% Dd, and 15.3% dd, implicating that 61.5% of D– women will carry a D+ child. Prevalence/relative risk estimated per 100,000 RhD–, parae-1, first child D+			
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			
Rating: Moderate Description: The study appears to provide sound evidence for a non-randomised study but cannot be considered comparable to a well-performed randomised trial. Some concerns on reporting bias, due to missing data.			
RESULTS			
Population analysed	**Intervention**	**Comparator**	
Available	±12000	±8700	
Analysed (week 12)	12576	8645	
Analysed (week 30)	11519	8811	

Number of new sensitisations	69 [a]		80 [b]	
Outcome	**RAADP** **n/N** **% (95% CI)**	**No RAADP** **n/N** **% (95% CI)**	**Risk estimate (95% CI)**	***Statistical significance*** ***p-value***
RAADP (one-dose 1000 IU at Week 30) vs no RAADP				
Prevalence of Rh D alloimmunisation (detected at Week 12)	39/12576 [a] 0.31% (0.21, 0.41)	58/8645 [b] 0.67% (0.50, 0.84)	RR 0.46 (0.09, 0.84)	*Favours RAADP* *p = NR*
Prevalence of Rh D alloimmunisation (detected at Week 30)	29/11519 [a] 0.25% (0.16, 0.34)	21.5/8811 [b] 0.24% (0.14, 0.35)	RR 1.03 (0, 2.18)	*No significant difference* *p = NR*
Prevalence of Rh D alloimmunisation (detected at Week 12 and Week 30)	68/NR [a] 0.56% (0.43, 0.69)	79.5/NR [b] 0.92% (0.71, 1.12)	RR 0.61 (0.22, 1.01)	*No significant difference* *p = NR*
Risk of Rh D alloimmunisation in first trimester* *Assuming 95% RAADP coverage during the study period	0.33% (NR)	0.63% (NR)	RR 0.52 (0.10, 0.95)	*Favours RAADP* *p = NR*
Prevalence of severe HDFN (detected at Week 12)	12/12576 0.10% (0.00, 0.15)	14/8645 0.16% (0.08, 0.25)	RR 0.59 (0, 1.50)	*No significant difference* *p = NR*
Prevalence of severe HDFN (detected at Week 30)	1/11519 0.01% (0, 0.03)	6/8811 0.07% (0.01, 0.12)	RR 0.13 (0, 0.68)	*Favours RAADP* *p = NR*
Prevalence of severe HDFN (detected at Week 12 or Week 30	13/NR 0.10% (0.05, 0.16)	20/NR 0.23% (0.13, 0.33)	RR 0.45 (0.1, 1.08)	*No significant difference* *p = NR*
Prevalence of severe HDFN* (detected at Week 12 or Week 30) **excluding cases with postnatal (n=13) and antenatal (n=6) prophylaxis status unknown*	0.11%	0.22%	RR 0.51 (0.09, 0.92)	*Favours RAADP* *p = NR*
Risk of developing HDFN once sensitisation occurred (detected at Week 12)	12/39 (30.8%)	14/58 (24.1%)	NR RR 1.27 (0.66, 2.46)[c]	*NR*
Risk of developing HDFN once sensitisation occurred (detected at Week 30)	1/29 (3.45%)	6/21.5 (27.9%)	NR RR 0.13 (0.02, 0.98)[c]	*Favours RAADP* *p = 0.02*
Risk of developing HDFN once sensitisation occurred (detected at Week 12 or Week 30)	13/68 (19%)	20/79.5 (25%)	NR RR 0.76 (0.41, 1.42)[c]	*No significant difference* *p = NR*
EXTERNAL VALIDITY				
Generalisability (relevance of the study population to the Guidelines target population)				
The study was conducted in the Netherlands, where, due to Rh IgG scarcity, prophylaxis is restricted to women without a living child. The analysis was limited to antenatal prophylaxis in parae-1 Rh D negative women. All subsequent pregnancies did not receive RAADP. The prevalence at week 12 would remain unchanged, however, the prevalence at week 30 may be different.				
Applicability (relevance of the evidence to the Australian health care system)				

The study used a single dose of 1000 IU at 30 weeks for parae-1 Rh D negative women, which is different to the dose regime given to the Australian population.
Additional comments
The authors estimated that the total group at risk of D immunisation and fell in the historic control group was 93% in 1999, 20% in 2002, and 10% in 2004. The authors estimated that the NNT to prevent one anti-D alloimmunisation in the 12th week of pregnancy is 523. To prevent one case of subsequent HDFN is 1526. Including possible subsequent pregnancies, the NNT to prevent one anti-D alloimmunisation in the 12th week of pregnancy is 357. To prevent one case of subsequent HDFN is 1255.

CI, confidence interval; HDFN, haemolytic disease of the fetus and newborn; IgG, immunoglobulin; ITT, intention to treat; IU, international units; IUT, intrauterine transfusion; NNT, number needed to treat; PP, per-protocol; RAADP, routine antenatal anti-D prophylaxis; RCT, randomised controlled trial; RR, relative risk; SD, standard deviation

a. Receipt of postnatal and/or antenatal prophylaxis was unknown in seven cases (n = 2, week 12; n = 5, week 30). In the prevalence calculation, 5 cases were considered as received because anti-D was detected at week 12 or the husband was typed as homozygously D positive, and two cases were estimated as 0.5 (i.e. 2*0.5=1, meaning one valid event not counted)

b. Receipt of postnatal prophylaxis was unknown in eight cases (n = 5, week 12; n = 3, week 30). In the prevalence calculation, 7 cases were considered as received because anti-D was detected at week 12 or the husband was typed as homozygously D positive, and one was estimated as 0.5 (i.e. 1*0.5=0.5, meaning 0.5 valid event not counted).

c. Calculated post-hoc using RevMan 5.3. M-H Fixed effect.

E2 Question 2

E2.1 Level I – Systematic review of RCTs

<table>
<tr><td colspan="4">STUDY DETAILS: SR/MA</td></tr>
<tr><td colspan="4">Citation</td></tr>
<tr><td colspan="4">Karanth 2013
Karanth, L., Jaafar, S. H., Kanagasabai, S., Nair, N. S., & Barua, A. (2013). Anti-D administration after spontaneous miscarriage for preventing Rhesus alloimmunisation. Cochrane Database of Systematic Reviews (3), CD009617.</td></tr>
<tr><td colspan="4">Affiliation/Source of funds</td></tr>
<tr><td colspan="4">Cochrane pregnancy and Childbirth Group</td></tr>
<tr><td>Study design</td><td>Level of evidence</td><td>Location</td><td>Setting</td></tr>
<tr><td>Systematic review and meta-analysis of Level II studies</td><td>Level I</td><td>Visscher 1972 (US)</td><td>Obstetrics and maternity</td></tr>
<tr><td colspan="2">Intervention</td><td colspan="2">Comparator</td></tr>
<tr><td colspan="2">Administration of anti-D Ig after spontaneous miscarriage, therapeutic evacuation of the uterus, early pregnancy complications up to 24 weeks' gestation irrespective of parity .</td><td colspan="2">No received anti-D or who have received a placebo after spontaneous miscarriage, therapeutic termination of pregnancy or early pregnancy complications up to 24 weeks of gestation.</td></tr>
<tr><td colspan="4">Population characteristics</td></tr>
<tr><td colspan="4">Rh negative mothers who have experienced spontaneous miscarriage, therapeutic evacuation of the uterus, early pregnancy complications up to 24 weeks gestation, irrespective of parity, ABO compatibility or size of fetomaternal haemorrhage.</td></tr>
<tr><td colspan="2">Length of follow-up</td><td colspan="2">Outcomes measured</td></tr>
<tr><td colspan="2">Not specified</td><td colspan="2">- Development of a positive Kleihauer Betke test.
- Development of RhD alloimmunisation in subsequent pregnancy.
- Detection of atypical blood group antibodies by positive indirect Coombs test after six months of exposure (non-prespecified outcome).
- Need for increased surveillance for suspected fetal blood sampling and fetal transfusions in subsequent pregnancies.
- Neonatal morbidity such as neonatal anaemia, jaundice, bilirubin encephalopathy, erythroblastosis, prematurity, hypoglycaemia (low blood sugar) in subsequent pregnancies.
- Maternal adverse events of anti-D administration including anaphylactic reaction.</td></tr>
<tr><td colspan="4">INTERNAL VALIDITY</td></tr>
<tr><td colspan="4">Overall risk of bias (descriptive)</td></tr>
<tr><td colspan="4">Rating: Low
Description: Cochrane review. The systematic review provides an accurate and comprehensive summary of the results of the available studies that address the question of interest.
Included study: The study was assessed to be methodologically weak. Method of randomisation was not clear and incomplete reporting suggests high risk of attrition and reporting bias. The study did not have statistical power to show any difference between the intervention and non-intervention groups with regards to maternal sensitisation during a miscarriage event, with or without surgical evacuation of the products of conception.</td></tr>
<tr><td colspan="4">RESULTS:</td></tr>
</table>

Outcome **No. patients** **(No. trials)**	**Anti-D** **n/N (%)**	**Placebo** **n/N (%)**	**Risk estimate (95% CI)**	***Statistical significance*** ***p-value*** **Heterogeneity** **I^2 (*p*-value)**
Rh D IgG versus placebo				
Rh D isoimmunisation in subsequent pregnancies following Anti-D administration N=9 (1 study)	0/3 (0)	0/6 (0)	Not estimable	Not estimable
Antibody D titre at 6 months following administration N=48 (1 study)	0/19 (0)	0/29 (0)	Not estimable	Not estimable
Positive Kleihauer test after miscarriage before 14 weeks gestation	No studies identified	No data	No data	No data
Positive Kleihauer test after miscarriage following 14 weeks gestation	No studies identified	No data	No data	No data
Health of infant in subsequent pregnancy	No studies identified	No data	No data	No data
Need for increased fetal surveillance for suspected isoimmunisation in subsequent pregnancy	No studies identified	No data	No data	No data
Adverse reactions	No studies identified	No data	No data	No data
EXTERNAL VALIDITY				
Generalisability (relevance of the study population to the Guidelines target population)				
The generalisability of this evidence is unclear. The population characteristics are likely the same as those of the target population but difference due to time periods are possible. Includes women with miscarriage beyond the first trimester.				
Applicability (relevance of the evidence to the Australian health care system)				
The applicability of this evidence is unclear. Only one study with a high risk of bias was identified from 1972. It is likely that obstetric and maternity practice has changed since that time.				
Additional comments				
Only one study identified (Visscher 1972) involving 48 women who had a miscarriage between 8 to 24 weeks of gestation. The study compared 300 µg anti-D Ig im injection (n=19) with 1 cc gamma globulin placebo (n=29). Of the 19 women in the treatment group, 14 had therapeutic dilatation & curettage (D&C) and five had spontaneous miscarriage; of the 29 women in the control group, 25 had therapeutic D&C and four had spontaneous miscarriage. Authors conclusion: There are insufficient data available to evaluate the practice of anti-D administration in an unsensitised Rh negative mother after spontaneous miscarriage.				

CI, confidence interval; im, intramuscular; RCT, randomised controlled trial; RR, relative risk; SD, standard deviation

E2.2 Level I - Systematic review of observational and cohort studies

STUDY DETAILS: SR/MA			
Citation			
NICE 2014 National Institute for Health and Care Excellence (2014). Evidence Update 71 – Ectopic pregnancy and miscarriage (December 2014) National Collaborating Centre for Women's and Children's Health. (2012). Ectopic pregnancy and miscarriage: Diagnosis and initial management in early pregnancy of ectopic pregnancy and miscarriage (December 2012). Royal College of Obstetricians and Gynaecologists			
Affiliation/Source of funds			
National Institute for Health and Care Excellence			
Study design	**Level of evidence**	**Location**	**Setting**
Systematic review of Level II and Level III studies	Level I-III	US (Visscher 1972, Gavin 1972) Hungary (Simonovits 1974)	Obstetrics and maternity care
Intervention		**Comparator**	
Anti-D prophylaxis The group identified eight studies. Five were non-comparative, descriptive studies reporting the incidence of sensitisations in women receiving no anti-D rhesus prophylaxis following first trimester obstetric events. The remaining three papers (Simonovits 1974, Gavin 1972, Visscher 1972) were comparative studies examining the effect of anti-D rhesus prophylaxis on outcomes and are considered relevant to this review.		No anti-D prophylaxis or placebo	
Population characteristics			
Rh D negative women who had a miscarriage, threatened miscarriage or ectopic pregnancy in the first trimester. *Studies identified:* - Threatened miscarriage - no studies found - Ectopic pregnancy - no studies found - Miscarriage – 1 study (Visscher 1972) - Incomplete miscarriage and medical termination of pregnancy* (Gavin 1972) - Medical termination of pregnancy* (Simonovits 1974) * Denoted as therapeutic abortion by the study authors			
Length of follow-up		**Outcomes measured**	
Not specified		Clinically significant rate of sensitisation Occurrence of Rh Disease in a subsequent pregnancy	
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			
Rating: Moderate Description: The systematic review has more than one weakness but no critical flaws. It *may* provide an accurate summary of the results of the available studies that were included in the review. Included studies: The group recognised that all evidence available was of very low quality. None of the studies were very large and it is unlikely that any were sufficiently powered to detect a statistically significant difference.			
RESULTS:			

Outcome **No. patients (No. trials)**	**Anti-D** **n/N (%)**	**No therapy or placebo** **n/N (%)**	**Risk estimate (95% CI)**	***Statistical significance*** ***p*-value** **Heterogeneity[a]** **I^2 (*p*-value)**

Rh D IgG (any dose) versus placebo or no treatment				
Incidence of sensitisation at 4–6 months following miscarriage/medical termination of pregnancy N=105 (2 studies) (Gavin 1972, Visscher 1972)	0/40 (0)	2/65 (3.1)	Not calculable	
Measured by Enzyme-Coombs screening procedure N=48 (1 study) (Visscher 1972)[b]	0/19 (0)	0/29 (0)	Not calculable	
Measured by Indirect Coombs test N=57 (1 study) (Gavin 1972)	0/21 (0)	2/36 (5.6)	RR 0.34 (0.02, 6.69) 37 fewer per 1000 (54 fewer to 316 more)	*No significant difference*
Evidence of sensitisation in subsequent pregnancy N=250 (2 studies) (Simonovits 1974, Visscher 1972)	1/99 (1.0)	2/151 (1.3)	Not calculated	
Measured by papain-treated cells (intervention), indirect Coombs test and papain-treated cells or not reported (control) N=241 (1 study) (Simonovitis 1974)	1/96 (1.0)[c]	2/145 (1.4)	RR 0.76 (0.0, 8.21) 3 fewer per 1000 (from 13 fewer to 99 more)	*No significant difference*
Measured by Enzyme-Coombs screening procedure N=9 (1 study) (Visscher 1972)	0/3 (0)	0/6 (0)	Not calculable	
EXTERNAL VALIDITY				
Generalisability (relevance of the study population to the Guidelines target population)				
The identified studies include a population that is similar to the guidelines target population. None of the studies identified included Indigenous Australians or Torres Strait Islanders				
Applicability (relevance of the evidence to the Australian health care system)				
The applicability of the identified studies is unclear. The studies were conducted in the US (Visscher 1972, Gavin 1972) and Hungary (Simonovits 1974).				
Additional comments				

The NICE Guidelines did not find any updated evidence (search date 8 July 2014). The review also looked at what dose would be appropriate and identified three RCTs that examined different doses of anti-D prophylaxis to women following first trimester medical termination of pregnancy.

The group recognised that there is little harm associated with the provision of anti-D rhesus prophylaxis. By contrast, the group recognised that there is a clear health benefit in avoiding sensitisation if possible.

RECOMMENDATIONS

81. Offer anti-D rhesus prophylaxis at a dose of 250 IU (50 micrograms) to all rhesus negative women who have a surgical procedure to manage an ectopic pregnancy or a miscarriage.

82. Do not offer anti-D rhesus prophylaxis to women who:

- receive solely medical management for an ectopic pregnancy or miscarriage or
- have a threatened miscarriage or
- have a complete miscarriage or
- have a pregnancy of unknown location.

83. Do not use a Kleihauer test for quantifying fetomaternal haemorrhage.

CI, confidence interval; IU, international units; NR, not reported; RCT, randomised control trial; RR, relative risk; SD, standard deviation

a. Heterogeneity defined as follows: (i) no significant heterogeneity if $P_{het} > 0.1$ and I2 < 25%; (ii) mild heterogeneity if I2 < 25%; moderate heterogeneity if I2 between 25–50%; substantial heterogeneity I2 >50%

b. Visscher 1972 is an RCT comparing 300 mcg anti-D prophylaxis with placebo (IgG) in 48 patients, with additional prospective case series of nine women who did not receive any prophylaxis.

c. This woman delivered an Rh+ baby at the end of her second pregnancy and tested negative 6 months before birth; therefore she is likely to have been sensitised in her second, full-term pregnancy)

E2.3 Level II – Randomised controlled trials

No studies identified

E2.4 Level III – Comparative observational studies

No studies identified

E3 Question 3

E3.1 Level I – Systematic review of diagnostic studies

STUDY DETAILS: Systematic review of diagnostic studies			
Citation			
Geifman-Holtzman 2006 Geifman-Holtzman, O., Grotegut, C. A., & Gaughan, J. P. (2006). Diagnostic accuracy of noninvasive fetal Rh genotyping from maternal blood-A meta-analysis. *American Journal of Obstetrics and Gynecology,* 195(4), 1163-1173.			
Affiliation/Source of funds			
Not stated Division of Maternal-Fetal Medicine, Department of Obstetrics, Gynecology and Reproductive Sciences, and Department of Biostatistics, Temple University School of Medicine, Philadelphia, PA			
Study design	**Level of evidence**	**Location**	**Setting**
Systematic review and meta-analysis of diagnostic studies	Level I	Not stated	Obstetrics and maternity
Index test	**Exon(s) sequenced**	**Internal control(s)**	**Reference standard or comparator**
Fetal Rh type determination in maternal blood, plasma or serum	Not specified	Not specified	Confirmation of fetus/newborn type
Population characteristics			
Pregnant Rh negative women who could be alloimmunised			
Number of studies		**Outcomes measured**	
37 publications performing 44 study protocols 3261 samples		Diagnostic accuracy, sensitivity, specificity, PPV, NPV, accuracy at gestational age	
Method of analysis			
Case reports and studies with less than 10 patients and subjects with more than one sample were excluded from the data analysis. To model the between study heterogeneity and synthesise the results a weighted random effects linear model using binomial distribution was used. The random effects composite (and 95% CI) was calculated. In addition, a hierarchical random effects Bayesian analysis to provide a composite estimate using Markov chain Monte Carlo simulation was used. Medians of the Bayesian composite effects are presented with 95.7 and 2.5 percentile estimates. Diagnostic accuracies based on trimester were determined from studies where gestational age was given and the source of the fetal DNA (blood, serum, etc.). Analysis was done using a comparison between rates for each trimester or source, based on a z-tests for independent proportions.			
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			
Rating: Serious Description: More than one critical flaw with non-critical weaknesses. The review has more than one critical flaw and *should not* be relied on to provide an accurate and comprehensive summary of the available studies. Key flaws included inadequate justification for excluding individual studies, risk of bias of included studies not reported or included in the analysis and potential sources of conflict of interest including any funding received not provided. Included studies: Descriptions of the included studies risk of bias assessment was not included. The authors did contact individual authors to try and remove duplicate samples. It was noted that 16 included studies reported 100% diagnostic accuracy in their fetal RhD genotyping, and many authors excluded samples because of absence of detectable DNA or inability to verify fetal or neonatal blood type, suggesting possible reporting biases.			

RESULTS							
Outcome	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**	**LR+ % (95% CI)**	**LR- % (95% CI)**	**Diagnostic accuracy % (95% CI)**
Diagnostic performance (n correct/N total) NIPT against birth blood sample							
N = 3261 (all studies)	--	--	--	--	--	--	91.4% (NR)
N = 3184 (after exclusion of studies with less than 10 patients and subjects with more than 1 sample)	--	--	--	--	--	--	91.7% (NR)
N = 3078 (all exclusions [a])	--	--	--	--	--	--	94.8%
N = 3078 (37 studies) Random effects model	95.4 (90.6, 97.8)	98.6 (96.4, 99.5)	99.0 (97.9, 99.6)	92.1 (80.9, 97.0)	--	--	--
N = 3078 (37 studies) Bayesian model	96.7 (92.5, 98.9)	98.9 (96.7, 99.9)	99.4 (98.4, 99.9)	92.7 (81.8, 97.9)	--	--	--
Diagnostic performance (n correct/N total) by gestational age [b] (N = 924, no. studies not reported)							
The diagnostic accuracies in the first trimester compared with the accuracies of the second trimester and third trimester were significantly different (p = 0.041). There was no statistically significant difference between second and third trimester (p > 0.05)							
1st trimester 218/240	--	--	--	--	--	--	90.8 (86.3, 94.0)
2nd trimester 350/412	--	--	--	--	--	--	85.0 (81.1, 88.2)
3rd trimester 232/272	--	--	--	--	--	--	85.3 (80.4, 89.2)
Diagnostic performance (n correct/N total) by fetal DNA/RNA source (N = 3078, no. studies not reported)							
The diagnostic accuracy using cffDNA from maternal serum, plasma or blood were significantly different compared to using DNA or RNA from fetal cells in maternal blood (p < 0.001)							
Maternal blood, fetal cells (DNA) 42/62	--	--	--	--	--	--	67.7 (54.5, 78.7)
Maternal blood, fetal cells (RNA) 100/131	--	--	--	--	--	--	76.3 (68.0, 83.1)
Maternal blood, free DNA 90/98	--	--	--	--	--	--	91.8 (84.1, 96.2)
Maternal plasma, free DNA 2293/2377	--	--	--	--	--	--	96.5 (95.6, 97.2)
Maternal serum, free DNA 394/410	--	--	--	--	--	--	96.1 (93.6, 97.7)

Diagnostic performance (n correct/N total) of alloimmunised patients (N = 3078, no. studies not reported)							
783/3078 (25.44% of total included patients)	--	--	--	--	--	--	91.8% (NR)
EXTERNAL VALIDITY							
Generalisability (relevance of the study population to the Guidelines target population)							
The evidence is directly generalisable to the Australian population with some caveats. The overall population included 25% alloimmunised women, which is higher than would be expected in the Guidelines target population. This difference was not considered to sufficiently influence test accuracy. There was no restriction on gestational age, and 70% of samples did not have a gestational age reported.							
Applicability (relevance of the evidence to the Australian health care system)							
The evidence is not applicable to the Australian health care context. This is a meta-analysis of studies published between 1999 and 2005. RNA/DNA extraction and sequencing methodologies have improved since that time. There were many different types of source material used, including both maternal (blood, plasma, serum) and fetal (circulating cells giving RNA or DNA, or cffDNA). The NIPT test used in Australia is not likely to use whole blood and will be based on cff DNA. It is assumed the tests will, at a minimum, use RT-qPCR for two exons to identify fetal Rh D status and include an internal control.							
Additional comments							
Most studies used fetal DNA obtained from maternal plasma or serum (2787 out of 3078 samples). There was no difference detected between serum and plasma (statistics not reported). Inaccuracies that were reported included failure to find DNA in the sample, insufficient specimen, and inability to verify fetal type. The discussion also states that "the results of this meta-analysis demonstrated that the diagnostic test of determining fetal RhD type using FfDNA [Free fetal DNA] in maternal blood is also high at 94.8%". Although it is unclear if this accuracy also includes sources that include circulating fetal cells. The authors note differences in ethnicities affect the incidence of RhD negativity, however, patient characteristics of included studies were not provided.							

--. data not reported; cffDNA, cell free fetal DNA; CI, confidence interval; DNA, deoxyribonucleic acid; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NIPT, non-invasive prenatal testing; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; RNA, ribonucleic acid; RT-PCR, real-time polymerase chain reaction.

a. A total of 183 (5.6%) samples were excluded from the meta-analysis. Of these, 49 were duplicates (1.5%), 28 (0.86%) were reported in studies of less than 10 patients. Of these 77 excluded, 79.22% (61/77) were correctly diagnosed. There were 106 (3.3%) samples excluded by study authors that were also excluded in the meta-analysis. (6 not enough specimen, 56 no DNA detected, 44 results unable to be verified of RhD gene rearrangements were suspected).

b. The number of samples reporting gestational age was = 924 (30%). The number of samples not reporting gestational age was = 2154. The gestational ages at which the test was performed was between 8–42 weeks. No definition of 1st, 2nd, or 3rd trimester was provided.

STUDY DETAILS: Systematic review of diagnostic studies			
Citation			
Mackie 2016 Mackie, F. L., Hemming, K., Allen, S., Morris, R. K., & Kilby, M. D. (2017). The accuracy of cell-free fetal DNA-based non-invasive prenatal testing in singleton pregnancies: a systematic review and bivariate meta-analysis. *BJOG: An International Journal of Obstetrics and Gynaecology*, 124(1), 32-46.			
Affiliation/Source of funds			
FLM is funded by the Richard and Jack Wiseman Trust (registered charity 1036690) The authors provided statements declaring no conflicts of interest.			
Study design	**Level of evidence**	**Location and study date**	**Setting**
Systematic review of diagnostic cohort studies	Level I	Studies published between 1997 to 13 April 2015. No language limits. Included studies: Argentina, Australia, Austria, Belgium, Brazil, China, Czech-Republic, Denmark, France, Germany, Ireland, Italy, Korea, Kuwait, Morocco, Netherlands, Pakistan, Spain, Switzerland, Turkey, UK, USA,	Obstetrics and maternity
Index test	**Exon(s) sequenced**	**Internal control(s)**	**Reference standard or comparator**
NIPT based on cffDNA in maternal blood	Not specified	Not specified	Birth outcome (blood sample)
Population characteristics			
Women with a singleton pregnancy, any gestation.			
Number of participants or samples		**Outcomes measured**	
30 studies (10290 tests) Prevalence (%) of Rh D, median (IQR): 69 (61 to 72)		Sensitivity, specificity, LR+, LR-, OR	
Method of analysis			
2x2 data was used to calculate sensitivity and specificity with 95%CI. Heterogeneity was explored using Forest plots and Hierarchical summary or receiver operating characteristic curves. Summary measures (including sensitivity, specificity, diagnostic odds ratio, LR+, LR-, 95% CI) were calculated using a bivariate logistic regression model with an unstructured correlation. This allows for correlation between sensitivity and specificity from the same study, and for the sensitivities and specificities to have different random effects. Subgroup analysis and meta-regression was planned *a priori* to assess effects of study level covariates. Subgroup analyses were used to assess the influence of categorical covariates due to model convergence difficulties			
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			
Rating: Moderate Description: More than one non-critical weakness – the systematic review has more than one weakness but no critical flaws. It *may* provide an accurate summary of the results of the available studies that were included in the review. Included studies: Risk of bias was assessed using the QUADAS-2 tool and presented as summary figure. Overall, studies were at low risk of bias, with key concerns related to patient selection bias and index test bias. Assessments specific to the studies in Rh D women is not sufficiently discussed. The authors noted that 13/30 studies reported inconclusive results: the majority had no reason given; RHD gene variant; insufficient number of markers present from prespecified cut-off; test failure; or low fetal fraction. Multiple pregnancies can pose specific challenges for NIPT (e.g. twin fetuses may have discordant Rh D status). Excluding them from the analyses may have introduced patient selection bias.			

RESULTS							
Outcome	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**	**LR+ (95% CI)**	**LR- (95% CI)**	**Diagnostic accuracy % (95% CI)**
Diagnostic performance NIPT against birth blood sample							
N = 10290 (30 studies) bivariate logistic regression model	99.3 (98.2, 99.7)	98.4 (96.4, 99.3)	NR	NR	61 (22, 167)	0.007 (0.003, 0.186)	OR 8466 (1877, 38183)
Diagnostic performance, by method of detection against birth blood sample							
RT-qPCR N = 9295 (22 studies)	99.7 (98.7, 99.9)	98.9 (96.4, 99.7)	NR	NR	90 (20, 383)	0.003 (0.001, 0.013)	OR 25978 (3125, 215980)
Conventional PCR N = 275 (4 Studies)	92.4 (83.2, 96.8)	95.4 (80.4, 99.1)	NR	NR	20 (4, 96)	0.079 (0.034, 0.1883)	OR 254 (41, 1576)
Mass spectrometry N = 1052 (4 studies)	Not calculable	Not calculable	Not calculable	Not calculable	Not calculable	Not calculable	OR will not converge[a]
EXTERNAL VALIDITY							
Generalisability (relevance of the study population to the Guidelines target population)							
The evidence is generalisable to the target population with some caveats. The include studies enrolled Rh D negative pregnant women, however, women with multiple pregnancies were excluded. Some included studies may not be directly applicable in terms of Rh D prevalence/ethnicity.							
Applicability (relevance of the evidence to the Australian health care system)							
The evidence is probably applicable to the Australian health care system with some caveats. This is a meta-analysis of studies published between 1997 and 2015 so some of the included studies would include RNA/DNA extraction and sequencing methodologies that have since improved. The NIPT test used in Australia is not likely to use whole blood and will be based on cff DNA. It is assumed the tests will, at a minimum, use RT-qPCR for two exons to identify fetal Rh D status and include an internal control.							
Additional comments							
Some of the included studies are overlapping with other meta-analyses included in this review. PPV and NPV were not provided due to variation in disease prevalence among the included studies. *Authors conclusion* The findings support the use of NIPT as a diagnostic test for rhesus status because of the nature of these conditions and the populations being tested.							

cffDNA, cell free fetal DNA; CI, confidence interval; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NIPT, non-invasive prenatal testing; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value; PCR, polymerase chain reaction; RT-qPCR, real-time quantitative PCR

a. Bivariate meta-analysis model might not converge when there are a small number of studies, or when there are zero cells (i.e. sensitivity or specificity is close to 100)

STUDY DETAILS: Systematic review of diagnostic studies			
Citation			
Saramago 2018 Saramago, P., Yang, H., Llewellyn, A., Walker, R., Harden, M., Palmer, S., Griffin, S., & Simmonds, M. (2018). High-throughput non-invasive prenatal testing for fetal rhesus D status in RhD negative women not known to be sensitised to the RhD antigen: A systematic review and economic evaluation. *Health Technology Assessment*, 22(13).			
Affiliation/Source of funds			
Commissioned by the HTA assessment programme of the National Institute for Health Research. Project number 15/17/02. Centre for Health Economics, University of York; Peninsula Technology assessment group, University of Exeter Medical School; Centre for Reviews and dissemination, University of York			
Study design	**Level of evidence**	**Location and study date**	**Setting**
Systematic review, meta-analysis and economic evaluation	Level I	Various Studies conducted in Denmark, the Netherlands, Spain, Sweden and UK	Obstetrics and maternity
Index test	**Exon(s) sequenced**	**Internal control(s)**	**Reference standard or comparator**
High throughput free-cell DNA tests (various platforms) All studies used maternal plasma as the sample source	Various All used at least two exons (usually exon 5, 7) except one study (exon 4 only)	Various All used at least two controls (targeting RHD positive and RHD negative DNA) except one (GAPDH)	Serological cord blood testing at birth or any other suitable postnatal blood
Population characteristics			
Clinical effectiveness: RhD negative pregnant women at GW 10–26. Majority where Caucasian. Three studies stated RAADP given at 28 and 30 weeks. *Diagnostic Accuracy:* Pregnant women who are RhD negative and not known to be sensitised to RhD antigen. Median GW 10–28. Only three studies stated multiple pregnancies were included (Finning 2008, Grande 2013, Wikman 2012). Majority where Caucasian, but one study recruited a large proportion of people with African ethnicity (19.3%). .			
Number of participants or samples		**Outcomes measured**	
Clinical effectiveness: N = 34428 (7 studies). Two comparative (NIPT vs no NIPT) and five non-comparative studies. Number of participants and samples ranged from 284 to 15126 (2 studies reported number of women (n = 23473), others reporting number of blood samples). *Diagnostic accuracy:* N = 42491 (8 studies). Number of participants and samples ranged from 282 to 18383 (2 studies reported number of women (n = 18882), others reporting number of blood samples). Excludes samples pre-GW8		*Clinical effectiveness:* Incidence of sensitisations, uptake of NIPT and RAADP, update of postnatal anti-D, reduction anti-D use, adverse events (allergic reactions, transmission of blood-borne disease, false paternity, HR-QoL) *Diagnostic accuracy:* FNR, FPR, inconclusive results, ROC Implementation rates	
Method of analysis			

Clinical effectiveness

Narrative synthesis owing to considerable heterogeneity in outcomes and study designs. The authors also conducted a simulation study (Monte Carlo), with a focus on parameters of relevance to the UK population and health care system. The simulation results are subject to a Monte Carlo error of ± 0.002%.

Diagnostic accuracy

Data was extracted from 2x2 tables, estimates of sensitivity, specificity, false positive rates and false negative rates where calculated and presented on forest plots and in ROC curves to examine the variability in diagnostic test accuracy within and between studies.

Hierarchical bivariate model fitted to produce summary ROC curves. Meta-analysis performed using standard random effects.

Heterogeneity explored with respect to gestational age at time of NIPT, type of NIPT, ethnicity

Heterogeneity for other clinical outcomes was assessed using the I^2-statistic value and visual inspection of forest plots. Subgroup analyses and meta-regression were used where feasible.

Sensitivity analysis explored the impact of including/excluding uninterpretable results and test accuracy in UK studies only.

INTERNAL VALIDITY

Overall risk of bias (descriptive)

Rating: Low

Description: No critical flaws. The systematic review provides an accurate and comprehensive summary of the results of the available studies that address the question of interest.

Clinical effectiveness

Included studies:

Both comparative studies judged to have significant limitations. Tibald (2013) had high risk of patient selection bias due to confounding and missing data. This study is not relevant to this review as it compared targeted RAADP with postnatal anti-D only (historical control). Banch Clausen (2014) was at critical risk of bias across all outcomes due to patient selection bias and insufficient adjustment for potential confounders.

Diagnostic accuracy

Included studies:

Despite some gaps in reporting, most studies were considered to have a low risk of bias for all four domains (patient selection, index test, reference standard, and flow and timing). Two studies (Akolekar 2011, Thurik 2015) were judged as having a high risk of bias due to patient selection bias and missing data. In Thurik (2015), 80% of participants had missing birth serology, in Akolekar (2011) inconclusive results were excluded from their analyses, thereby potentially inflating their diagnostic accuracy estimates.

Exclusion of multiple pregnancies from the analyses may have introduced patient selection bias, although it was deemed unlikely that this bias would substantially affect diagnostic accuracy estimates.

NIPT as an automated procedure was deemed to have a limited risk of human error, and multiple controls were used for RHD assays in all studies except one (Wikman 2012). Cord blood serology was the reference standard in all studies. The index test of NIPT was conducted independently of the reference standard and the results of one were considered unlikely to influence the results of the other, so the risk of incorporation bias was considered low.

Three studies (Chitty 2014, Finning 2008, Wikman 2012) stated that their diagnostic threshold was prespecified during the conduct of the screening programme. None of the studies reported whether or not there were any adverse events from the index test or reference standard.

RESULTS

Outcome **No. patients (No. trials)**	***targeted* RAADP** **n/N (%)** **% (95% CI)**	***universal* RAADP** **n/N (%)** **% (95% CI)**	**Risk estimate (95% CI)**	***Statistical significance*** ***p-value*** **Heterogeneity[a]** **I^2 (*p*-value)**
Targeted RAADP vs universal RAADP				
Incidence of Rh D alloimmunisations	The review authors identified one study (Tibald, 2013) involving 8347 women. The study compared targeted RAADP with historical control group (receiving postnatal anti-D only) therefore is not relevant to this review.			

Utilisation of anti-D 3 non-comparative studies identified.	Soothill (2015) observed a 6% (95%CI 4, 8) reduction per month, equating to 29% reduction in total use within 6 months of NIPT among 529 women at three maternity hospitals in the UK. This corresponded to 35% of Rh D negative women not receiving anti-D unnecessarily. Banch Clausen (2014) reported 37.1% (4706/12668) of Rh D negative women avoided unnecessary anti-D within 2 years of NIPT screening programme in Denmark Grande (2013) noted 5% (5/95) women with Rh D negative fetus requested anti-D despite NIPT in a single centre in Spain.		
Adverse events	No studies identified		
Targeted RAADP vs universal RAADP (simulation study) [a] * assumes women who do not receive NIPT (for any reason) would still be offered RAADP			
Incidence of Rh D alloimmunisation (assuming cord blood serology used to guide postpartum anti-D)	284 per 100,000	281 per 100,000	3 additional sensitisations per 100,000
Incidence of Rh D alloimmunisation (no postpartum anti-D to test negative women)	294 per 100,000	281 per 100,000	13 additional sensitisations per 100,000
Utilisation of anti-D (% Rh D negative women who receive)	65.9	99	*assumes 99% compliance
Unnecessary administration of anti-D (% women with Rh D negative fetus)	5.7	38.9	
Missed beneficial administration of anti-D (% women with Rh D positive fetus)	1.2	0.6	
Targeted RAADP vs universal RAADP (simulation study) * assumes women who do not receive NIPT (for any reason) are also not offered RAADP			
Incidence of Rh D alloimmunisation (assuming cord blood serology used to determine postpartum anti-D)	296 per 100,000	281 per 100,000	15 additional sensitisations per 100,000
Incidence of Rh D alloimmunisation (no postpartum anti-D to test negative women)	309 per 100,000	281 per 100,000	28 additional sensitisations per 100,000
Utilisation of anti-D (% Rh D negative women who receive)	62.7	99	36.9% reduction in anti-D use *assumes 99% compliance
Unnecessary administration of anti-D (% women with Rh D negative fetus)	4.5	38.9	

Missed administration of anti-D (% women with Rh D positive fetus)	3.2		0.6				
2x2 table with inconclusive results (where available)							
N = 42491	**Reference standard positive** n = 26143 (61.53%)		**Reference standard negative** n = 16348		**Inconclusive results** n = 889 (2 studies NR)		
Index text positive n = NR	NR		NR				
Index text negative n = NR	NR		NR				
Index test inconclusive n = 889 (2 studies NR)							
Outcome N (no. of studies)	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**	**LR+ (95% CI)**	**LR– (95% CI)**	**Diagnostic accuracy % (95% CI)**
Diagnostic performance of NIPT against birth blood sample (bivariate meta-analysis)[b]							
N=? (8 studies) Inconclusive results treated as test positive	99.66 (99.24, 99.85)	96.14 (94.18, 97.46)	NR	NR	NR	NR	NR
	Subgroup analysis (timing of NIPT): Meta-regression not performed as no linear trend observed. FNR after 11 weeks gestations were consistent, irrespective of timing, but were higher before 11 weeks gestation. No consistent pattern observed with FPRs. Subgroup analysis (number of inconclusive results): Meta-regression not performed as no linear trend observed. A trend towards reduced number of inconclusive results after GW 11. Subgroup analysis (ethnicity): Not feasible as relevant data not reported. Subgroup analysis (type of machine used to perform NIPT): Not feasible a relevant data confounded by study location.						
N=? (6 studies) Inconclusive test results treated as test positive Excluding Thurik 2015 and Grande 2013	99.62 (99.06, 99.85)	95.63 (93.22, 97.21)	NR	NR	NR	NR	NR
N=? (8 studies) Not including women with inconclusive results	99.65 (99.18, 99.85)	98.74 (98.17, 99.13)	NR	NR	NR	NR	NR
N=? (3 studies) UK only studies	99.79 (99.52, 99.91)	94.27 (92.9, 95.42)	NR	NR	NR	NR	NR
EXTERNAL VALIDITY							
Generalisability (relevance of the study population to the Guidelines target population)							
The evidence is generalisable to the target population with some caveats. Studies enrolled Rh D negative pregnant women but some may not be directly applicable in terms of RHD prevalence							
Applicability (relevance of the evidence to the Australian health care system)							

The evidence is probably applicable to the Australian health care system with some caveats. Only high throughput studies were included. This may overestimate the sensitivity of the test.
Additional comments
Clinical effectiveness The simulation study assumes offering RAADP at GW28. Where postpartum anti-D was administered, cord serology was assumed to be 100% accurate. There are no adverse events. *Diagnostic accuracy* Some of the included studies are overlapping with other meta-analyses included in this review. There was evidence of inconsistency across studies. The I^2-statistic for heterogeneity was 75% for the FNR and 99% for the FPR. The high heterogeneities are in part a consequence of the high accuracy of the test and the large size of the studies (and consequently small within-study variance), because I^2 increases as the average within-study variance declines). They do not necessarily indicate any clinically meaningful differences between studies. The heterogeneity in FPRs is likely to be a consequence of differing reporting and handling of inconclusive test results. Reasons for inconclusive results varied and included insufficient DNA (n= 36), RHD variant (n = 200), maternal Weak D (n = 93), suspected maternal RHD gene or high level maternal DNA (n = 57), fetal variant (n = 45), sample or technical problems (n = 30), weak PCR sample (n = 83), missing second sample (n = 18), or not reported. The authors calculated that approximately 6.7% (95%CI 3.7, 11.7) of women in the UK will have an inconclusive test result, and that the majority with an inconclusive test result have an Rh D positive infant (ranged from 38.5% to 85.7% of samples).

CI, confidence interval; FNR, false negative rate; FPR, false positive rate; HR-QoL, health-related quality of life; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NIPT, non-invasive prenatal testing; NPV, negative predictive value; PPV, positive predictive value; RAADP, routine antenatal anti-D prophylaxis; ROC, receiver operating characteristic

a. estimated to be 641 per 100,000 in the absence of RAADP

b. In all analyses, women whose NIPT was conducted at or before 11 weeks gestation were excluded when possible because of concerns that the diagnostic accuracy is poorer before 11 weeks and that the test should not be conducted. Some tests were performed between 8–11 weeks gestation in two studies. Data converted from FNR (at risk of sensitisation) and FPR (unnecessary anti-D). PPV and NPV were not provided

STUDY DETAILS: Systematic review of diagnostic studies							
Citation							
Zhu 2013 Zhu, Y. J., Zheng, Y. R., Li, L., Zhou, H., Liao, X., Guo, J. X., & Yi, P. (2014). Diagnostic accuracy of non-invasive fetal RhD genotyping using cell-free fetal DNA: A meta-analysis. *Journal of Maternal-Fetal and Neonatal Medicine,* 27(18), 1839-1844.							
Affiliation/Source of funds							
No conflicts of interest or funding declared. Department of Obstetrics and Gynecology, Research Institute of Surgery, Daping Hospital, Third Military Medical University, Chongqing, PR China							
Study design		**Level of evidence**		**Location and study date**		**Setting**	
Systematic review and meta-analysis of diagnostic studies		Level I		Studies published in English up to 2013 No restrictions on study location		Obstetrics and maternity	
Index test		**Exon(s) sequenced**		**Internal control(s)**		**Reference standard or comparator**	
Fetal Rh D blood group identification using maternal whole blood (or serum, plasma) and cffDNA.		Various		Various		Determination of fetus or newborn RhD blood type was provided	
Population characteristics							
Rh D negative pregnant women							
Number of participants or samples				**Outcomes measured**			
37 articles which included 46 protocols. 11,129 samples (352 inconclusive samples excluded)				Diagnostic accuracy, sensitivity, specificity PPR, NPR, diagnostic accuracy by gestational accuracy			
Method of analysis							
Data was analysed using meta-DiSc v1.4. The random effects model was calculated with a 95% confidence interval. The overall diagnostic accuracy, sensitivity and specificity for RhD determination from maternal blood were calculated for all studies that met inclusion criteria. Diagnostic accuracy based on trimester was analysed. Accuracy, ROC curve, sensitivity, specificity and positive and negative predictive ratios were calculated.							
INTERNAL VALIDITY							
Overall risk of bias (descriptive)							
Rating: Serious Description: More than one critical flaw with non-critical weaknesses. The review has more than one critical flaw and *should not* be relied on to provide an accurate and comprehensive summary of the available studies. Key flaws relating to risk of bias of included studies not reported or included in the analysis and potential sources of conflict of interest including any funding received not provided. Included studies: no risk of bias of included studies included in this review. Studies included in the review are not referenced. It is unclear if any effort was made to ensure duplicate sample results are not included.							
RESULTS							
N = 11129		**Reference standard positive** n = 6941 (62.4%)		**Reference standard negative** n = 3836		**Inconclusive results** n = 352	
Index text positive		6864		89			
Index text negative		77		3747			
Index test inconclusive						Not included in the analysis	
Outcome	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**	**LR+ % (95% CI)**	**LR- % (95% CI)**	**Diagnostic accuracy % (95% CI)**
Diagnostic performance (n correct/N total) NIPT against birth blood sample							

N = 11129 (46 studies)	--	--	--	--	--	--	95.3 (NR)
N = 10777 (46 studies)[a] Random effects model	99 (99, 99) I^2 = 80.5%	98 (97, 98) I^2 = 78.0%	98.7 (NR)	98.0 (NR)	--	--	98.5 (NR) 10611/10777
Diagnostic performance (n correct/N total) by gestational age (N = 6670, no. studies not reported)							
1st trimester 882/898	--	--	--	--	--	--	99.0 (NR)
2nd trimester 3282/3322	--	--	--	--	--	--	98.3 (NR)
3rd trimester 2418/2450	--	--	--	--	--	--	96.4 (NR)
EXTERNAL VALIDITY							
Generalisability (relevance of the study population to the Guidelines target population)							
The evidence is assumed directly generalisable to the Australian population with some caveats. The generalisability to the study population is unclear as details of included studies is not provided. There was no restriction on gestational age and no mention of alloimmunisation status.							
Applicability (relevance of the evidence to the Australian health care system)							
The evidence is assumed applicable to the Australian health care context with some caveats. The applicability of this study is unclear. The sample collection and diagnostic sequencing methods are not well described.							
Additional comments							
Some of the included studies are overlapping with other meta-analyses included in this review. PPV and NPV are assumed to be Positive predictive ratio/negative predictive ratio. Most studies (number not provided) used maternal plasma, particularly those published after 2003. Highest diagnostic accuracy occurred in 1st trimester							

--. data not reported; CI, confidence interval; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; ROC, Receiver operating characteristic

a. Authors stated they removed substandard samples. It is assumed this means inconclusive samples were excluded from the analysis. Only 44 studies listed in the meta-analysis, it is not clear which two protocols were not included.

E3.2 Level II – Consecutive patients with a valid reference standard

STUDY DETAILS: Diagnostic study			
Citation			
De Haas 2016 De Haas, M., Thurik, F. F., Van Der Ploeg, C. P. B., Veldhuisen, B., Hirschberg, H., Soussan, A. A., Woortmeijer, H., Abbink, F., Page-Christiaens, G. C. M. L., Scheffer, P. G., & Van Der Schoot, C. E. (2016). Sensitivity of fetal RhD screening for safe guidance of targeted anti-D immunoglobulin prophylaxis: Prospective cohort study of a nationwide programme in the Netherlands. *BMJ* (Online), 355			
Affiliation/Source of funds			
The study received no specific funding. The authors declared no specific conflicts of interest that could appear to influence the study outcomes. University of Amsterdam, Amsterdam; Leiden University Medical Center, Leiden; Sanquin Research, Amsterdam; University Medical Center, Utrecht; Netherlands Organization for Applied Scientific Research, Department of Child Health, Leiden; National Institute for Public Health and the Environment, Service for vaccine provision and prevention programs, Bilthoven; National Institute for Public Health and the Environment, Center for population screening, Bilthoven			
Study design	**Level of evidence**	**Location and study date**	**Setting**
Prospective observational study	Level II	Nationwide screening program, The Netherlands 4 Jul 2011–7 Oct 2012	Obstetrics and maternity
Index test	**Exon(s) sequenced**	**Internal control(s)**	**Reference standard or comparator**
Duplex RT-qPCR of cffDNA isolated from 1 mL of maternal plasma taken at GW 27–29	RHD exon 5, exon 7	Not specified. A non-human sequence introduced into the assay as an internal control for DNA isolation to further reduce the false negative rate.	Cord blood serology
Population characteristics			
RhD negative pregnant women who are not alloimmunised and a request for fetal RHD typing at Sanquin Blood Supply were eligible. Women with multiple pregnancies were excluded as were those with blood samples drawn prior to GW27. At total of 62 women were pregnant twice during the study period. Mean (SD) maternal age, years; 30.8 (4.8), Ethnicity (%): European (90.4), Mediterranean (4.1), Black Creole (0.8), Asian (0.4), Hindustani (0.3), Other (4.0)			
Number of studies or samples		**Outcomes measured**	
N=25789 32622 blood samples were sent to the laboratory. 382 samples were from Rh D positive women and 18 samples show weak reactivity. After exclusion of these 400 cases, fetal RHD testing was performed for 32222 pregnancies. Cord blood samples missing in 6433 women.		Sensitivity, specificity, PPV, NPV, diagnostic accuracy	
Method of analysis			
All PCR tests were performed in triplicate, with a prediction algorithm based on the six amplification signals used for scoring. Based on the combination of scored cycle threshold values, the computer algorithm provided the following conclusions: 'fetus RhD positive', 'fetus RhD negative', or 'no result', combined with or without advice to repeat the test. Performance was of the fetal RHD test was monitored every four weeks (independent of sample size) to enable adaptations to the antenatal screening programme if the false negativity rate would exceed the preset limit of 0.25%. Data on ethnicity, parity, and gestational age at time of blood sampling from a linked and anonymised dataset. All maternal or newborn samples in which the fetal RHD PCR test or the cord blood serology suggested the presence of an RHD variant were comprehensively analysed for research purposes.			

INTERNAL VALIDITY

Overall risk of bias (descriptive)

Rating: Unclear

Description: The exclusion of multiple pregnancies may favour diagnostic accuracy of the index test and presents an unclear risk of patient selection bias. The conduct of the test was monitored, and the algorithm adjusted to set the false negative rate at 0.25%, this introduces bias in favour of the test. Reference standard was conducted and interpreted with a low risk of bias. A high proportion of patients (20%) did not have cord blood samples taken and are not included in the analysis.

RESULTS

2x2 table with inconclusive results counted as test positive[a]

N = 25789	**Reference standard positive** n = 15825 (61.36%)	**Reference standard negative** n = 9964 (38.64%)	**Inconclusive results** n = 100
Index text positive n = 16041 (62.2%)	15816	225[b]	
Index text negative n = 9748 (37.8%)	9[c]	9739	
Index test inconclusive			Included as test positive

Outcome	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**	**LR+ (95% CI)**	**LR- (95% CI)**	**Diagnostic accuracy % (95% CI)**
Diagnostic performance NIPT against birth blood sample[d]							
N = 25789	99.94% (99.89-99.97)	97.74 (97.43, 98.02)	98.60 (98.40, 98.77)	99.91 (99.82, 99.95)	44.2593	0.0006	99.09 (NR)

EXTERNAL VALIDITY

Generalisability (relevance of the study population to the Guidelines target population)

Study excludes women with multiple pregnancies, therefore there are low concerns the study is not directly applicable to the Australian setting. Rh D prevalence = 61.36%

Applicability (relevance of the evidence to the Australian health care system)

Centralised setting may not be directly applicable to the Australian setting.

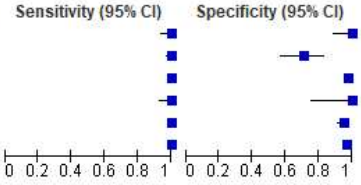
Additional comments

*More than 98% of women who participated in the antenatal screening programme participated in the fetal RHD testing

This study population overlaps with the population reported by Thurik 2015 (and De Haas 2012) that was included in Saramago 2018.

The study included here as supplementary data provides data on sensitivity and specificity according to ethnicity.

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
De Haas 2016 Asian	54	0	0	31	1.00 [0.93, 1.00]	1.00 [0.89, 1.00]
De Haas 2016 Creole	116	15	0	37	1.00 [0.97, 1.00]	0.71 [0.57, 0.83]
De Haas 2016 European	11839	147	6	7486	1.00 [1.00, 1.00]	0.98 [0.98, 0.98]
De Haas 2016 Hindustani	53	0	0	13	1.00 [0.93, 1.00]	1.00 [0.75, 1.00]
De Haas 2016 Mediterranean	602	14	0	263	1.00 [0.99, 1.00]	0.95 [0.92, 0.97]
De Haas 2016 Other	540	10	1	309	1.00 [0.99, 1.00]	0.97 [0.94, 0.98]



cffDNA, cell free fetal DNA; CI, confidence interval; DNA, deoxyribonucleic acid; GW, gestational week; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NIPT, non-invasive prenatal testing; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; RT-PCR, real-time polymerase chain reaction.

a. Inconclusive results were minimised by reporting RhD positive results if any RHD sequences were detected in maternal plasma, and in cases in which a pregnant woman was suspected of carrying an RHD variant allele. Inconclusive fetal RHD test results issued only when the presence of an RHD variant gene in the mother was suggested.

b. 100 of these samples contained variant RHD genes (mother, n=55; newborn n=45), with further testing indicating 22 newborns had a false negative serology (imperfect reference standard); 10 cases were serology sample mix ups; 71 cases had false positive fetal RHD test results owing to the strict scoring algorithm, which mainly aimed to prevent false negative cases, and the remaining 44 had five or six positive amplification signals suggesting vanishing twins as potential cause for discrepancy (Thurik 2015).

c. 6 samples due to low/no fetal DNA, three due to technical failures

d. LR+ and LR– calculated post-hoc using RevMan 5.3.

STUDY DETAILS: Diagnostic study			
Citation			
Haimila 2017 Haimila, K., Sulin, K., Kuosmanen, M., Sareneva, I., Korhonen, A., Natunen, S., Tuimala, J., & Sainio, S. (2017). Targeted antenatal anti-D prophylaxis program for RhD negative pregnant women - outcome of the first two years of a national program in Finland. *Acta Obstetricia et Gynecologica Scandinavica*, 96(10), 1228-1233.			
Affiliation/Source of funds			
Finnish Red Cross Blood Service, Helsinki, Finland			
Study design	**Level of evidence**	**Location and study date**	**Setting**
Prospective observational	Level II	Finland, Feb 2014 – Jan 2016	Maternity care centres and delivery hospitals
Index test	**Exon(s) sequenced**	**Internal control(s)**	**Reference standard or comparator**
NIPT (RT-qPCR) screening of cffDNA in maternal plasma at 24–26 weeks gestation	RHD exon 5, exon 7	None used (p. 1232)	Postnatal serology of cord or heel stick samples at local laboratories
Population characteristics			
All non-immunised RhD negative women at GW 24–26 weeks. Maternal blood samples were taken mainly at GW 24–26 but accepted from Week 20 onwards. No exclusions.			
Number of studies or samples		**Outcomes measured**	
10814 women were screened.		Sensitivity, specificity, false negative rate, and false positive rate.	
Method of analysis			
RT-PCR was used to sequence the genes, with a prediction algorithm used for scoring. In addition to the analysis software algorithm being based on strict thresholds for result interpretation, two persons separately evaluated the results visually to avoid false negative results. All the data were electronically transferred, and reports generated automatically, which not only minimised human error but also saved resources. The algorithm is not reported. Birth result was compared with the fetal one. Discordant results were reported and the newborn samples sent to the FRC Blood Service laboratory for genotyping, and postnatal prophylaxis was administered.			
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			
Rating: Low Description: No concerns with patient selection or conduct and interpretation of the reference standard. Minor concerns the algorithm used to interpret results minimises rate of false negative results which favours the index test. The algorithm is not provided. Exclusion of inconclusive results from the analysis favours the index test but details are provided differences are negligible.			
RESULTS			
2x2 table (not including inconclusive results)			
N = 10814	**Reference standard positive** n = 7081 (65.48%)	**Reference standard negative** n = 3647 (33.72%)	**Inconclusive results**
Index text positive n = 7087 (65.5%)	7080	7	
Index text negative n = 3641 (33.7%)	1b	3640	
Index test inconclusive[a] n = 86 (0.8%)	53	32	Maternal variant (n = 60) Fetal variant (n = 13) Unknown (n = 13)
2x2 table (inconclusive results counted as index test positive)[a]			

N = 10813		**Reference standard positive** n = 7134 (65.98%)		**Reference standard negative** n = 3679		**Inconclusive results**	
Index text positive n = 7172 (66.33%)		7133		39			
Index text negative n = 3641 (33.67%)		1		3640			
Outcome	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**	**LR+ (95% CI)**	**LR– (95% CI)**	**Diagnostic accuracy % (95% CI)**
Diagnostic performance NIPT against birth blood sample							
N = 10814[d]	99.99 (99.92, 99.99)	99.81 (99.60, 99.92)	99.90 (99.80, 99.96)	99.97 (99.85, 99.99)	NR	NR	99.93 (99.85, 99.97)
N=10813[a]	100 (100, 100)	99 (99.0, 99.0)	99.46 (NR)	99.97 (NR)	94.3201	0.0001	99.63 (NR)
EXTERNAL VALIDITY							
Generalisability (relevance of the study population to the Guidelines target population)							
The evidence is directly generalisable to the Australian population with some caveats.							
Applicability (relevance of the evidence to the Australian health care system)							
The evidence is applicable to the Australian health care context with few caveats							
Additional comments							
Women who tested positive were given routine antenatal anti-D of 1250-1500 IU at 28–30 weeks gestation. Unnecessary prophylaxis avoided in 3641/10814 (33.7%) of cases Unnecessary prophylaxis administered to 39/10814 (0.4%) of women. The turnaround time from sample enrolment to reporting was 5 days. The study assessed 83.2% (estimated) of the expected number of non-sensitised RhD negative pregnant women. Over the study period compliance increased from 69.7% in the first year (during roll-out of the programme), to 97.3% in the second year, and 98.3% in the last 6 months of the study period.							

cffDNA, cell free fetal DNA; CI, confidence interval; DNA, deoxyribonucleic acid; IU, international units LR+, positive likelihood ratio; LR-, negative likelihood ratio; NIPT, non-invasive prenatal testing; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; RT-PCR, real-time polymerase chain reaction;

a. includes 86 inconclusive results (53 positive, 32 negative, and 1 with birth serology results not available); 69% (60/86) were due to mothers' RHD null variants, 15% (13/86) were due to fetuses' RHD variants, and 15% (13/86) due to a haemolytic sample and weak or variable amplification.

b. sample mix up or low fetal DNA concentration

c. three due to technical errors, two due to fetal RHD variants, and two probably due to contamination

d. not including inconclusive results

STUDY DETAILS: Diagnostic study			
Citation			
Hyland 2017 Hyland, C. A., Millard, G. M., O'Brien, H., Schoeman, E. M., Lopez, G. H., McGowan, E. C., Tremellen, A., Puddephatt, R., Gaerty, K., Flower, R. L., Hyett, J. A., & Gardener, G. J. (2017). Non-invasive fetal RhD genotyping for RhD negative women stratified into RHD gene deletion or variant groups: comparative accuracy using two blood collection tube types. *Pathology,* 49(7), 757-764.			
Affiliation/Source of funds			
Australian Red Cross Blood Service funded by the Australian government ; University of Queensland, South Brisbane, Qld; Royal Prince Alfred Hospital, Camperdown, Sydney, NSW; Mater Mothers' Hospital, South Brisbane, Qld; The University of Sydney, Sydney, NSW, Australia The authors stated no conflicts of interest to disclose.			
Study design	**Level of evidence**	**Location and study date**	**Setting**
Prospective cohort	Level II	Australia Dates not reported	Obstetric and maternity (two maternity hospitals with centralised testing)
Index test	**Exon(s) sequenced**	**Internal control(s)**	**Reference standard or comparator**
qPCR of cffDNA in maternal plasma. (Rotor-Gene Q, Qiagen) Comparison of sample collection from EDTA and BCT tubes.	RHD exon 5, exon 10	CCR5 (a chemokine receptor gene, to verify that cfDNA (maternal and fetal) was extracted from each sample and to compare background cfDNA levels as a measure of maternal white cell lysis)	Cord blood sample
Population characteristics			
Rh-D negative pregnant women at any gestational age (median GW 19.29; range 9–37.1). Median BMI, recorded at each subject's first antenatal appointment, was 23.5 kg/m^2 (range 15–51 kg/m^2) and 15.8% of subject BMIs were >30 kg/m^2.			
Number of participants or samples		**Outcomes measured**	
665 women enrolled, three samples excluded (two haemolysed during transport, one was Rh D positive) 612/662 (92.4%) with cord blood outcomes. 15/662 (2.3%) flagged as potential RHD variants 647 included in the analysis Study does not specifically state consecutive patients enrolled. Authors contacted and confirmed that all consecutive eligible women were invited to participate.		True positive, false positive, false negative, true negative, inconclusive results.	
Method of analysis			
Sequenced using the TruSight One Sequencing Panel kit on a MiSeq platform (Illumina, USA). To verify extraction of cfDNA, the CCR5 signal was compared against a standard curve to give a quantitative value of cfDNA present (both maternal and fetal). Samples were run in triplicate, with a minimum of two samples with exon 5 & 10 positively amplified required to assign 'fetal RHD detected'. Samples with no more than one replicate amplified at exon 5 & 10 were assigned 'no fetal RHD detected'. Samples with only one exon detected were reported 'inconclusive'.			
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			
Rating: High Description: Unclear patient selection bias. The study does not discuss if Rh D alloimmunised women or those with multiple pregnancies were eligible for inclusion. Fifteen women with potential RHD maternal variants were excluded from the analysis, which would increase the apparent accuracy of the index test.			

RESULTS							
2x2 table with inconclusive results (where available)							
N = 599[a]		**Reference standard positive** n = 370 (61.8%)		**Reference standard negative** n = 229 (38.2%)		**Inconclusive results**	
Index text positive n=370 (61.8%)		370		1[b]			
Index text negative n=226 (37.7%)		0		226			
Index test inconclusive n = 2				2		In both samples, NIPT detected exon 10 but not exon 5. This was attributed to paternally inherited RHD variants.	
2x2 table (inconclusive results counted as index test positive)							
N = 599 [a]		**Reference standard positive** n = 370 (61.8%)			**Reference standard negative** n = 229 (38.2%)		
Index text positive n = 373		370			3		
Index text negative n = 226		0			226		
Outcome	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**	**LR+ (95% CI)**	**LR– (95% CI)**	**Diagnostic accuracy % (95% CI)**
Diagnostic performance NIPT against birth blood sample[c]							
N = 599	100 (99, 100)	98.69 (96, 100)	99.20	100	76.33	0.000	99.5%
EXTERNAL VALIDITY							
Generalisability (relevance of the study population to the Guidelines target population)							
The evidence is directly generalisable to the Australian target population with some caveats (multiple pregnancies not included)							
Applicability (relevance of the evidence to the Australian health care system)							
The evidence is directly applicable to the Australian health care context							
Additional comments							
Mean time between collection of sample and plasma processing was 61.7 h, (range 2.9–187.5 h, IQR, 26.5–77.98 h).							

cffDNA, cell free fetal DNA; CI, confidence interval; DNA, deoxyribonucleic acid; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NIPT, non-invasive prenatal testing; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; 15 patients with maternal variants were excluded before analysis.

a. Of 647 samples tested by NIPT, eight pregnancies resulted in fetal death (6/8 were RHD positive and 2/8 were RHD negative) and 40 had no birth serology available (28/40 were RHD positive and 12/40 were RHD negative). These data are not included in the analysis.

b. Suspected paternal RHD variant. RHD exons 5&10 detected using NIPT.

c. Calculated post-hoc in RevMan 5.3. Inconclusive results counted as index test positive

STUDY DETAILS: Diagnostic study			
Citation			
Macher 2012 Macher, H. C., Noguerol, P., Medrano-Campillo, P., Garrido-Mirquez, M. R., Rubio-Calvo, A., Carmona-Gonzalez, M., Martin-Sunchez, J., Perez-Simen, J. A., & Guerrero, J. M. (2012). Standardization non-invasive fetal RHD and SRY determination into clinical routine using a new multiplex RT-PCR assay for fetal cell-free DNA in pregnant women plasma: Results in clinical benefits and cost saving. *Clinica Chimica Acta*, 413(3-4), 490-494.			
Affiliation/Source of funds			
Supported by grants from the Instituto de Salud Carlos III (Red RETICEF RD06/00130001 and Evaluación de Tecnologías Sanitarias, P107/90175). No statement regarding potential conflicts of interests provided. The Virgen del Rocío University Hospital (IBiS/CSIC/SAS/University of Seville), Seville, Spain; Central University of Venezuela, Caracas, Venezuela			
Study design	**Level of evidence**	**Location and study date**	**Setting**
Prospective observational study	Level II	Seville, Spain 2010	Single institution Obstetrics and maternity
Index test	**Exon(s) sequenced**	**Internal control(s)**	**Reference standard or comparator**
RT-PCR of cffDNA in maternal plasma at GW 10–28 Cohort 1: Single TaqMan PCR Cohort 2: Multiplex TaqMan PCR	RHD exon 5, exon 7	SRY	Serological testing of cord blood at birth
Population characteristics			
RhD negative pregnant women presenting to a single institution. Baseline characteristics not provided.			
Number of participants or samples		**Outcomes measured**	
2127 RhD negative pregnant women, analysed in two consecutive cohorts. Cohort 1: 136 samples with two pregnancies aborting and 134 healthy newborns (25 by caesarean, 24 after 40–42 weeks gestation, and two mothers had twins). 58 of these pregnant women carried RhD negative fetuses. Cohort 2: 1993 samples were evaluated, with 1012 included in the analysis. The remaining pregnancies (n = 981) are ongoing.		Sensitivity, specificity, PPV, NPV, accuracy. Cost Concordance	
Method of analysis			
RT-PCR analysis using the TaqMan assay protocol was performed using two different instruments in parallel, LightCycler 2.0 and LightCycler 480 (Roche Diagnostics, Manheim, Germany) for single TaqMan PCR or Multiplex TaqMan PCR, respectively. Multiplex PCR allowed amplification of the three targets studied in a single run. DNA was automatically extracted using the MagNa Pure Compact instrument. A fetus was assessed as being RHD positive if all replicates of exon 5 and/or RHD exon 7 showed a positive PCR result and RHD negative when all replicates showed a negative PCR result. Samples not classified as either positive or negative were described as indeterminate. When a result was uncertain, the laboratory asked for a second sample from the same pregnant woman to repeat the determination. A result is considered uncertain when duplicates in PCR study do not match. If results in duplicate tests were inconclusive or all data for RHD and SRY were negative, PCR set-up was repeated, resulting in a maximum number of four RHD/SRY PCR procedures. Where it was not possible to obtain a second sample it was treated as an RhD positive result.			
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			

Rating: High Description: Some concerns with patient selection bias as exclusions and baseline characteristics not described. Conduct of the index test suggests repeat samples requested, but not clear if or how many uncertain results required re-testing. No description of inconclusive results. Not all patients included in the analysis, as 981 women still pregnant.							
2x2 table (not including inconclusive results)							
Cohort 1 **N = 134**[a]		**Reference standard positive** n = 76 (56.72%)		**Reference standard negative** n = 58 (43.28%)		**Inconclusive results**	
Index text positive n = 79 (58.96%)		76		3			
Index text negative n = 55 (41.04%)		0		55			
Index test inconclusive						NR (assumed counted as positive)	
2x2 table (not including inconclusive results)							
Cohort 2 **N = 1012**		**Reference standard positive** n = 619 (61.17%)		**Reference standard negative** n = 393 (38.83%)		**Inconclusive results**	
Index text positive n = 626 (61.86%)		619		7			
Index text negative n = 386 (38.14%)		0		386			
Index test inconclusive						NR (assumed counted as positive)	
RESULTS							
Outcome	**Sensitivity** **% (95% CI)**	**Specificity** **% (95% CI)**	**PPV** **% (95% CI)**	**NPV** **% (95% CI)**	**LR+** **(95% CI)**	**LR–** **(95% CI)**	**Diagnostic accuracy** **n/N (%)**
NIPT using a single exon against birth blood sample							
Cohort 1 single assay[b] N = 134	100 (95, 100)	94.8 (89, 99)	96.2	100	19.3333	0.0000	131/134 (97.8)
NIPT using multiplex PCR against birth blood sample							
Cohort 2 multiplex assay[b] N = 1012	100 (99, 100)	98.2 (96, 99)	98.9 (NR)	100 (NR)	56.1429	0.0000	1005/1012 (99.3)
EXTERNAL VALIDITY							
Generalisability (relevance of the study population to the Guidelines target population)							
The evidence is directly generalisable to the target population with some caveats (baseline characteristics not provided)							
Applicability (relevance of the evidence to the Australian health care system)							
The evidence is probably applicable to the Australian health care context with some caveats. (repeat testing and request for second sample may not be feasible)							
Additional comments							

The total study population included 9642 women, of which 20% were RhD negative.
The inconclusive results were not discussed. It is not clear if there were no inconclusive results, or if inconclusive results were resolved without being reported.
100% concordance reported between single and multiplex PCR (100 samples tested).
The authors noted 815 (38%) RhD negative pregnant women avoided unnecessary Anti D prophylaxis and the service avoided need for assays of the father as periodical controls.
The authors estimated total costs savings of more than €49,250 In the population studied.

cffDNA, cell free fetal DNA; CI, confidence interval; DNA, deoxyribonucleic acid; GW, gestational week; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NIPT, non-invasive prenatal testing; NPV, negative predictive value; NR, not reported; PCR, polymerase chain reaction; PPV, positive predictive value; SRY, sex-determining region Y; RHD, Rhesus D; RT-PCR, real-time polymerase chain reaction.

a. Not including two pregnancies that were aborted.

b. LR+ and LR–calculated post-hoc using in RevMan 5.3.

STUDY DETAILS: Diagnostic study			
Citation			
Manfroi 2018 Manfroi, S., Calisesi, C., Fagiani, P., Gabriele, A., Lodi, G., Nucci, S., Pelliconi, S., Righini, L., & Randi, V. (2018). Prenatal non-invasive foetal RhD genotyping: diagnostic accuracy of a test as a guide for appropriate administration of antenatal anti-D immunoprophylaxis. *Blood transfusion*, 1-11. doi:https://dx.doi.org/10.2450/2018.0270-17			
Affiliation/Source of funds			
Financially supported by a grant from the Regional Blood Centre of the Region of Emilia-Romagna (Italy) S. Orsola-Malpighi Polyclinic, Bologna; Ospedale degli Infermi, Rimini; Imola Hospital, Imola; Maggiore Hospital, Bologna; S. Anna Hospital, Ferrara; Maggiore Hospital, Bologna The authors declared no conflicts of interest			
Study design	**Level of evidence**	**Location and study date**	**Setting**
Prospective observational	Level II	Italy Feb 2016 to Jan 2018	Obstetrics and maternity Five regional immunohaematology and transfusion service centres
Index test	**Exon(s) sequenced**	**Internal control(s)**	**Reference standard or comparator**
RT-qPCR of cffDNA isolated from maternal plasma	RHD exon 5, exon 7, exon 10	Maize DNA used to validate fetal DNA extraction/ amplification. Rh D negative and RhD positive plasma controls provided with the kit (not described)	Rh D cord blood typing performed locally at birth
Population characteristics			
Rh D negative pregnant women, with Rh D positive partners or partners of unknown Rh D phenotype, presenting in hospitals at different gestational ages for antenatal immunohaematological tests (first or third trimester [approx. 28 weeks] screening) or for invasive diagnostic procedures. Majority of women were Caucasian (273/284, 96.1%) with participation of other ethnicities limited by language barriers. There were six twin pregnancies (2.1%) and 166 were multiparous (58.5%). Antibody screening performed at the beginning of third trimester of pregnancy was negative in 260/284 pregnant women and positive in 24/284 owing to passive anti-D from previous RhIg, administered after chorionic villous sampling in the first trimester. Postnatal screening, performed 6 months after delivery, was negative in all tested women (248/284).			
Number of participants or samples		**Outcomes measured**	
455 Rh D negative pregnant women were recruited; 31/455 collected samples were not tested due to various reasons. 31 samples excluded as these were in women before GW 24 and a further 26 excluded, with reasons not specified. One sample excluded as cord blood not available after stillbirth and a further 82 pregnancies were ongoing at the time of reporting. This leaves total 284 (62.4%) available samples included in the analysis.		Sensitivity, specificity, PPV, NPV, accuracy	
Method of analysis			

Extraction of fetal DNA initially using QIAamp®DNA DSP Blood Minit Kit (Qiagen), then QIAamp® DSP DNA virus kit, then finally with QIAamp®R Circulating Nucleic Acid Kit. Changes made due to high percentage of inconclusive results and low threshold cycle (Ct). Ct values <40 for exon 5 and <41 for exon 7 and 10 interpreted as positive signals.

RT-PCR was carried out in duplicate for RHD exons 5, 7 and 10 using the Free DNA Fetal Kit® RhD (Institut de Biotechnologies Jacques-Boy, Reims, France) and LightCycler® 480 Probes Master (Roche, Rotkreuz, Switzerland), generating six test results for each sample.

Genotype reported as RHD negative when all (6/6) RHD PCR reactions were negative, and RHD positive when at least five (5/6) PCR samples were positive. Inconclusive results were reported with RHD positive reactions observed in four or less (≤4/6) samples.

Inconclusive results were repeated to identify technical errors.

INTERNAL VALIDITY

Overall risk of bias (descriptive)

Rating: High

Description: Low concerns regarding conduct of the index test and reference standard. Serious concerns regarding patient selection (reasons for exclusions not provided) and not all patients included in the analysis (samples collected prior to GW 23 excluded). These concerns would favour the index text results. There are also concerns regarding applicability to target population.

RESULTS

2x2 table with inconclusive results (where available)

N = 284	**Reference standard positive** n = 196 (69%)	**Reference standard negative** n = 79 (27.8%)	**Inconclusive results**
Index text positive n = 198 (69.7%)	196	2	
Index text negative n = 77 (27.1%)	0	77	
Index test inconclusive n = 9 (3.2%)	4	5	Indeterminate results n = 5 Maternal variants n = 4

2x2 table (inconclusive results counted as index test positive)

N = 284	**Reference standard positive** n = 200 (70.4%)	**Reference standard negative** n = 84
Index text positive n = 207	200	7
Index text negative n =77	0	77

Outcome	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**	**LR+ (95% CI)**	**LR– (95% CI)**	**Diagnostic accuracy % (95%CI)**
Diagnostic performance of NIPT at GW 24–28 against birth blood sample							
N = 275[a]	100 (NR)	97.5 (94.0, 100)	99.0 (97.6,100).	100 (100,100)	NR	NR	99.3 (98.3, 100)
N = 306[b]	99.6 (98.7, 100)	NR	NR	NR	NR	NR	95.5 (93.3, 97.8)
N = 284[c]	100 (98, 100)	91.67 (84, 97)	96.62	100	12.00	0.0000	96.1 (93.9, 98.4)

EXTERNAL VALIDITY

Generalisability (relevance of the study population to the Guidelines target population)

The evidence is directly generalisable to the Australian population with some caveats. (predominantly Caucasians, some selection for Rh D positive partners) The majority of included women were Caucasian (96.1%), which may not be representative of the target population. The likelihood of a positive fetus in this patient population may be higher than the target population.
Applicability (relevance of the evidence to the Australian health care system)
The evidence is probably applicable to the Australian health care context with some caveats.
Additional comments
One false negative result reported in an 18 week sample.

cffDNA, cell free fetal DNA; CI, confidence interval; DNA, deoxyribonucleic acid; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NIPT, non-invasive prenatal testing; NPV, negative predictive value; NR, not reported; PCR, polymerase chain reaction; PPV, positive predictive value; RT-PCR, real-time polymerase chain reaction.

a. Data as reported by authors, not including inconclusive results

b. Including 31 samples taken prior to GW 23

c. Calculated post-hoc in RevMan 5.3 (inconclusive results counted as index test positive).

STUDY DETAILS: Diagnostic study			
Citation			
Moise 2016 Moise, K. J., Jr., Gandhi, M., Boring, N. H., O'Shaughnessy, R., Simpson, L. L., Wolfe, H. M., Baxter, J. K., Polzin, W., Eddleman, K. A., Hassan, S. S., Skupski, D. W., Ryan, G., Walker, M., Lam, G., Brown, R., Skoll, M. A., Robinson, C., Sheikh, A., Bronsteen, R., Plante, L. A., McLennan, G., Chikova, A., & Paladino, T. (2016). Circulating Cell Free DNA to Determine the Fetal RHD Status in All Three Trimesters of Pregnancy. *Obstet Gynecol,* 128(6), 1340-1346. clinicaltrials.gov #NCT00871195			
Affiliation/Source of funds			
Funding was provided by Sequenom, Inc. The authors declared no potential conflicts of interest. Baylor College of Medicine; The Ohio State University Wexner Medical Center; Columbia University Medical Center; the University of North Carolina School of Medicine; Sidney Kimmel Medical College at Thomas Jefferson University; Tristate Maternal-Fetal Medicine Association, Inc., Cincinnati, Ohio; Icahn School of Medicine at Mt Sinai; Wayne State University/Detroit Medical Center; New York Presbyterian Queens and Weill Cornell Medical College; Mount Sinai Hospital, University of Toronto; Evergreen Hospital, Seattle; Phoenix Perinatal Associates; McGill University, Montréal; the University of British Columbia; Medical University of South Carolina; Spectrum Health Hospitals, Grand Rapids; William Beaumont Hospital; Drexel University College of Medicine; Sequenom, Inc., and the Sequenom Center for Molecular Medicine.			
Study design	**Level of evidence**	**Location and study date**	**Setting**
Prospective observational	Level II	United States and Canada Sept 2009 – April 2011	17 study sites Obstetric and maternity
Index test	**Exon(s) sequenced**	**Internal control(s)**	**Reference standard or comparator**
PCR and MALDI-TOF (mass spectrometry) for cffDNA in maternal plasma	RHD exons 4, 5, and 7 and RHD pseudogene (37 bp insertion in exon 4)	TGIF gene	Birth serology using cord blood
Population characteristics			
Rh D negative pregnant women with no evidence of red cell alloimmunisation by initial antibody screen. Mean maternal age was 31.0 years (SD 5.19); median gravidity was 2 (range 1–13). Self-declared ethnicity was 86.9% Caucasian or white, 11.2% African American or black, 13.1% Hispanic or Latina, 0.8% Asian, and 1.2% other. Three samples taken at 11–13 6/7, 16–19 6/7, and 28–29 6/7 weeks of gestation. 1st trimester: median gestational age at which samples were obtained was 12.3 weeks (range 10.7–14.7 weeks). 2nd trimester: median gestational age was 18.0 weeks (range 15.1–24.4 weeks). 3rd trimester: median gestational age was 28.7 weeks (range 26.0–32.4 weeks).			
Number of participants or samples		**Outcomes measured**	
520 patients enrolled. (522 samples) 1st trimester (n = 467): 15 samples not tested due to handling errors, 34 samples excluded due to missing birth serology, 3 duplicates and three ineligible participants. 2nd trimester (n = 458): 9 samples not tested due to handling errors, 27 samples excluded due to missing birth serology, one duplicate and two ineligible participants. 3rd trimester (n= 425): nine samples not tested due to handling errors, five samples excluded due to missing birth serology, two duplicate and one ineligible participant.		Sensitivity, specificity, diagnostic accuracy	
Method of analysis			

MALDI-TOF mass spectrometry platform was used to detect fetal genetic sequences generated by the SensiGENE Fetal RHD Genotyping test.

If one or none of the three targets were detected, the sample was reported as Rh D negative. If all three targets were detected, the sample was reported as RHD positive. Detection of only two of the three targets was reported as inconclusive. Results were also considered inconclusive if an RHD gene variant precluded our ability to detect all three exons or if the RHD pseudogene was detected.

The quality metric of greater than 104 fetal copies was imposed for the acceptance of samples based on the limit of detection of the RHD assay. Samples found to contain less than 104 fetal copies were excluded from the final analysis as 'quantity not sufficient'.

All samples were genotyped with the Sample ID test, which utilized 44 single nucleotide polymorphisms selected with 45–55% heterozygosity. This allowed comparison of samples from the three trimesters to be genetically matched to confirm they were obtained from the same patient. The discriminatory power based on this panel was such that the probability of a random match occurring between any two unique samples was $1.8X10^{-9}$. Any mismatches occurring through mislabelling of samples from collection through processing were identified before unblinding the data.

INTERNAL VALIDITY

Overall risk of bias (descriptive)

Rating: High

Description: Low risk of patient selection bias. Some concerns regarding the conduct and interpretation of the index text. Samples were frozen and tested in batches, which is not as would occur in clinical practice. This may affect the results in favour of the index test. No concerns with conduct of the reference standard but serious concerns with patient flow due to missing data. Inconclusive results not included in the analysis which would favour the index test.

RESULTS

2x2 table with inconclusive results (where available)

1st trimester **N = 467**	**Reference standard positive** n = 324 (69.3%)	**Reference standard negative** n = 136 (29.1%)	**Inconclusive results** n = 26 (5.6%)
Index text positive n = 312 (66.8%)	323* (310 pregnancies) *includes one triplet and 11 twin results	2	
Index text negative n = 129 (27.6%)	1 *sampling error resulting from mislabelling	134* (128 pregnancies) *includes one triplet and four twin gestation serology results	
Index test inconclusive [a] n = 26 (5.6%)			RHD pseudogene present or suspected n = 22 RHD variants n = 4
2nd trimester **N = 458**	**Reference standard positive** n = 313 (68.3%)	**Reference standard negative** n = 137 (29.9%)	**Inconclusive results** n = 26
Index text positive n = 303 (66.1%)	313* (301 pregnancies) *includes one triplet and 10 twin gestation serology results	2	
Index text negative n = 129	0	135* (129 pregnancies) *includes one triplet and four twin gestation serology results	
Index test inconclusive [a] n = 26 (5.7%)			RHD pseudogene present or suspected n = 22 RHD variants n = 4
3rd trimester **N = 425**	**Reference standard positive** n = 286	**Reference standard negative** n = 127	**Inconclusive results** n = 26
Index text positive n = 278	286* (277 pregnancies) *includes nine twin gestation serology results	1	

Index text negative n = 121		0		126* (121 pregnancies) *includes one triplet and three twin gestation serology results			
Index test inconclusive [a] n = 26						RHD pseudogene present or suspected n = 21 RHD variants n = 5	
Outcome	**Sensitivity** **% (95% CI)**	**Specificity** **% (95% CI)**	**PPV** **% (95% CI)**	**NPV** **% (95% CI)**	**LR+** **(95% CI)**	**LR-** **(95% CI)**	**Diagnostic accuracy** **% (95% CI)**
Diagnostic performance of NIPT against birth blood sample (1st trimester)							
N = 441[b]	99.68 (98.22, 99.94)	98.46 (94.56, 99.58)	99.36	99.22	64.791	0.0033	99.32 (98.03, 99.77)
Diagnostic performance of NIPT against birth blood sample (2nd trimester)							
N= 432[b]	100 (98.74, 100)	98.47 (94.60, 99.58)	99.34	100	65.50	0.0000	99.53 (98.33, 99.87)
Diagnostic performance of NIPT against birth blood sample (3rd trimester)							
N = 399[b]	100 (98.63, 100)	99.18 (95.50, 99.96)	99.64	100	122.0	0.0000	99.75 (95.50, 99.96)
EXTERNAL VALIDITY							
Generalisability (relevance of the study population to the Guidelines target population)							
The evidence is directly generalisable to the Australian population with some caveats (prevalence of Rh D may be different)							
Applicability (relevance of the evidence to the Australian health care system)							
The evidence is not applicable to the Australian health care context (MALDI-TOF)							
Additional comments							
The data presented by the authors is unclear in terms of number of women enrolled (520) versus the number of samples tested in the 1st trimester (n = 522), the 2nd trimester (n = 497), and 3rd trimester (n = 442). The authors describe the births from 459 women (479 newborns) but it is not clear which were included in the samples tested at each trimester: - Rh D positive – 312 singletons, 22 twins (11x2), 3 triplets (1x3) = 324 women, 337 newborns. - Rh D negative – 129 singletons, 8 twins (4x2), 3 triplets (1x3) = 134 women, 140 newborns. - plus, one set of Rh D -negative and -positive twins It appears the sensitivity/specificity numbers do not include the inconclusive results and (correctly) are based on the number of pregnancies (not the number of samples) however, data are insufficient to recalculate with inconclusive results treated as Rh D positive.							

bp, base pair; cffDNA, cell free fetal DNA; CI, confidence interval; DNA, deoxyribonucleic acid; LR+, positive likelihood ratio; LR-, negative likelihood ratio; MALDI-TOF, matrix-assisted laser desorption/ionisation-time of flight; NIPT, non-invasive prenatal testing; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; RT-PCR, real-time polymerase chain reaction; SD, standard deviation

a. The authors indicate that 21 or 22 samples were inconclusive due to presence/suspected RHD pseudogene, with 7 of these newborns being Rh D negative and the remaining two-thirds (14 or 15) being Rh D positive. The 4 or 5 designated as RHD variants are not discussed, therefore it is not clear if the newborns would have typed as Rh D positive or negative.

b. Data as reported by study authors. Not including 26 inconclusive results. PPV, NPV, LR+, LR– calculated post-hoc using RevMan 5.3.

STUDY DETAILS: Diagnostic study

Citation

Picchiassi 2015

Picchiassi, E., Di Renzo, G. C., Tarquini, F., Bini, V., Centra, M., Pennacchi, L., Galeone, F., Micanti, M., & Coata, G. (2015). Non-Invasive Prenatal RhD Genotyping Using Cell Free Fetal DNA from Maternal Plasma: An Italian Experience. Transfusion medicine and hemotherapy : *offizielles Organ der Deutschen Gesellschaft fur Transfusionsmedizin und Immunhamatologie,* 42(1), 22-28.

Affiliation/Source of funds

Partially funded by the Umbria Region (Del. n 958 28/07/2008), the European Commission for 'Special Non-Invasive Advances in Fetal and Neonatal Evaluation' Network of Excellence (LSHBCT-2004–503243) and Sally De Micheli Foundation.

University Hospital of Perugia, Perugia, Italy

The authors declared they have no conflicts of interest.

Study design	Level of evidence	Location and study date	Setting
Prospective observational	Level II	Italy 2010–2013	Single centre, outpatient clinic Obstetrics and maternity
Index test	**Exon(s) sequenced**	**Internal control(s)**	**Reference standard or comparator**
RT-qPCR of cffDNA in maternal plasma	RHD exon 5, exon 7	TERT used as reference gene to confirm the presence and quality of total (fetal and maternal) DNA in each sample	Birth serology

Population characteristics

216 RhD negative women in their first trimester (up to 14 weeks gestation).

Median maternal age was 33 years (range 22–44) in those with Rh D negative fetus (n = 68) and 32 years (range 22–43) in those with Rh D positive fetus (n = 125). 97.4% (188/193) identified as Caucasian.

Samples were collected between GW 10^{+0} –14^{+6}. A second sample was collected in the 2nd trimester (between GW 18^{+0}–25^{+6}) in 13 women.

Number of participants or samples	Outcomes measured
216 enrolled birth serology missing in 23 (10.6%) women, leaving 193 (89.4%) included in the analysis.	sensitivity, specificity, PPV, NPV, diagnostic accuracy ROC

Method of analysis

qPCR analysis was performed using Real-Time PCR 7300 detection system (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). Reactions were performed in duplex and each sample analysed in triplicate. ROC curves were elaborated in order to establish which analysis reached the best diagnostic accuracy, and their areas were compared with DeLong method. All analyses were performed using MedCalc Software, Rel. 9.2.1.0

108 samples reanalysed in a second qPCR (95 from same sample, 13 from sample collected in 2nd trimester) and data combined to give six replicates.

The fetus was defined as RHD positive when both exons were positive, otherwise the fetus was considered RHD negative.

For samples tested in one PCR assay, one single exon was defined as positive if 2/3 or 3/3 replicates generated amplification products; if no replicate or only 1/3 had a positive amplification,

the exon assay was considered negative.

For samples tested in two different PCR assays, an exon was defined as positive if at least 3/6 replicates generated amplification products; if no replicate or only 1/6 or 2/6 had a positive amplification, the exon assay was considered negative.

A cycle threshold of <42 was interpreted as a positive signal.

INTERNAL VALIDITY

Overall risk of bias (descriptive)

Rating: Unclear

Description: Unclear risk of bias as reporting of patient exclusions and inconclusive results (if any) is not clear. Repeat testing of samples also presents concerns in terms of applicability in clinical practice.

RESULTS							
2x2 table with inconclusive results (where available)							
N = 193		**Reference standard positive** n = 125 (64.77%)		**Reference standard negative** n = 68		**Inconclusive results**	
Index text positive n = 120		116		4			
Index text negative n = 73		9		64			
Index test inconclusive						None reported	
Outcome	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**	**LR+ (95% CI)**	**LR– (95% CI)**	**Diagnostic accuracy % (95% CI)**
Diagnostic performance of NIPT in first trimester against birth serology							
N=193[a]	92.8 (86.9, 96.2)	94.1 (85.8, 97.7)	96.7 (93.5, 99.9)	87.7 (80.1, 95.2)	15.7760	0.0765	93.3 (88.8, 96.0)
EXTERNAL VALIDITY							
Generalisability (relevance of the study population to the Guidelines target population)							
The evidence is directly generalisable to the Australian target population with some caveats. The majority of women were Caucasian, but the majority of samples were from first trimester (GW10–13), which may lower the sensitivity of the test.							
Applicability (relevance of the evidence to the Australian health care system)							
The evidence is applicable to the Australian health care context with some caveats. (testing at or near GW 12–14 is more applicable to desired timeframe for testing in Australia).							
Additional comments							
Samples were considered 1st trimester since 178/193 patients were at weeks 10–13 of gestation and nine samples were at week 14 of gestation The authors also assessed if repeat testing will improve diagnostic accuracy, either using a 2nd qPCR analysis from 95 plasma samples (group 1) or 13 new plasma samples (group 2) from repeated blood sampling in the 2nd trimester (data not presented here). The comparison found accuracy improved (six replicates vs three replicates) but the effect was not significant.							

cffDNA, cell free fetal DNA; CI, confidence interval; DNA, deoxyribonucleic acid; GW, gestational week; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NIPT, non-invasive prenatal testing; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; RT-PCR, real-time polymerase chain reaction; qPCR, quantitative PCR

a. Data as reported by authors. Inconclusive results not included (not clear if any). LR+ and LR– calculated post-hoc using RevMan 5.3.

E3.3 Level III-1 – Non-consecutive patients with a valid reference standard

STUDY DETAILS: Diagnostic study			
Citation			
Jakobsen 2018 Jakobsen, M. A., Rosbach, H. K., Dellgren, C., Yazer, M., & Sprogoe, U. (2018). Results of noninvasive prenatal RhD testing in Gestation Week 25 are not affected by maternal body mass index. *Transfusion*. doi:10.1111/trf.14827			
Affiliation/Source of funds			
No source of funds or conflicts of interest disclosed Odense University Hospital, Odense, Denmark; University of Pittsburgh, Pittsburgh, Pennsylvania; University of Southern Denmark, Odense, Denmark.			
Study design	**Level of evidence**	**Location and study date**	**Setting**
Retrospective post-hoc analysis of a cohort study	Level III-1 (diagnostic)	Region of Southern Denmark 2011 to 2013	Obstetrics and maternity
Index test	**Exon(s) sequenced**	**Internal control(s)**	**Reference standard or comparator**
NIPT of cffDNA in maternal plasma Gestational week 25	RHD Exon 5, exon 10	CCR5	Neonatal serology
Population characteristics			
RhD negative pregnant women who had undergone NIPT testing and had results available. The median BMI at GW 12 was 24.2 (10th and 90th percentiles, 20.1–32.4) 261 of 1618 (16%) had a BMI of 30 or more indicating that they were obese (WHO). The median BMI in this group of 261 obese women was 33.6 (10th and 90th percentiles, 30.5-41.1).			
Number of participants or samples		**Outcomes measured**	
A total of 1618 pregnancies in 1588 RhD negative women were included (from approximately 4500 eligible pregnancies). Neonatal RhD type was available for 1649 neonates. (This equalled a total of 1618 births of which 31 were twins). There were 30 women who delivered twice (at different time points) during the study period and their BMIs for both pregnancies were included. Thus, there were 1618 individual BMIs from 1588 women included in this study.		Diagnostic accuracy, with comparison based on BMI	
Method of analysis			
Results from NIP RHD performed in Gestation Week 25 were correlated to maternal BMI in Week 12. The accuracy of NIP RHD result was determined by correlation with serologic RhD types of the neonates. Both the fraction of fetal to maternal cffDNA and the BMI of the pregnant women were approximated log-normally distributed. Comparison of BMI between groups was carried out using Wilcoxon rank-sum test and the correlation between log BMI and log (fraction of fetal to maternal cffDNA) was carried out using linear regression. A twin birth was regarded as Rh D positive if at least one of the twin neonates serologically typed as Rh D positive.			
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			
Rating: Unclear Description: Concerns with patient selection bias, as not all eligible pregnancies included (1618/~4500).			
RESULTS			
2x2 table with inconclusive results (where available)			
N = 1618	**Reference standard positive** n = 987 (61%)	**Reference standard negative** n = 631 (39%)	**Inconclusive results**
Index text positive n = 987 (61%)	978	9	

Index text negative n = 582 (36%)		4 [a]		578			
Index test inconclusive n = 49 (3%)		5		44			
2x2 table (inconclusive results counted as index test positive)							
N = 1618		**Reference standard positive** n = 987			**Reference standard negative** n = 631		
Index text positive n = 1036		983			53		
Index text negative n = 582		4			578		
Outcome	**Sensitivity** **% (95% CI)**	**Specificity** **% (95% CI)**	**PPV** **% (95% CI)**	**NPV** **% (95% CI)**	**LR+** **(95% CI)**	**LR–** **(95% CI)**	**Diagnostic accuracy** **% (95% CI)**
Diagnostic performance NIPT against birth blood sample[a]							
N = 1618 [b]	99.3 (NR)	99.1 (NR)	NR	NR	NR	NR	NR
N = 1618 [c]	99.59 (99, 100)	91.60 (89, 94)	94.88	99.31	11.8574	0.0044	96.48
EXTERNAL VALIDITY							
Generalisability (relevance of the study population to the Guidelines target population)							
The evidence is directly generalisable to the Australian target population with some caveats (prevalence of RHD may vary)							
Applicability (relevance of the evidence to the Australian health care system)							
The evidence is directly applicable to the Australian health care context							
Additional comments							
There was no difference in median BMI between the pregnancies with inconclusive (n = 49) or FN (n = 4) NIP RHD test results (n = 53 pregnancies, median BMI = 23.7) compared to those who had TP (n = 978) or TN (n = 578) NIP RHD test results (n = 1556, median BMI = 24.2; Wilcoxon Signed rank-sum; p = 0.80). Among the obese women, 8/261 were inconclusive. There was no significant difference in the rate of inconclusive or FP NIP RHD results among the obese women compared to the nonobese women (Chi^2, p = 0.83). The median cffDNA fraction from 150 randomly selected NIP RHD TP pregnancies was 5.47% in Gestation Week 25 (10th and 90th percentiles: 0.64%-27.2%). There was no statistically significant correlation between the pregnant women's BMI and cffDNA fraction (r^2 = 0,012; p = 0.17).							

cffDNA, cell free fetal DNA; CI, confidence interval; DNA, deoxyribonucleic acid; GW, gestational week; LR+, positive likelihood ratio; LR–, negative likelihood ratio; NIPT, non-invasive prenatal testing; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; RT-PCR, real-time polymerase chain reaction.

a. The BMIs of the four women whose NIP RHD result was FN were in the range of 22 to 29.9, thereby classifying them as between healthy weight to overweight according to the WHO classification, but not obese.

b. Data as reported by authors.

c. Calculated in RevMan 5.3. Inconclusive results were counted as index test positive

STUDY DETAILS: Diagnostic study			
Citation			
Orzińska 2015 Orzińska. A., Guz, K., Debska, M., Uhrynowska, M., Celewicz, Z., Wielgo, M., Brojer, E. (2012). 14 Years of Polish Experience in Non-Invasive Prenatal Blood Group Diagnosis. *Transfus Med Hemother*, 42: 490-494.			
Affiliation/Source of funds			
Supported by the Polish-Norwegian Research Programme conducted by The National Centre for Research and Development within the framework of the Norwegian Financial Mechanism 2009–2014, project agreement No. Pol-Nor/203111/69/2013. Institute of Haematology and Transfusion Medicine (IHTM), Warsaw, Poland; 2nd Department of Obstetrics and Gynaecology Medical Centre of Postgraduate Education, Warsaw, Poland; Pomeranian Medical University, Szczecin, Poland; Medical University of Warsaw, Warsaw, Poland The authors declared they have no conflicts of interest.			
Study design	**Level of evidence**	**Location and study date**	**Setting**
Retrospective cohort	Level III-1	Poland 2000–2014	Single reference laboratory (IHTM) Obstetrics and maternity
Index test	**Exon(s) sequenced**	**Internal control(s)**	**Reference standard or comparator**
RT-PCR of cffDNA in maternal plasma GW 5–39	RHD exon 7, exon 10, intron 4 (between 2000–2011) RHD exon 5, exon 7 (from 2012)	SRY (or bi-allelic polymorphism from the father) and CCR5	Birth serology from cord blood or material from swabs
Population characteristics			
Pregnant women at median 19 weeks gestation with suspected alloimmunisation. Includes 536 Rh D negative women, 24 Rhc-negative, 26 RhE-negative, 43 K-negative, and 42 HPA-1a-negative women (N = 671). Among the Rh D negative women, 160 had no anti-D, 282 had anti-D, 76 anti-D+C, 13 anti-D+E, 5 anti-G, and 13 anti-E. Baseline characteristics not provided.			
Number of participants or samples		**Outcomes measured**	
536 RhD negative pregnant women. In 407 cases they were able to collect data on the RhD phenotype or genotype of the newborn (missing 129, 24%) *Only data from RhD negative women reported here.		True positive, true negative, false positive, false negative.	
Method of analysis			
DNA was extracted automatically from 3–4 mL maternal plasma using easyMag Nuclisens (Biomerieux) The target gene was detected using RT-qPCR with Taqman primers on ABI Prism7700 (Applied Biosystems) up to 2013, then LightCycler II 480 (Roche Diagnostics). Primers/probes were updated in 2012 for multiplex PCR. Samples were tested in triplicate for gene/alleles, in duplicate for SRY, and once only for CCR5. The fetus was reported as Rh D positive if all PCR results for gene/allele encoding antigen were positive (Ct<40) and Rh D negative when there was no signal of amplification after up to 45 cycles in any of the replicates. If there were any discrepancies in the results, the test was repeated from the second tube or additional blood collection at a later stage of pregnancy was recommended.			
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			

Rating: High

Description: Serious concerns regarding patient selection, attrition bias and reporting of results. Difficult to diagnose cases (RHD variants, weak Rh D, patient with insufficient DNA collected) are excluded from the analysis favouring the index test.

Data from 255 women is reported in a previous publication (Brojer 2005) and included in the meta-analysis by Geifman-Holtzman 2006. Here they note 25 samples (9.8%) were excluded due to the absence of detectable DNA and diagnostic accuracy was 99.6%. This suggests not all FP or FN data are reported here.

The authors also note an RHD gene was present in the maternal genome of seven women, with the status of the neonate was not reported in 6/7, indicating inconclusive results were not included in analysis.

RESULTS

2x2 table with inconclusive results (where available)

N = 407		**Reference standard positive** n = 308		**Reference standard negative** n = 99		**Inconclusive results** n = NR	
Index text positive n = 308		308		0			
Index text negative n = 99		0		99			
Index test inconclusive n = NR							
Outcome	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**	**LR+ (95% CI)**	**LR– (95% CI)**	**Diagnostic accuracy % (95% CI)**
Diagnostic performance of NIPT against birth blood sample							
N = 407[a]	100 (100, 100)	100 (100, 100)	100.00	100.00	not calculable	0.0000	100

EXTERNAL VALIDITY

Generalisability (relevance of the study population to the Guidelines target population)

The evidence is not directly generalisable to the Australian target population but could be sensibly applied.

The study included women with suspected alloimmunisation (regardless of genotype), however, this is not expected to alter the confidence in results.

Applicability (relevance of the evidence to the Australian health care system)

The evidence is probably applicable to the Australian health care context with some caveats.

Additional comments

Women who were alloimmunised were included in this study. Other genotypes including RHCE*c, RHCE*E, RHCE*C, KEL*01 and HPA*1A were also tested.

cffDNA, cell free fetal DNA; CI, confidence interval; DNA, deoxyribonucleic acid; GW, gestational week; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NIPT, non-invasive prenatal testing; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; RT-PCR, real-time polymerase chain reaction.

a. Data as reported by authors. Calculated post-hoc in RevMan 5.3. Inconclusive results were not included.

STUDY DETAILS: Diagnostic study			
Citation			
Papasavva 2016 Papasavva, T., Martin, P., Legler, T. J., Liasides, M., Anastasiou, G., Christofides, A., Christodoulou, T., Demetriou, S., Kerimis, P., Kontos, C., Leontiades, G., Papapetrou, D., Patroclos, T., Phylaktou, M., Zottis, N., Karitzie, E., Pavlou, E., Kountouris, P., Veldhuisen, B., van der Schoot, E., & Kleanthous, M. (2016). Prevalence of RhD status and clinical application of non-invasive prenatal determination of fetal RHD in maternal plasma: a 5 year experience in Cyprus. *BMC research notes,* 9, 198. doi:http://dx.doi.org/10.1186/s13104-016-2002-x			
Affiliation/Source of funds			
The Cyprus Institute of Neurology and Genetics, Cyprus; International Blood Group Reference Laboratory, Bristol Institute for Transfusion Sciences, NHS Blood and Transport; Department of Transfusion Medicine, University Medical Center Göttingen; Sanquin Blood Supply, The Netherlands; The authors declared they had no conflicts of interest.			
Study design	**Level of evidence**	**Location and study date**	**Setting**
Retrospective cohort	Level III-1	Cyprus	Single referral centre covering all of Cyprus, Obstetrics and maternity
Index test	**Exon(s) sequenced**	**Internal control(s)**	**Reference standard or comparator**
RT-PCR of cffDNA in maternal plasma After GW 16	RHD exon 4, exon 5, exon 10	SRY and CCR5	Serology at birth
Population characteristics			
RhD negative pregnant women			
Number of participants or samples		**Outcomes measured**	
73 patients		True positive, false positive, true negative, false negative	
Method of analysis			
RT multiplex PCR used to amplify the regions of RHD gene in exons 4, 5, and 10, using four replicates per exon per sample. Amplification of the SRY gene on the Y chromosome is used to confirm the presence of male fetal DNA. Genomic DNA from the parents also included for genotyping in order to confirm the phenotype. During each run, genomic DNA from RhD negative female as well as RhD positive male were used for negative and positive control respectively. DNA sample from a person having the RHD pseudogene was also incorporated in the assay to exclude the possibility of someone carrying the pseudogene. To identify RHD variants in two pregnant women, the MLPA assay was performed. The fragments were analysed using a 3130 Genetic Analyzer (Applied Biosystems) The data were analysed using the R programming language (version 3.1.2). Descriptive statistics were utilised for the analysis, while the CI were defined using the exact binomial test (function binom.test in R). In cases where no negative effects were observed, such as the method's accuracy, the 95 % CI was estimated using the rule of three. If no RHD signals were obtained for exons 4, 5 and 10, the fetus was determined to be RhD negative. When at least two positive signals were obtained from each RHD exon plus a total of three more positive signals from any exon the fetus was determined as RhD positive. No grey zone results were observed.			
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			
Rating: Unclear Description: Some concerns with patient selection bias with baseline characteristics and exclusion criteria not clearly reported. Unclear reporting of inconclusive results			
RESULTS			
2x2 table with inconclusive results (where available)			

N = 73		**Reference standard positive** n = 53		**Reference standard negative** n = 18		**Inconclusive results**	
Index text positive n = 53		53		0			
Index text negative n = 18		0		18			
Index test inconclusive n = 2						Maternal weak D phenotype following discrepancies observed during testing	
Outcome	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**	**LR+ (95% CI)**	**LR– (95% CI)**	**Diagnostic accuracy % (95% CI)**
Diagnostic performance NIPT against birth blood sample							
N = 71[a]	100 (93, 100)	100 (81, 100)	100.00	100.00	Not calculable	0.0000	100 (95.3, 100)
EXTERNAL VALIDITY							
Generalisability (relevance of the study population to the Guidelines target population)							
The evidence is not directly generalisable to the Australian target population and it is difficult to judge if it is sensible to apply. (only women with Rh D positive partners, low prevalence rate of Rh D negative phenotype)							
Applicability (relevance of the evidence to the Australian health care system)							
The evidence is probably applicable to the Australian health care context with some caveats. The lower the prevalence of the Rh D negative phenotype did not appear to affect the accuracy of the test (the Cypriot population has a reported prevalence rate half that in Australia) but this study selected for women with Rh D positive partners only and may not be a true reflection of the conduct of the test in Australia.							
Additional comments							
The study also assessed the frequency of the RhD status in the Cypriot population, using 445 random samples. Rh D negative prevalence in the Cypriot population was estimated to be 7.2% (95% CI 4.97, 10.00) compared to authors figures of ~15% in Caucasians. Only women who had a positive fetus (74.6%) were administered anti-D, however, no dose, timing or subsequent testing for Rh D alloimmunisation data was reported. Two women had RHD sequences identified. NIPD cannot be performed on these samples since RHD sequences are present in the mother's genome, the authors did not report outcomes for these two women. This study may provide evidence that weak D prevalence is higher in the Cypriot population, however, this needs to be determined by further investigation. The exon 4 and 5 assays are designed to amplify only RHD and not RHD*Ψ.							

cffDNA, cell free fetal DNA; CI, confidence interval; DNA, deoxyribonucleic acid; LR+, positive likelihood ratio; LR-, negative likelihood ratio; MLPA, multiplex ligation-dependent probe amplification; NIPT, non-invasive prenatal testing; NPV, negative predictive value; NR, not reported; PCR, polymerase chain reaction; PPV, positive predictive value; RT-PCR, real-time polymerase chain reaction.

a. Calculated post-hoc using RevMan 5.3. Not including two samples that were maternal weak D type 1 and type 11. Fetal RHD prediction not possible for these women. The serology of the newborns for these women were not provided.

STUDY DETAILS: Diagnostic study							
Citation							
Ryan 2017 Ryan, H., Lambert, M., Mulvany, J., Kelly, S., Madigan, G., McDermott, R., Murphy, D., O'Donovan, B., Banch Clausen, F., Donnelly, J., Fitzgerald, J., & Ni Loingsigh, S. (2017). The identification of maternal RHD variant alleles in RhD negative pregnant women during the validation of Fetal RHD Screen in Ireland. *Transfusion Medicine*, 27(Supplement 2), 40.							
Affiliation/Source of funds							
Source of funds not stated. Irish Blood Transfusion Service, Dublin , Ireland; National Maternity Hospital, Dublin, Ireland; The Rotunda Maternity Hospital, Dublin, Ireland; Rigshospitalet, Copenhagen, Denmark No declaration regarding conflicts of interest provided.							
Study design		**Level of evidence**		**Location and study date**		**Setting**	
Cross-sectional		Level III-3		Ireland Dates not stated		Two large maternity hospitals Obstetrics and maternity	
Index test		**Exon(s) sequenced**		**Internal control(s)**		**Reference standard or comparator**	
Multiplex RT-qPCR of cffDNA in maternal plasma		RHD exons 7, exon 10		GAPDH		Not stated	
Population characteristics							
Rh D negative pregnant women, ethnicity unknown; mean gestation 13 weeks							
Number of participants or samples				**Outcomes measured**			
323				Sensitivity, specificity, inconclusive			
Method of analysis							
Successful cfDNA extraction confirmed by GAPDH. Low cycle threshold (Cp) values for an RHD signal, relative to GAPDH were recognised as potential maternal RHD sequences. Further analysis of possible maternal RHD sequences was performed by SSP-PCR (Inno-Train) on maternal genomic DNA to identify specific RHD alleles. Algorithm not described. RHD screen results reported as RHD positive fetus, RHD negative fetus or inconclusive (technical and maternal RHD).							
INTERNAL VALIDITY							
Overall risk of bias (descriptive)							
Rating: Not assessed. (conference abstract/poster) Description: Insufficient information to assess risk of bias.							
RESULTS							
2x2 table with inconclusive results (where available)							
N = 323		**Reference standard positive** n = NR		**Reference standard negative** n = NR		**Inconclusive results**	
Index text positive n = NR		NR		2[a]			
Index text negative n = NR		NR		NR			
Index test inconclusive n = 12						Maternal weak D[b] n = 7 Maternal variants[c] n = 5	
Outcome	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**	**LR+ (95% CI)**	**LR– (95% CI)**	**Diagnostic accuracy % (95% CI)**
Diagnostic performance NIPT (GW 13) against birth blood sample							

N = 323	100 (98.87, 100)	97.59 (95.26, 99.92)	NR	NR	NR	NR	NR
EXTERNAL VALIDITY							
Generalisability (relevance of the study population to the Guidelines target population)							
The evidence is directly generalisable to the Australian target population with some caveats (prevalence of RHD may vary)							
Applicability (relevance of the evidence to the Australian health care system)							
The evidence is probably applicable to the Australian health care context with some caveats.							
Additional comments							
Targeting RHD exons 7 and 10 enables detection of all RHD alleles including, hybrid and partial RHD (except DHAR) and RHDΨ. Serologically RhD negative women with variant RHD alleles will continue to receive antenatal anti-D. This precludes determination of the fetal RHD type however, given the current ethnic mix in Irish Maternity Hospitals, the frequency of such maternal RHD variants is expected to be low, with limited additional anti-D given.							

cffDNA, cell free fetal DNA; CI, confidence interval; DNA, deoxyribonucleic acid; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NIPT, non-invasive prenatal testing; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; RT-PCR, real-time polymerase chain reaction.

a. The authors suggest that the two false positives may be due to fetal variants, but further analysis was not possible.

b. Presence of maternal RHD sequences indicated. Fetal RHD prediction not possible for these women (2.17% of total);

c. Variants identified, RHD*06.01, RHD*06.02, RHD*11, RHD*01N.05 and RHD*01N.03 (3 individuals).

d. Data as reported by authors.

STUDY DETAILS: Diagnostic study			
Citation			
Sørenson 2018 Sorensen, K., Kjeldsen-Kragh, J., Husby, H., & Akkok, C. A. (2018). Determination of fetal RHD type in plasma of RhD negative pregnant women. Scandinavian Journal of Clinical and Laboratory Investigation((Husby) Department of Obstetrics, Oslo University Hospital, Oslo, Norway), 1-6.			
Affiliation/Source of funds			
The authors declared no potential conflict of interest. Source of funding not reported. Oslo University Hospital, Oslo, Norway; University and Regional Laboratories Region Skåne, Lund, Sweden;			
Study design	**Level of evidence**	**Location and study date**	**Setting**
Retrospective cohort	Level III-1	Norway 2011–2013	Dept of Obstetrics, Oslo University Hospital and surrounding GPs and health centres Obstetrics and maternity
Index test	**Exon(s) sequenced**	**Internal control(s)**	**Reference standard or comparator**
RT-PCR of cffDNA in maternal plasma at GW 16–36 (median 24)	RHD exon 7, exon 10	GAPDH	Serology at birth
Population characteristics			
RhD negative women presenting for routine ultrasound. Baseline characteristics (age, ethnicity, parity etc.) not reported.			
Number of participants or samples		**Outcomes measured**	
373 samples from 281 patients. 283 samples collected fresh, 90 samples from frozen. Two samples were received from 92 women (GW 18 and 24).		Sensitivity, specificity,	
Method of analysis			
Automatic DNA extraction from 1mL thawed plasma performed with the Magna Pure LC (Roche, Mannheim, Germany). The presence of the RHD gene was investigated in the real-time PCR instrument, LightCycler 480 (Roche). Samples were examined in triplicate. All PCR set-ups included RHD positive (80, 8 and 3 RHD gene copies) and RHD negative controls obtained from diluted DNA isolated from whole blood. Cycle threshold (Ct) value was calculated using Avs Quant/2nd Derivative Max mode. If 2–3 samples positive and $Ct_{RHD}>Ct_{GAPDH}$, then the predicted phenotype was RhD positive and prophylaxis recommended. If 0–1 samples positive then the predicted phenotype was RhD negative and prophylaxis was not recommended. If 2–3 samples were positive but $Ct_{RHD}\leq Ct_{GAPDH}$, then the predicted phenotype was inconclusive and prophylaxis recommended. Any inconclusive results were further investigated. A matched-paired t-test was used to compare the mean RHD Ct values in samples taken in 18 and 24 weeks of gestation (two samples from each woman). Sensitivity and specificity were calculated in Stata			
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			
Rating: Unclear Description: Low concerns with patient selection bias or patient flow. Some concerns conduct regarding conduct of the reference standard. It is not known if it was carried out without knowledge of the index test.			
RESULTS			
2x2 table (including inconclusive results)			
N = 373 samples (281 women)	**Reference standard positive** n = 178 pregnancies	**Reference standard negative** n = 103 pregnancies	**Inconclusive results**

Index text positive n = 234 samples (177 pregnancies)		233 samples (176 pregnancies)		1			
Index text negative n = 127 samples (95 pregnancies)		0		127 samples (95 pregnancies)			
Index test inconclusive n = 12 samples (9 women)		7 pregnancies		2 pregnancies		maternal weak D (n = 9)[a]	
2x2 table (inconclusive results counted as index test positive)							
N = 373 samples **(281 women)**		**Reference standard positive** n = 183 (65.12%)			**Reference standard negative** n = 98 (34.88%)		
Index text positive n = 186 pregnancies (66.19%)		183 pregnancies			3 pregnancies		
Index text negative n = 95 pregnancies (33.81%)		0			95 pregnancies		
Outcome	**Sensitivity** **% (95% CI)**	**Specificity** **% (95% CI)**	**PPV** **% (95% CI)**	**NPV** **% (95% CI)**	**LR+** **(95% CI)**	**LR–** **(95% CI)**	**Diagnostic accuracy** **% (95% CI)**
Diagnostic performance NIPT against birth blood sample							
N = 373[b] (samples)	100 (98.4, 100)	99.2 (95.7, 100)	NR	NR	NR	NR	NR
N= 281[c] (pregnancies)	100 (98, 100)	96.94 (91, 99)	98.39	100.00	32.6667	0.0000	278/281 (98.93%)
EXTERNAL VALIDITY							
Generalisability (relevance of the study population to the Guidelines target population)							
The evidence is directly generalisable to the Australian target population with some caveats (prevalence of RHD may vary)							
Applicability (relevance of the evidence to the Australian health care system)							
The evidence is probably applicable to the Australian health care context with some caveats.							
Additional comments							
Based on these results antenatal prophylaxis would have been recommended for 186/281 (66%) women predicted carrying an RhD positive fetus or with an inconclusive result (177+9). Three (1.1%) of these women would have received unnecessary antenatal prophylaxis (the false positive and two inconclusive); while 95 women (34%) would have avoided unnecessary antenatal prophylaxis. A comparison of the mean Ct values, showed a statistically significantly lower Ct value at GW 24 compared with the mean Ct value at GW 18 (36.6 vs 37.1, p<.0002) (n = 55 women; the number of women with an RHD positive result out of 92).							

cffDNA, cell free fetal DNA; CI, confidence interval; DNA, deoxyribonucleic acid; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NIPT, non-invasive prenatal testing; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; RT-PCR, real-time polymerase chain reaction.

a. Low Ct values indicate the presence of a maternal RHD gene hampering determination of the RhD type of the fetus. All the inconclusive and false positives were investigated and all revealed a maternal RHD gene.

b. Data as reported by authors, not including inconclusive results

c. Calculated post-hoc using RevMan 5.3. Inconclusive results counted as index test positive.

E4 Question 4

E4.1 Level I – Systematic review of RCTs

Source: Shea et al. 2007. BMC Medical Research Methodology 7:10 doi:10.1186/1471-2288-7-10 http://amstar.ca/Amstar_checklist.php

No studies identified

E4.2 Level I – Systematic review of observational and cohort studies

Source: Shea et al. 2017. BMJ 358: j4008 doi:10.1136 (https://doi.org/10.1136/bmj.j4008)

No studies identified

E4.3 Level II – Prospective cohort studies

STUDY DETAILS: Cohort study			
Citation			
MacKenzie 2006 MacKenzie, I. Z., Roseman, F., Findlay, J., Thompson, K., Jackson, E., Scott, J., Reed, M. (2005). The kinetics of routine antenatal prophylactic intramuscular injections of polyclonal anti-D immunoglobulin. *BJOG* 2006; 113:97–101.			
Affiliation/Source of funds			
Funding was provided by Oxford Rhesus Therapy Unit The authors are affiliated with National Blood Service and the University of Oxford, UK.			
Study design	**Level of evidence**	**Location**	**Setting**
Prospective observational study	Level II	Maternity unit and antenatal serology laboratory in a district teaching hospital, UK	Obstetrics and maternity
Intervention		**Comparator**	
Intramuscular injections of anti-D IgG 500 IU at 28 and 34 weeks of gestation (D-Gam Bio Products Laboratory, Elstree, Hertfordshire, UK) Anti-D prophylaxis provided for all potentially sensitising events (N=0) and also given postnatally to mothers with Rh D positive newborns (N=26/43).		NA	
Population characteristics			
Rh D negative nulliparae and multiparae women who were 20 weeks pregnant with an initial negative RhD antibody screen. Women who had received anti-D IgG after 20 weeks of gestation during the current pregnancy for potential sensitising events were excluded, as were women whose partners were known to be RhD negative.			
Length of follow-up		**Outcomes measured**	
Serum samples taken at 4, 7, 14, 21, 28, 42, 56, 70, and 84 days after the first dose or until delivery.		The kinetic profile and duration of detectable anti-D IgG in maternal serum following both injections, correlated with maternal weight	
Method of analysis			
Linear regression analysis			
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			
Rating: Serious Description: The study has important problems and cannot be considered comparable to a well-performed randomised trial. Small cohort with insufficient reporting of outcome data to provide useful information relating to association between BMI and persistence of anti-D or incidence of Rh D alloimmunisation.			
RESULTS			

Population analysed	**Intervention**		**Comparator**	
Available	45			
Analysed	43			
Outcome	**Intervention n/N (%)**	**Comparator n/N (%)**	**Risk estimate (95% CI)**	***Statistical significance p-value***
Peak anti-D IgG serum value measured seven days after first dose	Correlation with BSA (GW 28)		R^2=0.299	*Significant inverse relationship favouring low BSA* *p = 0.002*

Peak anti-D IgG serum value measured seven days after first dose	Correlation with maternal body weight (GW28)			R^2=0.171	*Significant inverse relationship favouring low maternal body weight* *p = 0.006*
Persistence of anti-D IgG at 12 weeks (84 days or more) after the first injection	BSA < 1.80 m^2 5/9 (56%)	BSA 1.80–1.99 m^2 3/6 (50%)	BSA > 2.00 m^2 3/6 (50%)	NR	*No significant difference* *p = NR*
EXTERNAL VALIDITY					
Generalisability (relevance of the study population to the Guidelines target population)					
The population is similar to the Guidelines target population.					
Applicability (relevance of the evidence to the Australian health care system)					
The dose (500 IU) used in this study is different to that used in the Australian health care system (i.m. 625 IU at 28 and 34 weeks).					
Additional comments					
Despite correlations between weight and BSA, there was no influence on duration of persistent IgG.					

BSA, body surface area; CI, confidence interval; IU, international units; m, metre; NR, not reported; RCT, randomised controlled trial; SD, standard deviation; UK, United Kingdom

STUDY DETAILS: Cohort			
Citation			
Woelfer 2004 Woelfer, B., Schuchter, K., Janisiw, M., Hafner, E., Philipp, K., Panzer, S. (2004). Postdelivery levels of anti-D IgG prophylaxis in D-mothers depend on maternal body weight. Transfusion, 2004;44:512-517.			
Affiliation/Source of funds			
Supported by a Grant from Medizinisch-Wissenschaftlicher Fonds des Bürgermeisters der Bundeshauptstadt Wien Conducted in the Department of Obstetrics and Gynecology Donauspital Vienna and the Clinic for Blood Group Serology, University of Vienna, Austria			
Study design	**Level of evidence**	**Location**	**Setting**
Cohort study of consecutive patients	Level II	Vienna, Austria	Obstetrics and maternity
Intervention		**Comparator**	
Postpartum 1500 IU anti-D im within 72 hours of delivery (300 mcg, Rhesogam, Centeon Pharma, Vienna, Austria)		NA	
Population characteristics			
26 Rh D negative women who had delivered an Rh D positive child Median age: 28.5 yrs (range 23 to 43); primiparae (n=10), multiparae (n=16); median gestational at birth 40 weeks (range 37 to 43); vaginal delivery (n=15), caesarean section (n=11); no RBC transfusions			
Length of follow-up		**Outcomes measured**	
14 days following administration		Anti-D serum concentration (flow cytometry)	

Method of analysis				
Linear models with repeated measure. Caesarean section was considered a potential confounder. Multiple and univariate linear regression analyses were applied during each measurement. Analyses were applied to women with a BMI less than or equal to 27 kg/m^2 (n=12) and greater than 27 kg/m^2 (n=14). The dependency on serum levels and BMI was revealed by local linear regression analysis utilising the locally weighted least-squares method (Fig. 1)				
INTERNAL VALIDITY				
Overall risk of bias (descriptive)				
Rating: Moderate (for the outcome of anti-D levels) Description: The study appears to provide sound evidence for a non-randomised study but cannot be considered comparable to a well-performed randomised trial. Small cohort with insufficient longer-term data to provide useful information relating to association between BMI and incidence of Rh D alloimmunisation in a subsequent pregnancy.				
RESULTS				
Population analysed	**BMI ≤27 kg/m^2**		**BMI >27 kg/m^2**	
Available	12		14	
Analysed	12		14	
Outcome	**BMI ≤27 kg/m^2**	**BMI >27 kg/m^2**	**Risk estimate (95% CI)**	***Statistical significance p-value***
Serum concentration of anti-D (ng/mL)[a], median (1IQR, 3IQR)				
1 Day	39.6 (30.0, 64.3)	32.9 (16.4, 45.7)		*Increasing BMI associated with lower serum anti-D over time* $p < 0.001$
2 Days	99.6 (77.5, 152.5)	54.6 (34.6, 92.5)		
3 Days	114.3 (91.1, 128.9)	94.3 (55.7, 113.9)		
2 weeks	143.6 (99.3, 70.7)	96.4 (61.4, 111.4)		
Serum concentration of anti-D (ng/mL), mean (95% CI)[b]				
1 Day	64.3 (46.7, 81.8)	Calculations based on the general linear model found each kg/m^2 BMI higher than 27 kg/m^2 reduced the anti-D Ig G serum concentration	MD 4.2 (6.4, 2.0)	$p = 0.002$
2 Days	109.3 (87.2, 131.4)		MD 6.0 (10.1, 1.8)	$p = 0.011$
3 Days	154.4 (118.8, 190.1)		MD 7.6 (14.0, 1.2)	$p = 0.025$
2 weeks	158.0 (100.9, 215.1)		MD 8.4 (15.8, 1.1)	$p = 0.030$
EXTERNAL VALIDITY				
Generalisability (relevance of the study population to the Guidelines target population)				
The generalisability of the study population is unclear. The selected population is similar to those in the Australian population.				
Applicability (relevance of the evidence to the Australian health care system)				
The timing and dose (postpartum 1500 IU anti-D im within 72 hours of delivery) used in this study is different to that used in the Australian health care system (antenatal im 625 IU at 28 and 34 weeks plus a dose determined on the birth of Rh positive fetus).				
Additional comments				
Total anti-D Ig G levels increased over time, with no further increase after 2 weeks (p<0.001). Median serum concentrations noted in graph (figure 2) however, not stated in text or tables, therefore are estimated. Fetomaternal haemorrhage was not determined at birth.				

BMI, body mass index; CI, confidence interval; IgG, immunoglobulin; im, intramuscular; IQR, interquartile range; IU, international units; kg, kilogram; m, metre; mL, millilitre; ng, nanogram; ITT, intention to treat; MD, mean difference;RBC, red blood cell; RCT, randomised controlled trial; SD, standard deviation not calculable; yrs, years

a. Data estimated from Figure 2 using Webplotdigitiser and rounded to two decimal places.

b. Mean anti-D Ig G levels were skewed to underestimate in women with BMI < 32 kg/m2 and overestimate in women with BMI > 36 kg/m^2

E4.4 Level III – All or none, retrospective cohort studies

STUDY DETAILS: Case-control			
Citation			
Koelewijn 2009 Koelewijn J. M., de Haas, M., Vrijkotte, T. G. M., van der Schoot, C. E., Bonsel, G. J. (2009). Risk factors for RhD immunisation despite antenatal and postnatal anti-D prophylaxis. BJOG 2009; 116:1307–1314.			
Affiliation/Source of funds			
Funding from OPZI (detection and prevention of pregnancy immunisation)-project, the nationwide evaluation of pregnancy screening in the Netherlands. Performed by Sanquin Research and the Academic Medical Centre of the University of Amsterdam, was financed by the Health Care Insurance Board.			
Study design	**Level of evidence**	**Location**	**Setting**
Case-control	Level III	The Netherlands	Nationwide evaluation of the Dutch antenatal anti-D-prophylaxis programme (primary and clinical care setting)
Intervention		**Comparator**	
1000 IU anti-D antenatally at 30 weeks gestation 1000 IU anti-D postnatally within 48 hours of birth of Rh+ child		Cases – Rh D negative parae-1 who were Rh D alloimmunised despite adequate antenatal and postnatal anti-D prophylaxis during their previous pregnancy Controls –Rh D positive and Rh D negative parae-1 women who delivered after introduction of Dutch anti-D programme, from sample of controls for previous study. The women had a negative screening result i.e. were not alloimmunised.	
Population characteristics			
Rh D negative pregnant primiparae women with Rh D antibodies detected in first trimester screening. 37 cases identified in a nationwide study covering 1999, 2002, and 2004. Five additional cases identified in 2000 and 2003. Parae-1 who had a negative first trimester screening test but had a positive screening at or after the 30th week screening, were not included.			
Length of follow-up		**Outcomes measured**	
Cases were identified over a 5-year period (1999–2004) Controls were selected over a 10-month period (Sept 2002–Jun 2003).		Potential risk factors associated with RhD alloimmunisation including those related to increased FMH, decreased levels of anti-D Ig or altered immune response (maternal weight, ethnicity and age, paternal ethnicity, gender of the child, twin pregnancy, invasive prenatal diagnostic procedures, external version, postmaturity (>42 weeks of completed gestation), mode of delivery, surgical removal of the placenta, pregnancy-related RBC transfusion).	
Method of analysis			
Univariate analysis of risk factors (Pearson's chi-square test, Fisher's exact test (n < 5) or Students t-test, depending on the measurement level in the variable). Multivariate logistic regression with univariate factors ($P < 0.10$) offered stepwise into the model. Estimated the remnant risk of immunisation in the next pregnancy for all combinations of significant risk factors by multiplying the odds ratio predicted by the model with the baseline immunisation prevalence of 0.310% in women without significant risk factors and adequate antenatal and postnatal prophylaxis. To restore the proportion of primary care pregnancies in the control group (298/339 = 88%) to the population proportion for primiparae of 72%, the authors weighted the primary care controls with 0.35. These weighted data were used in all analyses. Missing values (<1%) were not substituted			

INTERNAL VALIDITY				
Overall risk of bias (descriptive)				
Rating: Moderate Description: The study appears to provide sound evidence for a non-randomised study but cannot be considered comparable to a well-performed randomised trial. Of key concern is an over-representation of women from the primary setting (midwives, GPs) vs obstetric setting (3:1) in the controls compared with cases. Weighted data were used in the analysis.				
RESULTS				
Population analysed	**Case**		**Controls**	
Available	42		339	
Analysed	42		339 (weighted n=146)[a]	
Outcome	**Cases** **n/N (%)** **Mean ± SD**	**Controls** **n/N (%)** **Mean ± SD**	**Risk estimate (95% CI)**	***Statistical significance*** ***p*-value**
Univariate analysis				
Body mass index	23.8 ± 4.5	24.0 ± 4.5	NR	*No significant difference* *p = 0.84*
Body weight in kg	67.6 ± 11.5	69.6 ± 13.3	NR	*No significant difference* *p = 0.42*
Body weight >75 kg	NR (21.9)	NR (23.8)	NR	*No significant difference* *p = 0.82*
Body weight >100 kg	NR (3.1)	NR (3.3)	NR	*No significant difference* *p = 0.71*
Invasive prenatal diagnostic procedures in previous pregnancy	NR (2.4)	NR (0.7)	NR	*No significant difference* *p* = 0.39
Caesarean section in previous pregnancy	NR (23.8)	NR (13.0)	NR	*No significant difference* *p* = 0.09
Assisted vaginal delivery in previous pregnancy	NR (23.8)	NR (15.8)	NR	*No significant difference* *p* = 0.23
Surgical removal of placenta in previous pregnancy	NR (6.3)	NR (4.7)	NR	*No significant difference* *p* = 0.50
Abortion (after spontaneous miscarriage or medical termination of pregnancy)	NR (11.9)	NR (13.0)	NR	*No significant difference* *p* = 0.85
Multivariate analysis (R^2 = 0.150)				
Postmaturity (≥ 42 weeks of completed gestation)			OR 3.07 (1.02, 9.20)	*Significant association* *p = NR*
Caesarean section or assisted vaginal delivery			OR 2.23 (1.04, 4.74)	*Significant association* *p = NR*
Maternal age at delivery (years)			OR 0.89 (0.80, 0.98)	*Significant association* *p = NR*
Pregnancy-related RBC transfusion			OR 3.51 (0.97, 12.7)	*No significant association* *p = NR*

Twins			*Not assessed* *(0 event rate in the control group)*	

EXTERNAL VALIDITY

Generalisability (relevance of the study population to the Guidelines target population)

The participants in this study were Rh D negative primiparae women who had received adequate prophylaxis.

The patient population in Australia also includes multigravidae patients. It is not known if the prevalence of high BMI is similar to that of the Australian population.

Applicability (relevance of the evidence to the Australian health care system)

The women in this study received a different dosing regimen (1000 IU at 30 weeks) to those in the Australian setting (im 625 IU at 28 and 34 weeks).

Additional comments

The prevalence of new Rh D alloimmunisation's estimated as 0.31% (39/12 576) (including all Rh D negative parae-1 who delivered their first child after introduction of RAADP).

Factors shown to be significant risk factors associated with RhD immunisation in parae-1 with RhD antibodies detected in first trimester screening in the univariate analysis were:

- maternal age at delivery in previous pregnancy (p = 0.02)
- postmaturity (≥ 42 weeks of completed gestation) (p = 0.01)
- non-spontaneous delivery (assisted vaginal delivery or caesarean section) (p = 0.03),
- pregnancy-related RBC transfusion (p = 0.02)
- twins (p = 0.05)

In addition to the above weight-related factors, potential risk factors with no significant association ($p > 0.05$) with RhD immunisation in parae-1 with RhD antibodies detected in first trimester screening in the univariate analysis were:

- maternal blood group A/AB, maternal or paternal non-Dutch ethnicity, gender, invasive prenatal diagnostic procedures, external version in a previous pregnancy, miscarriage or termination of pregnancy after the first completed pregnancy

BMI, body mass index; CI, confidence interval; GP, general practitioner; im, intramuscular; IU, international units; OR, odds ratio; RAADP, routine antenatal anti-D prophylaxis; RBC, red blood cell; RCT, randomised controlled trial; SD, standard deviation

a. By design, the controls under primary care were overrepresented (with lower prevalence of potential risk factors for example previous medical intervention), which could overestimate the effect of potential risk factors. The authors therefore weighted the primary care controls (0.35) to restore the proportion of primary care pregnancies to the control group. All *p*-values are based on n=146.

STUDY DETAILS: Analysis of factors in single arm of RCT

Citation

Bichler 2003

Bichler, J., Schondorfer, G., Pabst, G., Andresen, I. (2003). Pharmacokinetics of anti-D IgG in pregnant RhD negative women. BJOG, Vol. 110, pp. 39-45

Affiliation/Source of funds

Source of funds/conflicts of interest not declared

Study design	**Level of evidence**	**Location**	**Setting**
Open-label randomised study	Level III-2	Bavaria, Germany	Gynaecological practices, multicentre

Intervention	**Comparator**
Anti-D IgG 1500 IU intravenous (iv) at 28 weeks gestation [Rhophylac]	Anti-D IgG 1500 IU intramuscular (im) at 28 weeks gestation [Rhophylac]

Population characteristics

Rh D negative pregnant women not previously alloimmunised

Length of follow-up	**Outcomes measured**
11 weeks following antenatal prophylaxis, 6 months following birth of Rh D positive infant	Peak serum concentration of anti-D via flow cytometry (pharmacokinetics) with respect to patient weight

INTERNAL VALIDITY

Overall risk of bias (descriptive)

Rating: Critical

Description: The study is too problematic to provide any useful evidence on the outcome of interest.

RESULTS

Population analysed	**Intervention (n)**		**Comparator (n)**	
Randomised	6		8	
Efficacy analysis (ITT)	6		8	
Efficacy analysis (PP)	5 (one woman was excluded as she was found to be RhD^{weak}-positive)		8	
Safety analysis	6		8	
Outcome	**Rh D IgG 1500 IU iv** **n/N (%)** **Mean ± SD**	**Rh D IgG 1500 IU im** **n/N (%)** **Mean ± SD**	**Risk estimate (95% CI)**	***Statistical significance p*-value**
Rh D IgG peak serum concentration up to seven days after administration (ng/mL)	36.1 ± 2.6	19.8 ± 8.7		
Rh D IgG peak serum concentration (C_{max}) (ng/mL)	70.9 ± 8.2 (range 62.0 to 84.4)	22.1 ± 12.0 (range 6.9 to 46.1)	Outliers in i.m. group were two women with body weight > 80 kg (see below) Bioavailability of i.m. estimated at 77.8% (41, 96)	
Adverse events	Three adverse events in two patients. Considered not related to the study drug.	Four adverse events in three patients. Considered not related to the study drug.		
Rh D IgG 1500 IU i.m.				
Rh D IgG peak serum concentration (C_{max}) (ng/mL)	Body weight > 80 kg (n=2) Patient 9: 6.9 ng/mL Patient 12: 10.0 ng/mL	Body weight < 80 kg (n=6) Mean: 26.6 ng/mL	Despite low peak Rh D IgG serum, the women had quantifiable Rh D IgG levels up to last scheduled blood sample (weeks 9 and 11 respectively).	

EXTERNAL VALIDITY

Generalisability (relevance of the study population to the Guidelines target population)

The study population is generalisable to the guidelines target population. The study included Rh D negative unsensitised women who were unselected for previous births.

Applicability (relevance of the evidence to the Australian health care system)

The dose (one administration of 1500 IU at 28 weeks) and one route (iv) used in this study is different to that used in the Australian health care system (im 625 IU at 28 and 34 weeks).

Additional comments

The bioavailability of im anti-D IgG had a large confidence interval, influenced by low results observed in patients 9 and 12 who weighed more than 80kg. The authors concluded that for overweight women the iv administration of Rhophylac may be more advantageous. There were no women who were over 80 kg in the iv group

CI, confidence interval; im, intramuscular; iv, intravenous; IgG immunoglobulin; IU, international units; kg, kilogram; mL, millilitre; MRA, magnetic resonance angiography; MRI, magnetic resonance imaging; NA, not applicable; ng, nanogram; NR, not reported; PP, per-protocol; RBC, red blood cell; RCT, randomised controlled trial; TCD, transcranial Doppler

Appendix F GRADE Evidence to Decision tables

F1 Question 1a

Should universal RAADP (1 or 2 doses) vs. placebo or no universal RAADP be used for Rh D negative pregnant women with no preformed anti-D?	
PROBLEM:	Maternal Rh antibodies develop during pregnancy when an Rh negative woman carries an Rh positive fetus. It occurs when fetal red blood cells (RBCs) enter the maternal circulation and antibodies are produced towards the fetal Rh antigen. Small fetomaternal haemorrhages at birth and silent transplacental haemorrhages in the antenatal period are believed to be the key source of fetal RBCs entering the maternal circulation (Bowman, 2003, Chilcott et al., 2003, McBain et al., 2015). The maternal response to the fetal RBCs is known as 'sensitisation' or alloimmunisation. No apparent adverse health outcomes occur in the mother as a result of this sensitisation, but haemolytic disease of the fetus and newborn (HDFN) can arise in an Rh positive fetus (usually in subsequent pregnancies). HDFN occurs when maternal Rh antibodies cross the placenta into the baby's circulation and mediate destruction of the baby's RBCs. This destruction causes fetal anaemia (a shortage in RBCs, required to carry oxygen), and can lead to bilirubinaemia (elevated levels of bilirubin, a waste product of the degraded RBCs) and jaundice (yellowing of the skin and whites of the eyes). In severe cases the HDFN causes kernicterus (a form of brain damage) or hydrops fetalis (gross oedema or accumulation of fluid leading to fetal death) (Bowman, 2003, McBain et al., 2015, Zwiers et al., 2018). In the absence of intervention, HDFN affects 1% of neonates, and is a significant cause of perinatal mortality, morbidity and long-term disability (Bowman, 2003, Chilcott et al., 2003). In Australia, about 17% of people are Rh negative.[1] It is highest in those who are of European origin (16%), less common in those of African origin (7%) and rare in Indigenous peoples and those of East Asian origin (<1%). In the United Kingdom it is estimated 10% of live births are Rh D positive infants delivered to Rh D negative women (Chilcott et al., 2003); however, this number may be higher in the Australian setting (Hyland et al., 2013). [1] http://resources.transfusion.com.au/cdm/ref/collection/p16691coll1/id/233
OPTION:	universal RAADP (1 or 2 doses)
COMPARISON:	placebo or no universal RAADP
MAIN OUTCOMES:	Incidence of Rh D alloimmunisation (any timepoint, RCTs); Incidence of Rh D alloimmunisation (any timepoint, observational studies); Incidence of Rh D alloimmunisation (in subsequent pregnancy); Incidence of Rh D alloimmunisation (during pregnancy); Incidence of Rh D alloimmunisation (at birth of Rh positive newborn or within three days of delivery); Incidence of Rh D alloimmunisation (up to 12-months postnatal follow-up); Incidence of a positive test for fetomaternal haemorrhage (at 32 to 35 weeks gestation); Incidence of a positive test for fetomaternal haemorrhage (at birth of Rh positive newborn); Adverse neonatal events: jaundice; Adverse neonatal events: prevalence of severe HDFN (perinatal mortality, need for IUT and/or exchange transfusion); Adverse maternal events attributed to anti-D;
SETTING:	Obstetrics and maternity, primary
PERSPECTIVE:	Australian Health Care Setting
BACKGROUND:	The National Health and Medical Research Council's (NHMRC) *1999 Guidelines on the Prophylactic Use of Rh D Immunoglobulin (Anti-D) in Obstetrics* were updated by the National Blood Authority (NBA) in 2003, with the aim of updating the guidance on antenatal prophylaxis. The updated 2003 Anti-D Guidelines aimed to inform clinicians, other health professionals, and policy makers of new recommendations for the staged implementation of full antenatal prophylaxis in Australia. The 2003 Anti-D Guidelines also included policy intent as a national program to address the issue of sufficiency of supply of product and were intended to be reviewed within five years, according to the availability of Rh D immunoglobulin. To ensure the Anti-D Guidelines continue to reflect current evidence and best clinical practice, the NBA undertook a scoping exercise in September 2016 to identify the extent of newly available evidence and areas of concerns. Through a collaborative partnership with the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) and other relevant stakeholders, it was agreed that a new evidence-based guideline would be developed.

	One key area of concern identified in the scoping report was *"Does the available evidence still support universal routine antenatal prophylaxis?"* (i.e., all pregnant women who are Rh D negative with no preformed anti-D).
CONFLICT OF INTERESTS:	See Clinical Guidance.

ASSESSMENT

Problem

Is the problem a priority?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ No ○ Probably no ○ Probably yes ● Yes ○ Varies ○ Don't know	Rh D negative women who are exposed to Rh D positive fetal red cells in the antenatal period and those who deliver a Rh D positive baby are at risk of developing anti-D antibodies. These antibodies can cross the placenta and cause fetal red cell destruction. This can result in fetal and neonatal anaemia and neonatal jaundice; in severe cases, these can cause serious morbidity or mortality. In the absence of Rh D immunoprophylaxis, it is estimated that Rh D alloimmunisation occurred in 14% to 17% of Rh D negative women who delivered a Rh D positive baby (1) After the introduction of routine postnatal prophylaxis, the rate of Rh D alloimmunisation dropped to around 1% to 2% (2, 3). The addition of antenatal Rh D immunoprophylaxis further reduced the risk of Rh D alloimmunisation to approximately 0.1% to 0.2% (2, 4). Recent literature and international practice guidelines support this review of the indications for, and dosing of Rh D immunoprophylaxis.	Maintenance of supply of RhD immunoglobulin is a global issue. No alternatives to the blood product (Rh D immunoglobulin) are available or anticipated within the near future. Boosting donors to maintain the supply of Rh D immunoglobulin poses potential clinical risks with ethical concerns and places a considerable burden on those donors.

Desirable Effects

How substantial are the desirable anticipated effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Trivial ○ Small ○ Moderate ● Large ○ Varies ○ Don't know	Reducing the incidence of Rh D alloimmunisation is important because it is the most critical intermediate step for reducing the incidence of HDFN (and consequent risk of serious fetal or neonatal morbidity or death). Prevention of Rh D alloimmunisation protects the mother from the need for invasive interventions that are needed if HDFN causes significant anaemia in an Rh-positive fetus as well as potential clinical complications that affect her own health.	

	Administration of Rh D immunoglobulin has an excellent safety record. Theoretical risks associated with exposure to blood product will always remain. A 2013 report (5) noted that the majority (63%) of errors associated with Rh D IgG related to omission or late administration. For research evidence on Desirable and Undesirable anticipated effects, as well as the certainty of this evidence, see the GRADE Evidence Profile.	

Undesirable Effects

How substantial are the undesirable anticipated effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Large ○ Moderate ○ Small ● Trivial ○ Varies ○ Don't know	Reducing the incidence of Rh D alloimmunisation is important because it is the most critical intermediate step for reducing the incidence of HDFN (and consequent risk of serious fetal or neonatal associated serious morbidity or death). Prevention of Rh D alloimmunisation protects the mother from the need for invasive interventions that are needed if HDFN causes significant anaemia in an Rh-positive fetus as well as potential clinical complications that affect her own health. Administration of Rh D immunoglobulin has an excellent safety record. Theoretical risks associated with exposure to blood product will always remain. A 2013 report (5) noted that the majority (63%) of errors associated with Rh D IgG related to omission or late administration. For research evidence on Desirable and Undesirable anticipated effects, as well as the certainty of this evidence, see the GRADE Evidence Profile.	

Certainty of evidence

What is the overall certainty of the evidence of effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
● Very low ○ Low ○ Moderate ○ High ○ No included studies	*See Appendix 1*	

Values

Is there important uncertainty about or variability in how much people value the main outcomes?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Important uncertainty or variability ○ Possibly important uncertainty or variability ○ Probably no important uncertainty or variability ● No important uncertainty or variability		

Balance of effects

Does the balance between desirable and undesirable effects favor the intervention or the comparison?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Favors the comparison ○ Probably favors the comparison ○ Does not favor either the intervention or the comparison ○ Probably favors the intervention ● Favors the intervention ○ Varies ○ Don't know	Fetal deaths due to HDFN are extremely low, largely due to improvements in fetomaternal medicine and maternity and neonatal care; however, there are considerable costs associated with caring for Rh D alloimmunised women and their babies, which can be avoided with prophylactic administration of antenatal Rh D IgG. The incidence of HDFN depends on the proportion of the population that is Rh D negative, which varies between ethnic groups. In Australia, about 17% of people are Rh D negative (6) but the proportion of Rh D positive babies born of Rh D negative women is not known.	In 2015 there were around 310,000 pregnancies in Australia (calculated based on ABS data, and estimated number of stillbirths and terminations) suggesting approximately 31,000 were at risk of Rh D alloimmunisation (assuming around 10% of all births are Rh D positive babies of Rh D negative women)(7). In the absence of antenatal prophylaxis (but assuming postnatal prophylaxis), around 1% to 2% would become sensitised (about 310 to 620 women). Assuming 80% of these women have a subsequent pregnancy, an estimated 256 to 496 Rh D negative women would require close monitoring in their second pregnancy. Of these, intrauterine transfusions would be required in about 10% to 12% of babies, with fetal anaemia and HDFN leading to approximately 18 to 35 fetal or neonatal deaths, 10 to 20 children with minor developmental issues and 4 to 8 children with major developmental issues. Note: the above is based on assumptions for the UK (similar prevalence of Rh D negative people).

Resources required

How large are the resource requirements (costs)?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
● Large costs ○ Moderate costs ○ Negligible costs and savings ○ Moderate savings ○ Large savings ○ Varies ○ Don't know	The 2003 guidelines recommend RAADP. Resource costs associated with this programme are considered justifiable (8).	

Certainty of evidence of required resources

What is the certainty of the evidence of resource requirements (costs)?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Very low ○ Low ○ Moderate ○ High ● No included studies		

Cost effectiveness

Does the cost-effectiveness of the intervention favor the intervention or the comparison?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Favors the comparison ○ Probably favors the comparison ○ Does not favor either the intervention or the comparison ○ Probably favors the intervention ○ Favors the intervention ○ Varies ● No included studies	The cost-effectiveness analysis in the 2003 guidelines indicated that both a postpartum program and a postpartum plus antenatal prophylaxis program appeared to remain well within the usual bounds of cost-effectiveness at a range of prices per vial of Rh D immunoglobulin. The Working Party concluded that antenatal prophylaxis appears to be a cost-effective addition to a postpartum program even at a relatively high price of Rh D immunoglobulin of $115 per vial.	

Equity

What would be the impact on health equity?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Reduced ○ Probably reduced ● Probably no impact ○ Probably increased ○ Increased ○ Varies ○ Don't know	RAADP has been implemented in Australia with no impact on health equity noted.	

Acceptability Is the intervention acceptable to key stakeholders?		
JUDGEMENT	**RESEARCH EVIDENCE**	**ADDITIONAL CONSIDERATIONS**
○ No ○ Probably no ○ Probably yes ● Yes ○ Varies ○ Don't know	RAADP has been implemented in Australia and is considered acceptable to key stakeholder.	

Feasibility Is the intervention feasible to implement?		
JUDGEMENT	**RESEARCH EVIDENCE**	**ADDITIONAL CONSIDERATIONS**
○ No ○ Probably no ○ Probably yes ● Yes ○ Varies ○ Don't know	RAADP has been implemented in Australia. The implementation of this programme is considered feasible.	

SUMMARY OF JUDGEMENTS

	JUDGEMENT						
PROBLEM	No	Probably no	Probably yes	**Yes**		Varies	Don't know
DESIRABLE EFFECTS	Trivial	Small	Moderate	**Large**		Varies	Don't know
UNDESIRABLE EFFECTS	Large	Moderate	Small	**Trivial**		Varies	Don't know
CERTAINTY OF EVIDENCE	**Very low**	Low	Moderate	High			No included studies

	JUDGEMENT						
VALUES	Important uncertainty or variability	Possibly important uncertainty or variability	Probably no important uncertainty or variability	**No important uncertainty or variability**			
BALANCE OF EFFECTS	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	Probably favors the intervention	**Favors the intervention**	Varies	Don't know
RESOURCES REQUIRED	**Large costs**	Moderate costs	Negligible costs and savings	Moderate savings	Large savings	Varies	Don't know
CERTAINTY OF EVIDENCE OF REQUIRED RESOURCES	Very low	Low	Moderate	High			**No included studies**
COST EFFECTIVENESS	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	Probably favors the intervention	Favors the intervention	Varies	**No included studies**
EQUITY	Reduced	Probably reduced	**Probably no impact**	Probably increased	Increased	Varies	Don't know
ACCEPTABILITY	No	Probably no	Probably yes	**Yes**		Varies	Don't know
FEASIBILITY	No	Probably no	Probably yes	**Yes**		Varies	Don't know

TYPE OF RECOMMENDATION

Strong recommendation against the option	Conditional recommendation against the option	Conditional recommendation for either the option or the comparison	Conditional recommendation for the option	**Strong recommendation for the option**
○	○	○	○	●

CONCLUSIONS

Recommendation

The ERG **recommends** access to antenatal Rh D immunoglobulin for the prevention of Rh D alloimmunisation in Rh D negative pregnant women with no preformed anti-D antibodies. (*Strong recommendation, low to very low certainty of evidence about the size of effect*)

Related recommendation(s)

1. Should targeted RAADP (based on noninvasive prenatal screening) vs. universal RAADP be used in Rh D negative pregnant women with no preformed anti-D?

The ERG **recommends** that antenatal Rh D immunoprophylaxis in Rh D negative pregnant women with no preformed anti-D antibodies be targeted to those predicted to be carrying an Rh D positive fetus, based on NIPT for fetal *RHD*. This applies to both routine and sensitising event immunoprophylaxis, if the result of fetal *RHD* genotyping is available (see EOP3 and EOP7). (*Strong recommendation, low certainty of evidence about the size of the effect*)

If fetal Rh D status is not available or is uncertain, the ERG **recommends** that antenatal Rh D immunoprophylaxis be offered to Rh D negative pregnant women with no preformed anti-D antibodies. (*Strong recommendation, low certainty of evidence about the size of the effect*)

Expert opinion points:

D detected in the mother's blood is preformed (due to

EOP3 Rh D immunoglobulin should not be given to Rh D negative pregnant women with preformed anti-D antibodies. However, if it is unclear whether the anti-
sensitisation) or passive (due to administration of Rh D immunoglobulin in the past 12 weeks), the treating clinician should be consulted. If there is continuing doubt, Rh D immunoglobulin should be administered.

EOP7 A dose of Rh D immunoglobulin 625 IU should be offered to every Rh D negative woman with no preformed anti-D antibodies, unless NIPT for fetal *RHD* has predicted the fetus to be Rh D negative, to ensure adequate protection against alloimmunisation for the following indications after 12^{+6} weeks of pregnancy:

- genetic studies (chorionic villus sampling, amniocentesis and cordocentesis)
- abdominal trauma considered sufficient to cause FMH, even if FMH testing is negative
- each occasion of revealed or concealed antepartum haemorrhage. Where the woman suffers unexplained uterine pain the possibility of concealed antepartum haemorrhage (and the need for immunoprophylaxis) should be considered
- external cephalic version (successful or attempted)
- miscarriage or termination of pregnancy.

Justification

Although the comparative evidence is of low to very low certainty, large studies on the incidence of Rh D alloimmunisation show a reduction in risk since the introduction of antenatal immunoprophylaxis. There is evidence that the risk of fetomaternal haemorrhage of sufficient size to cause Rh D alloimmunisation increases in the third trimester (Urbaniak SJ, 1998). Antenatal prophylaxis reduces the incidence of a positive test for fetomaternal haemorrhage (*moderate certainty of evidence about the effect*), implying reduced risk of Rh D alloimmunisation through effective removal of fetal red cells.

Subgroup considerations

No subgroups considered.

Implementation considerations

None anticipated. Routine antenatal prophylaxis with Rh(D)immunoglobulin in Rh D negative women with no preformed anti-D antibodies has been available in Australia since the staged introduction starting in 2003.

Monitoring and evaluation

A reporting system similar to The Serious Hazards of Transfusion (SHOT) UK-wide system should be implemented. It should include errors and adverse events relating to administration of Rh(D)immunoglobulin and episodes of Rh D sensitisation despite immunoprophylaxis.

Research priorities

None

REFERENCES SUMMARY

1. Urbaniak SJ.,. The scientific basis of antenatal prophylaxis. BJOG; 1998.
2. McBain, R. D., Crowther, C. A., Middleton, P. Anti-D administration in pregnancy for preventing Rhesus alloimmunisation. Cochrane Database Syst Rev; Sep 3, 2015.
3. Qureshi, H. Massey, E. Kirwan, D. Davies, T. Robson, S. White, J. Jones, J. Allard,S. British Society for Haematology,. BCSH guideline for the use of anti-D immunoglobulin for the prevention of haemolytic disease of the fetus and newborn. Transfus Med; 2014.
4. Pilgrim, H., Lloyd-Jones, M., Rees, A. Routine antenatal anti-D prophylaxis for RhD-negative women: a systematic review and economic evaluation. Health Technology Assessment; 2009.
5. Bolton-Maggs P., Davies T., Poles D., Cohen H. Errors in anti-D immunoglobulin administration: retrospective analysis of 15 uears of reports to the UK confidential haemovigilance scheme. BJOG; 2013.
6. Blewett J., I need to know about D. Transfusion Fact Sheet; 2010.
7. National Institute of Health and Care Excellence, Routine antental anti-D prophylaxis for women who are rhesus D negative. NICE technology appraisal guidance [TA156]; 2008.
8. NHMRC, Guidelines on the Prophylactic Use of Rh D Immunoglobulin (Anti-D) in Obstetrics. 2003.

APPENDICES

Appendix 1

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo or no universal RAADP	Risk with universal RAADP (1 or 2 doses)				
Incidence of Rh D alloimmunisation (any timepoint, RCTs)	Study population		**RR 0.39** (0.09 to 1.63)	2297 (2 RCTs)	⨁⨁◯◯ LOW[a,b,c,d,e,f]	In Rh D negative women with no preformed anti-D, universal routine antenatal prophylactic Rh D immunoglobulin may reduce the incidence of Rh D alloimmunisation (1 or 2 doses, any timepoint) but we are uncertain about the size of the effect.
	14 per 1,000	**5 per 1,000** (1 to 22)				
Incidence of Rh D alloimmunisation (any timepoint, observational studies)	Study population		**RR 0.31** (0.18 to 0.54)	51987 (8 observational studies)	⨁◯◯◯ VERY LOW[b,e,g,h,i]	
	11 per 1,000	**3 per 1,000** (2 to 6)				
Incidence of Rh D alloimmunisation (in subsequent pregnancy)	Study population		**RR 0.43** (0.31 to 0.59)	31826 (6 observational studies)	⨁⨁◯◯ LOW[b,e,h,i,j]	In Rh D negative women with no preformed anti-D, universal routine antenatal prophylactic Rh D immunoglobulin may reduce the incidence of Rh D alloimmunisation (in a subsequent pregnancy) but we are uncertain about the size of the effect.
	8 per 1,000	**3 per 1,000** (2 to 5)				
Incidence of Rh D alloimmunisation (during pregnancy)	Study population		**RR 0.33** (0.08 to 1.37)	28357 (4 observational studies)[k]	⨁◯◯◯ VERY LOW[a,b,c,e,f,g,h,i]	In Rh D negative women with no preformed anti-D, universal routine antenatal prophylactic Rh D immunoglobulin may reduce the incidence of Rh D alloimmunisation (during pregnancy) but we are very uncertain about the size of the effect.
	6 per 1,000	**2 per 1,000** (0 to 8)				
Incidence of Rh D alloimmunisation (at birth of Rh positive newborn or within three days of delivery)	Study population		**RR 0.19** (0.08 to 0.45)	24622 (8 observational studies)[l]	⨁◯◯◯ VERY LOW[a,b,c,e,g,h,i]	In Rh D negative women with no preformed anti-D, universal routine antenatal prophylactic Rh D immunoglobulin may reduce the incidence of Rh D alloimmunisation (at birth or within three days of delivery of a Rh D positive newborn) but we are very uncertain about the size of the effect.
	14 per 1,000	**3 per 1,000** (1 to 6)				
	Study population					

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo or no universal RAADP	Risk with universal RAADP (1 or 2 doses)				
Incidence of Rh D alloimmunisation (up to 12-months postnatal follow-up)	15 per 1,000	**3 per 1,000** (2 to 4)	**RR 0.19** (0.13 to 0.29)	17372 (8 observational studies)[m]	⨁⨁◯◯ LOW[a,b,c,e,h,i,j]	In Rh D negative women with no preformed anti-D, universal routine antenatal prophylactic Rh D immunoglobulin may reduce the incidence of Rh D alloimmunisation (up to 12-months after the birth of an Rh D positive newborn) but we are uncertain about the size of the effect.
Incidence of a positive test for fetomaternal haemorrhage (at 32 to 35 weeks gestation) assessed with: Kleihauer test	Study population		**RR 0.60** (0.41 to 0.88)	1884 (1 RCT)	⨁⨁⨁◯ MODERATE[a,b,e,n]	In Rh D negative women with no preformed anti-D, universal RAADP (1 or 2 doses) likely reduces the incidence of a positive test for fetomaternal haemorrhage (assessed at 32-35 weeks gestation).
	70 per 1,000	**42 per 1,000** (29 to 62)				
Incidence of a positive test for fetomaternal haemorrhage (at birth of Rh positive newborn) assessed with: Kleihauer test	Study population		**RR 0.60** (0.46 to 0.79)	1189 (1 RCT)	⨁⨁⨁◯ MODERATE[a,b,c,e,n]	In Rh D negative women with no preformed anti-D, universal RAADP (1 or 2 doses) likely reduces the incidence of a positive test for fetomaternal haemorrhage (assessed at birth of an Rh D positive newborn).
	202 per 1,000	**121 per 1,000** (93 to 159)				
Adverse neonatal events: jaundice	Study population		**RR 0.26** (0.03 to 2.30)	1882 (1 RCT)	⨁⨁◯◯ LOW[a,b,c,e,f,n]	In Rh D negative women with no preformed anti-D, the effect of universal RAADP (1 or 2 doses) is uncertain.
	4 per 1,000	**1 per 1,000** (0 to 10)				
Adverse neonatal events: prevalence of severe HDFN (perinatal mortality, need for IUT and/or exchange transfusion)	Study population		**RR 0.51** (0.09 to 0.92)	21221 (1 observational study)	⨁◯◯◯ VERY LOW[n,o,p]	In Rh D negative women with no preformed anti-D, the effect of universal RAADP (1 or 2 doses) on severe adverse neonatal events is very uncertain.
	2 per 1,000	**1 per 1,000** (0 to 2)				
Adverse maternal events attributed to anti-D - not measured	None of the identified studies reported any serious adverse events. A few cases of mild pain, soreness, and itching at the		-	-	-	In Rh D negative women with no preformed anti-D, the effect of universal RAADP (1 or 2 doses) on adverse maternal events is unknown

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo or no universal RAADP	Risk with universal RAADP (1 or 2 doses)				
	injection site noted. One study reported marked flushing and mild chest pain that was attributed to a specific batch study drug. [1,2]					

1. Pilgrim, H., Lloyd-Jones, M., Rees, A. Routine antenatal anti-D prophylaxis for RhD-negative women: a systematic review and economic evaluation. Health Technology Assessment; 2009.
2. McBain, R. D., Crowther, C. A., Middleton, P. Anti-D administration in pregnancy for preventing Rhesus alloimmunisation. Cochrane Database Syst Rev; Sep 3, 2015.

a. One or more randomised studies with plausible bias that raises serious doubts about the results.
b. Missing data and exclusion of some patients may over-estimate the clinical effectiveness of RAADP.
c. Includes one quasi-randomised trial with high risk of selection bias.
d. No significant heterogeneity, with variability in effect estimates assessed as moderate (I2 statistic between 25-50%). Does not reduce confidence in results to inform decision making.
e. Obstetric practice and the baseline characteristics of the population may not be reflective of current practice however this was not considered too seriously affect the confidence in the observed effect and could be sensibly applied.
f. Low event rate and/or wide confidence intervals that cross the line of no effect. Confidence in the results is weak.
g. Significant heterogeneity with substantial variability in effect estimates (I2 statistic greater than 50%). Reduces confidence in the results to inform decision making.
h. One or more comparative observational studies with some important problems that seriously weaken the confidence in the results
i. Studies include historical and/or geographic controls and it is not clear if intervention and control groups are comparable at baseline.
j. No significant heterogeneity. I2 statistic equals 0%.
k. Includes 1 RCT and 1 quasi-RCT
l. Includes 1 RCT, 1 quasi-RCT and 6 Coh studies. 1 Coh study does not contribute any data.
m. Includes 1 RCT, 1 quasi-RCT and 6 Coh studies. 2 Coh studies do not contribute any data
n. One study only. Heterogeneity not assessed.
o. One or two comparative observational studies that appear to provide sound evidence for a non-randomised study but cannot be considered comparable to a well-performed RCT
p. Some concerns with reporting bias and missing data.

F2 Question 1b

Should RAADP (single-dose) vs. RAADP (two-dose) be used for Rh D negative pregnant women with no preformed anti-D?	
PROBLEM:	Maternal Rh antibodies develop during pregnancy when an Rh negative woman carries an Rh positive fetus. It occurs when fetal red blood cells (RBCs) enter the maternal circulation and antibodies are produced towards the fetal Rh antigen. Small fetomaternal haemorrhages at birth and silent transplacental haemorrhages in the antenatal period are believed to be the key source of fetal RBCs entering the maternal circulation (Bowman, 2003, Chilcott et al., 2003, McBain et al., 2015). The maternal response to the fetal RBCs is known as 'sensitisation' or alloimmunisation. No apparent adverse health outcomes occur in the mother as a result of this sensitisation, but haemolytic disease of the fetus and newborn (HDFN) can arise in an Rh positive fetus (usually in subsequent pregnancies). HDFN occurs when maternal Rh antibodies cross the placenta into the baby's circulation and mediate destruction of the baby's RBCs. This destruction causes fetal anaemia (a shortage in RBCs, required to carry oxygen), and can lead to bilirubinaemia (elevated levels of bilirubin, a waste product of the degraded RBCs) and jaundice (yellowing of the skin and whites of the eyes). In severe cases the HDFN causes kernicterus (a form of brain damage) or hydrops fetalis (gross oedema or accumulation of fluid leading to fetal death) (Bowman, 2003, McBain et al., 2015, Zwiers et al., 2018). In the absence of intervention, HDFN affects 1% of neonates, and is a significant cause of perinatal mortality, morbidity and long-term disability (Bowman, 2003, Chilcott et al., 2003). In Australia, about 17% of people are Rh negative (Blewitt 2010). It is highest in those who are of European origin (16%), less common in those of African origin (7%) and rare in Indigenous peoples and those of East Asian origin (<1%). In the United Kingdom it is estimated 10% of live births are Rh D positive infants delivered to Rh D negative women (Chilcott et al., 2003); however, this number may be higher in the Australian setting (Hyland et al., 2013).
OPTION:	RAADP (single dose)
COMPARISON:	RAADP (two-dose)
MAIN OUTCOMES:	Incidence of Rh D alloimmunisation; Incidence of a positive test for feto-maternal haemorrhage; Adverse neonatal events; Adverse maternal events; Serum anti-D levels at birth; Incidence of Rh D alloimmunisation (1 dose, any timepoint); Incidence of Rh D alloimmunisation (2 dose, any timepoint); Incidence of Rh D alloimmunisation (1 dose, estimated); Incidence of Rh D alloimmunisation (2 doses, estimated);
SETTING:	Obstetrics and maternity, primary setting
PERSPECTIVE:	
BACKGROUND:	The National Health and Medical Research Council's (NHMRC) *1999 Guidelines on the Prophylactic Use of Rh D Immunoglobulin (Anti-D) in Obstetrics* were updated by the National Blood Authority (NBA) in 2003, with the aim of updating the guidance on antenatal prophylaxis. The updated 2003 Anti-D Guidelines aimed to inform clinicians, other health professionals, and policy makers of new recommendations for the staged implementation of full antenatal prophylaxis in Australia. The 2003 Anti-D Guidelines also included policy intent as a national program to address the issue of sufficiency of supply of product and were intended to be reviewed within five years, according to the availability of Rh D immunoglobulin. To ensure the Anti-D Guidelines continue to reflect current evidence and best clinical practice, the NBA undertook a scoping exercise in September 2016 to identify the extent of newly available evidence and areas of concerns. Through a collaborative partnership with the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) and other relevant stakeholders, it was agreed that a new evidence-based guideline would be developed. One key area of concern identified in the scoping report was "Should universal routine antenatal prophylaxis be moved from a two-dose regimen to a one-dose regimen?" In June 2010, the Rh(D) Joint Consultative Committee (JCC) considered the available evidence and the relative advantages and disadvantages, and strongly supported the move. A trial on this comparison has been conducted in Australia and the results were presented at the HAA Meeting in November 2016. Pennell, C., Cheng, J., Veselinovic, B.P., et al. 2017. Single dose Anti-D prophylaxis in pregnancy: is it time to change? *Journal of Paediatrics and Child Health,* 53**,** 112-113.

CONFLICT OF INTERESTS:	See Clinical guidance

ASSESSMENT

Problem

Is the problem a priority?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ No ○ Probably no ○ Probably yes ● Yes ○ Varies ○ Don't know	Rh D negative women who are exposed to Rh D positive fetal red cells in the antenatal period and those who deliver a Rh D positive baby are at risk of developing anti-D antibodies. These antibodies can cross the placenta and cause fetal red cell destruction. This can result in fetal and neonatal anaemia and neonatal jaundice; in severe cases, these can cause serious morbidity or mortality. In the absence of Rh D immunoprophylaxis, it is estimated that Rh D alloimmunisation occurred in 14% to 16% of Rh D negative women who delivered a Rh D positive baby (1). After the introduction of routine postnatal prophylaxis, the rate of Rh D alloimmunisation dropped to around 1% to 2% (2, 3). The addition of antenatal Rh D immunoprophylaxis further reduced the risk of Rh D alloimmunisation to approximately 0.1% to 0.2% (4, 3). Recent literature and international practice guidelines support this review of the indications for, and dosing of Rh D immunoprophylaxis.	Maintenance of supply of RhD immunoglobulin is a global issue. No alternatives to the blood product (Rh D immunoglobulin) are available or anticipated within the near future. Boosting donors to maintain the supply of Rh D immunoglobulin poses potential clinical risks with ethical concerns and places a considerable burden on those donors.

Desirable Effects

How substantial are the desirable anticipated effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Trivial ○ Small ● Moderate ○ Large ○ Varies ○ Don't know	Desirable: Improved convenience, compliance, and a reduction in the potential negative effects associated with intramuscular injection. Undesirable: A theoretical increased risk of Rh D alloimmunisation due to concerns regarding lower Rh D immunoglobulin levels in maternal circulation as gestation advances in the late third trimester. Administration of Rh D immunoglobulin has an excellent safety record. Theoretical risks associated with exposure to blood product will always remain.	

	A 2013 report (5) noted that the majority (63%) of errors associated with Rh D IgG related to omission or late administration. For research evidence on Desirable and Undesirable anticipated effects, as well as the certainty of this evidence, see the GRADE Evidence Profile.	

Undesirable Effects

How substantial are the undesirable anticipated effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Large ● Moderate ○ Small ○ Trivial ○ Varies ○ Don't know	Desirable: Improved convenience, compliance, and a reduction in the potential negative effects associated with intramuscular injection. Undesirable: A theoretical increased risk of Rh D alloimmunisation due to concerns regarding lower Rh D immunoglobulin levels in maternal circulation as gestation advances in the late third trimester. Administration of Rh D immunoglobulin has an excellent safety record. Theoretical risks associated with exposure to blood product will always remain. A 2013 report (5) noted that the majority (63%) of errors associated with Rh D IgG related to omission or late administration. For research evidence on Desirable and Undesirable anticipated effects, as well as the certainty of this evidence, see the GRADE Evidence Profile.	

Certainty of evidence

What is the overall certainty of the evidence of effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
● Very low ○ Low ○ Moderate ○ High ○ No included studies	The relative risk of Rh D alloimmunisation in Rh D negative pregnant women with no preformed anti-D antibodies is similar with a single dose or two dose regimens. *See Appendix 1*	Very low quality evidence suggests improved compliance with a single dose (6, 5, 7), but the proportion of patients with undetectable anti-D at delivery is higher in women who received two doses of Rh D IgG, suggesting better coverage in the late third trimester with a two-dose regimen (6). The impact of improved compliance or persistence on patient outcomes is not known.

Values

Is there important uncertainty about or variability in how much people value the main outcomes?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Important uncertainty or variability ○ Possibly important uncertainty or variability ○ Probably no important uncertainty or variability ● No important uncertainty or variability		

Balance of effects

Does the balance between desirable and undesirable effects favor the intervention or the comparison?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Favors the comparison ○ Probably favors the comparison ● Does not favor either the intervention or the comparison ○ Probably favors the intervention ○ Favors the intervention ○ Varies ○ Don't know	There is no conclusive evidence to suggest a single dose of Rh D immunoglobulin (1500 IU) given at 28 weeks gestation is superior or inferior to a two-dose regimen (500-625 IU) given at 28 and 34 weeks gestation in terms of efficacy or safety..	Improved compliance with a single dose of Rh D immunoglobulin is probably balanced by improved persistence of anti-D levels observed with two doses.

Resources required

How large are the resource requirements (costs)?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Large costs ○ Moderate costs ○ Negligible costs and savings ○ Moderate savings ○ Large savings ○ Varies ● Don't know	Detailed cost analysis to be conducted. There are likely to be increased costs (and delay) associated with production of new formulation of Rh D immunoglobulin (1500 IU) (estimated 13% increased with targeted antenatal prophylaxis). There are also potential reduced costs associated with resources required with a single dose such as allied health, storage, transport, etc. Impact on supply: An additional 250 IU of Rh D Ig required per Rh D negative pregnancy per year (estimated to be 45,000 with universal prophylaxis or 30,000 with targeted prophylaxis).	Currently 8000 vials of 625 IU are issued per month (96 000 vials per year).

Certainty of evidence of required resources

What is the certainty of the evidence of resource requirements (costs)?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Very low ○ Low ○ Moderate ○ High ● No included studies		

Cost effectiveness

Does the cost-effectiveness of the intervention favor the intervention or the comparison?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Favors the comparison ○ Probably favors the comparison ● Does not favor either the intervention or the comparison ○ Probably favors the intervention ○ Favors the intervention ○ Varies ○ No included studies		

Equity

What would be the impact on health equity?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Reduced ○ Probably reduced ○ Probably no impact ○ Probably increased ○ Increased ○ Varies ● Don't know	A single dose is associated with improved compliance (6), therefore better adherence to the protocol may be observed in regional and remote areas and in women who do not seek or cannot access regular antenatal care. Follow-up for other maternity needs may be adversely affected.	A two-dose regimen may offer other benefits over a single-dose regimen, with the need for a second dose at 34 weeks' gestation offering increased incentive to attend antenatal appointments later in pregnancy

Acceptability

Is the intervention acceptable to key stakeholders?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ No ○ Probably no ● Probably yes ○ Yes ○ Varies ○ Don't know	A single dose regimen is more convenient for both patients and service delivery than two doses. This is because a single dose reduces the burden of an additional IM injection at 34 weeks gestation, which is expected to be acceptable to key stakeholders (GPs, specialist, midwives, and Rh D negative women). The cost-effectiveness of a single dose is unknown and true population compliance of a single dose is uncertain. A single dose of 1500 IU is an increase in total Rh D immunoglobulin usage relative to the	

	current recommended 625 IU x2 and there are concerns regarding Rh D immunoglobulin levels in maternal circulation as gestation advances in the late third trimester.	

Feasibility

Is the intervention feasible to implement?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ No ○ Probably no ● Probably yes ○ Yes ○ Varies ○ Don't know	The logistics of implementing a higher single dose (1500 IU) of Rh D immunoglobulin would require the supplier to manufacture and license a new product (to provide an acceptable volume to be injected). Additional supplies of plasma would be needed in setting up and testing the manufacturing processes, including stability and storage studies to meet the Australian Therapeutic Goods Administration (TGA) requirements. It is estimated it would be a number of years before a locally produced 1500 IU Rh D IgG product would be available. The Blood Service has provided the required amount of Rh(D)Ig G to service Australia's RAADP programme since 2006. This is facilitated through ongoing discussion with the supplier and donors. Local product is favoured due to the extreme rigor that has been employed by the Blood Service in the Rh D immunisation and boosting program and its exemplary safety record.	Sourcing a 1500 IU dose from overseas goes against the principles of voluntary donors and self-sufficiency which are included in the National Blood Agreement. Product from overseas may be less acceptable to key stakeholders and poses the risk associated with dependence on an overseas manufacturer. If targeted antenatal prophylaxis is introduced, this would reduce the pressure on plasma supply; thus, allowing plasma to become available for the necessary manufacture and testing.

SUMMARY OF JUDGEMENTS

	JUDGEMENT						
PROBLEM	No	Probably no	Probably yes	**Yes**		Varies	Don't know
DESIRABLE EFFECTS	Trivial	Small	**Moderate**	Large		Varies	Don't know
UNDESIRABLE EFFECTS	Large	**Moderate**	Small	Trivial		Varies	Don't know
CERTAINTY OF EVIDENCE	**Very low**	Low	Moderate	High			No included studies
VALUES	Important uncertainty or variability	Possibly important uncertainty or variability	Probably no important uncertainty or variability	**No important uncertainty or variability**			

	JUDGEMENT						
BALANCE OF EFFECTS	Favors the comparison	Probably favors the comparison	**Does not favor either the intervention or the comparison**	Probably favors the intervention	Favors the intervention	Varies	Don't know
RESOURCES REQUIRED	Large costs	Moderate costs	Negligible costs and savings	Moderate savings	Large savings	Varies	**Don't know**
CERTAINTY OF EVIDENCE OF REQUIRED RESOURCES	Very low	Low	Moderate	High			**No included studies**
COST EFFECTIVENESS	Favors the comparison	Probably favors the comparison	**Does not favor either the intervention or the comparison**	Probably favors the intervention	Favors the intervention	Varies	No included studies
EQUITY	Reduced	Probably reduced	Probably no impact	**Probably increased**	Increased	Varies	Don't know
ACCEPTABILITY	No	Probably no	**Probably yes**	Yes		Varies	Don't know
FEASIBILITY	No	Probably no	**Probably yes**	Yes		Varies	Don't know

TYPE OF RECOMMENDATION

Strong recommendation against the option	Conditional recommendation against the option	Conditional recommendation for either the option or the comparison	**Conditional recommendation for the option**	Strong recommendation for the option
○	○	○	●	○

CONCLUSIONS

Recommendation

The ERG **recommends** that administration of Rh D immunoglobulin 625 IU at 28 and 34 weeks of pregnancy[a] continue in Rh D negative pregnant women with no preformed anti-D antibodies unless NIPT for fetal *RHD*[b] has predicted that they are not carrying an Rh D positive fetus. The ERG does not currently suggest changing to a single dose of Rh D immunoglobulin 1500 IU. (*Weak recommendation, low to very low certainty of evidence about the size of effect*).

[a] A woman's pregnancy care schedule and clinical discretion may warrant the administration of Rh D immunoglobulin within 2 weeks before or after the recommended 28 and 34 weeks of pregnancy. However, if the second dose of Rh D immunoglobulin is given before 34 weeks and the pregnancy goes beyond the due date, the risk of inadequate anti-D coverage at birth increases.

[b] All women have an ABO/Rh D type and antibody screen performed early in pregnancy. Women who are Rh D negative should be retested at 28 weeks unless NIPT for fetal *RHD* has predicted that they are not carrying an Rh D positive fetus. The specimen should be collected before giving prophylactic Rh D immunoglobulin; however, the immunoglobulin can be given before the results are available (see RANZCOG Guideline on Routine antenatal assessment in the absence of pregnancy complications (C-Obs 3b).

Related recommendation(s)

1. Should universal RAADP (1 or 2 doses) vs. placebo or no universal RAADP be used in Rh D negative pregnant women with no preformed anti-D?

The ERG **recommends** access to antenatal Rh D immunoglobulin for the prevention of Rh D alloimmunisation in Rh D negative pregnant women with no preformed anti-D antibodies. (*Strong recommendation, low to very low certainty of evidence about the size of effect*)

2. Should targeted RAADP (based on noninvasive prenatal screening) vs. universal RAADP be used in Rh D negative pregnant women with no preformed anti-D?

The ERG **recommends** that antenatal Rh D immunoprophylaxis in Rh D negative pregnant women with no preformed anti-D antibodies be targeted to those predicted to be carrying an Rh D positive fetus, based on NIPT for fetal *RHD*. This applies to both routine and sensitising event immunoprophylaxis, if the result of fetal *RHD* genotyping is available (see EOP3 and EOP7). (*Strong recommendation, low certainty of evidence about the size of the effect*)

If fetal Rh D status is not available or is uncertain, the ERG **recommends** that antenatal Rh D immunoprophylaxis be offered to Rh D negative pregnant women with no preformed anti-D antibodies. (*Strong recommendation, low certainty of evidence about the size of the effect*)

Justification

Overall justification

It is uncertain whether a single dose of Rh D immunoglobulin is as effective as current recommended 625 IU Rh D immunoglobulin at 28 and 34 weeks. There are possible benefits to patients in terms of compliance and acceptability of a single dose.

There are concerns that a single dose administered at 28 weeks has inadequate duration of coverage, with evidence of undetectable anti-D levels at delivery in a higher proportion of women treated with a single dose compared with women who received two doses. However, the clinical significance on the incidence of Rh D alloimmunisation is uncertain and likely to be small.

Detailed justification

Certainty of evidence

There are no sufficient size studies to suggest a single dose of Rh D immunoglobulin (1500 IU) given at 28 weeks gestation is superior or inferior to a two-dose regimen (500-625 IU) given at 28 and 34 weeks gestation in terms of efficacy or safety.

Acceptability
A single injection at 28 weeks may reduce the burden on key stakeholders, by removing the need for a second injection at 34 weeks.

Subgroup considerations

No subgroups considered.

Implementation considerations

No changes to recommended dosing of Rh D immunoglobulin made therefore there are no implementation issues.

Monitoring and evaluation

A reporting system similar to The Serious Hazards of Transfusion (SHOT) UK-wide system should be implemented. It should include errors and adverse events relating to Rh D immunoglobulin adminstration and episodes of Rh D alloimmunisation despite immunoprophylaxis.

Research priorities

What are the consequences (if any) of moving to a 1500 IU single dose Rh D immunoglobulin regimen in terms of safety, efficacy, cost-effectiveness, and patient acceptability?

What is the correlation between low serum Rh D IgG levels in the late third trimester and incidence of Rh D alloimmunisation?

REFERENCES SUMMARY

1. Urbaniak SJ., . The scientific basis of antenatal prophylaxis. BJOG; 1998.
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APPENDICES

Appendix 1

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)
	Risk with RAADP (two-dose)	Risk with RAADP (single dose)			
Incidence of Rh D alloimmunisation - not reported	No evidence found.		-	-	-
Incidence of a positive test for feto-maternal haemorrhage - not reported			-	-	-[a,b]
Adverse neonatal events - not reported	-	-	-	-	-
Adverse maternal events - not reported	-	-	-	-	-
Serum anti-D levels at birth	Complete data not available (reported as abstract only). The proportion of patients with undetectable anti-D was 45.2% vs 14.2%; OR 5.0; 95% CI NR; p<0.001. Favouring the two-dose regimen		-	(1 RCT)	⨁◯◯◯ VERY LOW[a,b]
Incidence of Rh D alloimmunisation (1 dose, any timepoint)	Study population		**RR 0.31** (0.12 to 0.80)	36555 (4 observational studies)	⨁◯◯◯ VERY LOW[c,d,e,f,g,h]
	12 per 1,000	**4 per 1,000** (1 to 9)			
	Study population				

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)
	Risk with RAADP (two-dose)	Risk with RAADP (single dose)			
Incidence of Rh D alloimmunisation (2 dose, any timepoint)	10 per 1,000	**3 per 1,000** (2 to 5)	**RR 0.32** (0.20 to 0.51)	15264 (6 observational studies)[i]	⨁◯◯◯ VERY LOW[c,d,e,f,h,j,k]
Incidence of Rh D alloimmunisation (1 dose, estimated)	In a meta-regression model, Turner 2012 estimated an OR of 0.42 (95%CI 0.17, 0.73) for a single dose based on the relative effectiveness observed in published studies adjusted for bias and expert opinion. Using only studies relevant to the UK health system Pilgrim 2009 estimated the risk of sensitisation using a single dose to be 0.34% (0.28, 0.40). [1,2]		-	(10 observational studies)	⨁⨁◯◯ LOW[b,c,d,e,f,h,l]
Incidence of Rh D alloimmunisation (2 doses, estimated)	In a meta-regression model, Turner 2012 estimated an OR of 0.31 (95%CI 0.09, 0.65) for two-doses of RAADP based on the relative effectiveness observed in published studies adjusted for bias and expert opinion. Using only studies relevant to the UK health system, Pilgrim 2009 estimated the risk of sensitisation using two-doses to be 0.30% (95% CI 0.22, 0.38). [1,2]		-	(10 observational studies)	⨁⨁◯◯ LOW[b,c,d,e,f,h,l]

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2. Pilgrim, H., Lloyd-Jones, M., Rees, A. Routine antenatal anti-D prophylaxis for RhD-negative women: a systematic review and economic evaluation. Health Technology Assessment; 2009.

a. Study is reported in a conference abstract and it is difficult to judge internal bias. Not all outcomes reported.
b. One study only. Heterogeneity not assessed.
c. One or more randomised studies with plausible bias that raises some doubts about the results
d. Missing data and exclusion of patients may over-estimate the clinical effectiveness of RAADP
e. One or more comparative observational studies with some important problems that seriously weaken the confidence in the results
f. Studies include historical and/or geographic controls and it is not clear if intervention and control groups are comparable at baseline.
g. Significant heterogeneity with substantial variability in effect estimates (I2 statistic greater than 50%). Reduces confidence in the results to inform decision making.
h. Obstetric practice and the baseline characteristics of the population may not be reflective of current practice however this was considered to not seriously alter the confidence in the effect.
i. Includes 1 RCT and 1 quasi-RCT
j. No heterogeneity (I2 statistic = 0%). Does not reduce confidence in results to inform decision making.
k. Low event rate and/or wide confidence intervals that cross the line of no effect. Confidence in the results is weak.
l. Authors elicited expert opinion to estimate association between the relative and observed effectiveness for different dosing regimens

F3 Question 2

Should sensitising event prophylaxis vs. placebo or no sensitising event prophylaxis be used for Rh D negative women with no preformed anti-D with a first-trimester sensitising event?	
PROBLEM:	Current guidance for sensitising event prophylaxis in the first trimester includes: miscarriage, TOP, ectopic pregnancy, CV sampling however the evidence underpinning these recommendations is limited. Questions remain regarding the administration of Rh D immunoglobulin after the following sensitising events: abdominal trauma, molar pregnancy, ectopic pregnancy, spontaneous miscarriage, threatened miscarriage or medical TOP (with/without a curette).
OPTION:	sensitising event prophylaxis
COMPARISON:	placebo or no sensitising event prophylaxis
MAIN OUTCOMES:	Incidence of Rh D alloimmunisation (4-6 months after spontaneous miscarriage and/or therapeutic evacuation); Incidence of Rh D alloimmunisation (4-6 months after incomplete miscarriage or medical termination of pregnancy); Incidence of Rh D alloimmunisation (at subsequent pregnancy after spontaneous miscarriage and/or therapeutic evacuation); Incidence of Rh D alloimmunisation (at subsequent pregnancy after induced medical termination of pregnancy); Incidence of Rh D alloimmunisation (after abdominal trauma, molar pregnancy, ectopic pregnancy); Incidence of a positive test for feto-maternal haemorrhage; Adverse neonatal events (e.g. jaundice); Adverse maternal events attributed to anti-D;
SETTING:	Obstetrics and maternity, primary setting.
PERSPECTIVE:	National healthcare perspective
BACKGROUND:	The National Health and Medical Research Council's (NHMRC) *1999 Guidelines on the Prophylactic Use of Rh D Immunoglobulin (Anti-D) in Obstetrics* were updated by the National Blood Authority (NBA) in 2003, with the aim of updating the guidance on antenatal prophylaxis. The updated 2003 Anti-D Guidelines aimed to inform clinicians, other health professionals, and policy makers of new recommendations for the staged implementation of full antenatal prophylaxis in Australia. The 2003 Anti-D Guidelines also included policy intent as a national program to address the issue of sufficiency of supply of product and were intended to be reviewed within five years, according to the availability of Rh D immunoglobulin. To ensure the Anti-D Guidelines continue to reflect current evidence and best clinical practice, the NBA undertook a scoping exercise in September 2016 to identify the extent of newly available evidence and areas of concerns (Health Research Consulting, 2017). Through a collaborative partnership with the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) and other relevant stakeholders, it was agreed that a new evidence-based guideline would be developed. One key area of concern identified in the scoping report included: *"Should the list of first-trimester sensitising events be amended to include additional events?"*
CONFLICT OF INTERESTS:	

ASSESSMENT

Problem

Is the problem a priority?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ No ○ Probably no ○ Probably yes ● Yes ○ Varies ○ Don't know	Rh D negative women who are exposed to Rh D positive fetal red cells in the antenatal period and those who deliver a Rh D positive baby are at risk of developing anti-D antibodies. These antibodies can cross the placenta and cause fetal red cell destruction. This can result in fetal and neonatal anaemia and neonatal jaundice; in severe cases, these can cause serious morbidity or mortality. In the first trimester (< 12 weeks gestation), even in the absence of traumatic manoeuvres to the uterus, the reported risk of alloimmunisation following medical termination of pregnancy or blood loss is 1.5-2% (1) .	

Desirable Effects

How substantial are the desirable anticipated effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Trivial ○ Small ● Moderate ○ Large ○ Varies ○ Don't know	For research evidence on Desirable and Undesirable anticipated effects, as well as the certainty of this evidence, see the GRADE Evidence Profile.	International practice guidelines were reviewed for indications and dosing of Rh D immunoprophylaxis after potentially sensitising events in the first trimester.

Undesirable Effects

How substantial are the undesirable anticipated effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Large ○ Moderate ● Small ○ Trivial ○ Varies ○ Don't know	For research evidence on Desirable and Undesirable anticipated effects, as well as the certainty of this evidence, see the GRADE Evidence Profile.	

Certainty of evidence

What is the overall certainty of the evidence of effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
● Very low ○ Low ○ Moderate ○ High ○ No included studies	The evidence is very uncertain about the effect of sensitising event prophylaxis compared with placebo or no sensitising event prophylaxis on the incidence of Rh D alloimmunisation after spontaneous miscarriage, incomplete miscarriage, therapeutic evacuation, or medical termination of pregnancy in Rh D negative women. The effectiveness of sensitising event prophylaxis compared with placebo or no sensitising event prophylaxis on the incidence of Rh D alloimmunisation after abdominal trauma, molar pregnancy, or ectopic pregnancy is not known. *See Appendix 1*	

Values

Is there important uncertainty about or variability in how much people value the main outcomes?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Important uncertainty or variability ○ Possibly important uncertainty or variability ○ Probably no important uncertainty or variability ● No important uncertainty or variability		

Balance of effects

Does the balance between desirable and undesirable effects favor the intervention or the comparison?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Favors the comparison ○ Probably favors the comparison ○ Does not favor either the intervention or the comparison ○ Probably favors the intervention ● Favors the intervention ○ Varies ○ Don't know		

Resources required

How large are the resource requirements (costs)?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Large costs ○ Moderate costs ● Negligible costs and savings ○ Moderate savings ○ Large savings ○ Varies ○ Don't know	Recommendations about sensitising event prophylaxis are unchanged. No changes are resource costs ae expected.	

Certainty of evidence of required resources

What is the certainty of the evidence of resource requirements (costs)?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Very low ○ Low ○ Moderate ○ High ● No included studies		

Cost effectiveness

Does the cost-effectiveness of the intervention favor the intervention or the comparison?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Favors the comparison ○ Probably favors the comparison ○ Does not favor either the intervention or the comparison ○ Probably favors the intervention ○ Favors the intervention ○ Varies ● No included studies		

Equity

What would be the impact on health equity?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Reduced ○ Probably reduced ● Probably no impact ○ Probably increased ○ Increased ○ Varies ○ Don't know	Recommendations about sensitising event prophylaxis are unchanged. No impact on health equity are expected.	

Acceptability

Is the intervention acceptable to key stakeholders?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ No ○ Probably no ○ Probably yes ● Yes ○ Varies ○ Don't know	Sensitising event prophylaxis is considered acceptable to key stakeholders (pregnant women, caregivers, Blood Service, donors, etc.).	

Feasibility Is the intervention feasible to implement?		
JUDGEMENT	**RESEARCH EVIDENCE**	**ADDITIONAL CONSIDERATIONS**
○ No ○ Probably no ○ Probably yes ● Yes ○ Varies ○ Don't know	Sensitising event prophylaxis is feasible to implement.	

SUMMARY OF JUDGEMENTS

	JUDGEMENT						
PROBLEM	No	Probably no	Probably yes	**Yes**		Varies	Don't know
DESIRABLE EFFECTS	Trivial	Small	**Moderate**	Large		Varies	Don't know
UNDESIRABLE EFFECTS	Large	Moderate	**Small**	Trivial		Varies	Don't know
CERTAINTY OF EVIDENCE	**Very low**	Low	Moderate	High			No included studies
VALUES	Important uncertainty or variability	Possibly important uncertainty or variability	Probably no important uncertainty or variability	**No important uncertainty or variability**			
BALANCE OF EFFECTS	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	Probably favors the intervention	**Favors the intervention**	Varies	Don't know
RESOURCES REQUIRED	Large costs	Moderate costs	**Negligible costs and savings**	Moderate savings	Large savings	Varies	Don't know
CERTAINTY OF EVIDENCE OF REQUIRED RESOURCES	Very low	Low	Moderate	High			**No included studies**

	JUDGEMENT						
COST EFFECTIVENESS	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	Probably favors the intervention	Favors the intervention	Varies	**No included studies**
EQUITY	Reduced	Probably reduced	**Probably no impact**	Probably increased	Increased	Varies	Don't know
ACCEPTABILITY	No	Probably no	Probably yes	**Yes**		Varies	Don't know
FEASIBILITY	No	Probably no	Probably yes	**Yes**		Varies	Don't know

TYPE OF RECOMMENDATION

Strong recommendation against the option	Conditional recommendation against the option	Conditional recommendation for either the option or the comparison	Conditional recommendation for the option	**Strong recommendation for the option**
○	○	○	○	●

CONCLUSIONS

Recommendation

After the following sensitising events in the first 12 weeks of singleton or multiple pregnancy: miscarriage, termination of pregnancy (medical after 10 weeks gestation or surgical), ectopic pregnancy, molar pregnancy and chorionic villus sampling, the ERG **recommends** that a dose of Rh D immunoglobulin 250 IU be given to all Rh D negative women with no preformed anti-D antibodies to prevent RhD alloimmunisation. (*Strong recommendation, very low certainty of evidence about the size of effect*)

In Rh D negative women with an ongoing pregnancy who have uterine bleeding in the first 12 weeks of pregnancy, there is insufficient evidence to support the routine use of Rh D immunoglobulin. However, where the bleeding is repeated, heavy or associated with abdominal pain or significant pelvic trauma, immunoprophylaxis may be administered to women with no preformed antibodies. (*Qualified (weak) recommendation, expert consensus*)

In the setting of medical termination of pregnancy before 10 weeks of gestation, there is insufficient evidence to suggest the routine use of Rh D immunoglobulin (*Discretionary (weak) recommendation, expert consensus*) (see NICE Guidelines on abortion care (NG140); and Foregoing Rh testing and anti-D immunoglobulin for women presenting for early abortion: a recommendation from the National Abortion Federation's Clinical Policies Committee)

Expert opinion point:

At all times when Rh D immunoglobulin is being administered for a sensitising event, it should be given as soon as practical within 72 hours. If delayed beyond 72 hours, the dose should be given up to 10 days from the sensitising event, but may have lower efficacy.

Justification

The available evidence does not justify changes to the 2003 Guidelines. The expert group has clarified the wording around threatened miscarriage and has added guidance related to molar pregnancy. This guidance is consistent with International Guidelines.

Subgroup considerations

No subgroups considered.

Implementation considerations

None considered.

Monitoring and evaluation

Research priorities

What is the volume of fetal cells in maternal circulation after the following first trimester sensitising events – abdominal trauma, molar pregnancy, ectopic pregnancy, spontaneous miscarriage, threatened miscarriage or medical termination of pregnancy (with/without a curette)?

What volume of fetal cells in maternal circulation increases the risk of Rh D alloimmunisation?

REFERENCES SUMMARY

1. Committee on Practice Bulletins-Obstetrics. Practice Bulletin No. 181: Prevention of Rh D Alloimmunization. Obstet Gynecol; 2017.

APPENDICES

Appendix 1

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo or no sensitising event prophylaxis	Risk with sensitising event prophylaxis				
Incidence of Rh D alloimmunisation (4-6 months after spontaneous miscarriage and/or therapeutic evacuation) assessed with: Enzyme-Coombs screening	Study population		not estimable	48 (1 RCT)[1]	⨁◯◯◯ VERY LOW[a,b,c,d,e,f,g]	The evidence is very uncertain about the effect of sensitising event prophylaxis compared with placebo or no sensitising event prophylaxis on the incidence of Rh D alloimmunisation 4-6 months after spontenous miscarriage or therapeutic evacuation in Rh D negative women.
	0 per 1,000	**0 per 1,000** (0 to 0)				
Incidence of Rh D alloimmunisation (4-6 months after incomplete miscarriage or medical termination of pregnancy) assessed with: Indirect Coombs	Study population		**RR 0.34** (0.02 to 6.69)	57 (1 observational study)[2]	⨁◯◯◯ VERY LOW[a,c,d,h,i,j,k]	The evidence is very uncertain about the effect of sensitising event prophylaxis compared with placebo or no sensitising event prophylaxis on the incidence of Rh D alloimmunisation 4-6 months after after incomplete miscarriage or medical termination of pregnancy in Rh D negative women.
	56 per 1,000	**19 per 1,000** (1 to 372)				
Incidence of Rh D alloimmunisation (at subsequent pregnancy after spontaneous miscarriage and/or therapeutic evacuation) assessed with: Enzyme-Coombs screening	Study population		not estimable	9 (1 RCT)[1]	⨁◯◯◯ VERY LOW[a,b,c,d,e,f,g]	The evidence is very uncertain about the effect of sensitising event prophylaxis compared with placebo or no sensitising event prophylaxis on the incidence of Rh D alloimmunisation in a subsequent pregnancy after spontaneous miscarriage or therapeutic evacuation in Rh D negative women.
	0 per 1,000	**0 per 1,000** (0 to 0)				
Incidence of Rh D alloimmunisation (at subsequent pregnancy after medical termination of pregnancy) assessed with: Papain-treated cells or Indirect-coombs	Study population		**RR 0.76** (0.07 to 8.21)	241 (1 observational study)[3]	⨁◯◯◯ VERY LOW[c,d,h,i,k,l,m]	The evidence is very uncertain about the effect of sensitising event prophylaxis compared with placebo or no sensitising event prophylaxis on the incidence of Rh D alloimmunisation in a subsequent pregnancy after induced abortion in Rh D negative pregnant women
	14 per 1,000	**10 per 1,000** (1 to 113)				
Incidence of Rh D alloimmunisation (after abdominal trauma, molar pregnancy, ectopic pregnancy) - not reported	No comparative evidence found [4]		-	-	-	The effect of sensitising event prophylaxis compared with placebo or no sensitising event prophylaxis on the incidence of Rh D alloimmunisation after

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo or no sensitising event prophylaxis	Risk with sensitising event prophylaxis				
						abdominal trauma, molar pregnancy, or ectopic pregnancy in Rh D negative women is unknown.
Incidence of a positive test for feto-maternal haemorrhage - not reported	No comparative evidence found [5]		-	-	-	The effect of sensitising event prophylaxis compared with placebo or no sensitising event prophylaxis on the incidence of a positive test for feto-maternal haemorrhage after abdominal trauma, molar pregnancy, or ectopic pregnancy in Rh D negative women is unknown.
Adverse neonatal events (e.g. jaundice) - not reported	No comparative evidence found [5]		-	-	-	
Adverse maternal events attributed to anti-D - not reported	No comparative evidence found [5]		-	-	-	

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a. The evidence is not directly applicable to the target population or the Australian healthcare context and it is difficult to judge if it could be sensibly applied. Obstetric practice and the baseline characteristics of the population may not be reflective of current practice.
b. The study was conducted in the United States among RhD negative women with complete miscarriage (n=9) or incomplete miscarriage with curettage (n=48). An unknown proportion of women had miscarriage outside the first trimester (after GW 12) and the intervention was administered at a dose higher than recommended in Australia (1500 IU vs 625 IU).
c. Single study. Publication bias likely.
d. Single study. Heterogeneity not assessed.
e. Small study not sufficiently powered to detect a statistically significant difference.
f. One randomised study with plausible bias that raises serious doubts about the results.
g. Method of randomisation not reported and unclear if treatment allocation concealed. Some concerns with reporting bias and missing data.
h. Comparative study with some important problems that seriously weakens the confidence in the results.
i. Method of treatment allocation or blinding not reported. Some concerns with reporting bias and missing data.

j. The study was conducted in the United States among Rh D negative women who had medical termination of pregnancy (n=33) or were treated for incomplete miscarriage (n=24). Thirteen (22.8%) women were treated outside the first trimester (GW >13) and the dose of Rhogam was not stated.
k. Low event rate and/or wide confidence intervals that cross the line of no effect. Confidence in the results is weak.
l. The evidence is probably applicable to the Australian population and healthcare context with some caveats.
m. The study was conducted in Hungary among Rh D negative women in their second pregnancy whose first pregnancy was terminated in the first trimester by termination of pregnancy (method of termination not clear). The intervention was administered at the same dose as recommended in Australia (250 IU).

F4 Question 3a

Should targeted RAADP (based on noninvasive prenatal screening) vs. universal RAADP be used for Rh D negative pregnant women with no preformed anti-D?	
PROBLEM:	Maintenance of supply of RhD immunoglobulin is a global issue. No alternatives to the blood product (Rh D immunoglobulin) are currently available or anticipated within the near future. Boosting donors to maintain the supply of Rh D immunoglobulin poses potential clinical risks (with ethical concerns) and places a considerable burden on those donors.
OPTION:	targeted RAADP (based on noninvasive prenatal screening)
COMPARISON:	universal RAADP
MAIN OUTCOMES:	Incidence of Rh D alloimmunisation; Utilisation of anti-D; Incidence of a positive test for feto-maternal haemorrhage; Adverse neonatal events; Adverse maternal events attributed to anti-D (e.g. allergic response, infection);
SETTING:	Obstetrics and maternity, primary care
PERSPECTIVE:	National health care perspective
BACKGROUND:	The National Health and Medical Research Council's (NHMRC) *1999 Guidelines on the Prophylactic Use of Rh D Immunoglobulin (Anti-D) in Obstetrics* were updated by the National Blood Authority (NBA) in 2003, with the aim of updating the guidance on antenatal prophylaxis. The updated 2003 Anti-D Guidelines aimed to inform clinicians, other health professionals, and policy makers of new recommendations for the staged implementation of full antenatal prophylaxis in Australia. The 2003 Anti-D Guidelines also included policy intent as a national program to address the issue of sufficiency of supply of product and were intended to be reviewed within five years, according to the availability of Rh D immunoglobulin. To ensure the Anti-D Guidelines continue to reflect current evidence and best clinical practice, the NBA undertook a scoping exercise in September 2016 to identify the extent of newly available evidence and areas of concerns (Health Research Consulting, 2017). Through a collaborative partnership with the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) and other relevant stakeholders, it was agreed that a new evidence-based guideline would be developed. A key area of concern identified in the scoping report included *"To reduce unnecessary use of Rh D IgG, should noninvasive prenatal screening be used in the first trimester so that prophylaxis can be targeted?"*
CONFLICT OF INTERESTS:	

ASSESSMENT

Problem

Is the problem a priority?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ No ○ Probably no ○ Probably yes ● Yes ○ Varies ○ Don't know	Rh D immunoglobulin is produced from pooled plasma that is sourced and donated by Rh D negative people who have had been stimulated to produce D antibodies. It is a finite resource and needs to be used carefully maintained. No alternatives to the blood product (Rh D immunoglobulin) are currently available or anticipated within the near future. Boosting donors to maintain the supply of Rh D immunoglobulin poses potential clinical risks and places a considerable burden on those donors. Current protocols recommend antenatal immunoprophylaxis for all Rh D negative women, whereas this could be avoided in about one-third of women who do not delivery an Rh D positive child. This mean unnecessary exposure to blood product to around 5% of pregnant women (about 15,000 women per year), which raises important ethical issues (1, 2).	

Desirable Effects

How substantial are the desirable anticipated effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Trivial ● Small ○ Moderate ○ Large ○ Varies ○ Don't know	For research evidence on Desirable and Undesirable anticipated effects, as well as the certainty of this evidence, see the GRADE Evidence Profile.	The key desirable effect is reduced utilisation of Rh D immunoglobulin and less exposure to unnecessary blood product.

Undesirable Effects

How substantial are the undesirable anticipated effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
● Large ○ Moderate ○ Small ○ Trivial ○ Varies ○ Don't know	For research evidence on Desirable and Undesirable anticipated effects, as well as the certainty of this evidence, see the GRADE Evidence Profile.	The key undesirable effect is theoretical increased risk of Rh D alloimmunisation due to false negative test results incorrectly classifying some women as carrying an Rh D negative fetus.

Certainty of evidence

What is the overall certainty of the evidence of effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Very low ○ Low ○ Moderate ○ High ○ No included studies	*See Appendix 1*	

Values

Is there important uncertainty about or variability in how much people value the main outcomes?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Important uncertainty or variability ○ Possibly important uncertainty or variability ● Probably no important uncertainty or variability ○ No important uncertainty or variability	No uncertainty about the main outcomes (incidence of Rh D sensitisation, utilisation of Rh D immunoglobulin).	

Balance of effects

Does the balance between desirable and undesirable effects favor the intervention or the comparison?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Favors the comparison ○ Probably favors the comparison ○ Does not favor either the intervention or the comparison ○ Probably favors the intervention ● Favors the intervention ○ Varies ○ Don't know	Certain reduction in unnecessary use of Rh D immunoglobulin and reduced exposure to Rh D immunoglobulin balanced against a theoretical increased risk of Rh D alloimmunisation (no available evidence)	

Resources required

How large are the resource requirements (costs)?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Large costs ○ Moderate costs ○ Negligible costs and savings ○ Moderate savings ○ Large savings ○ Varies ● Don't know	One Australian study estimated net cost savings of AUD$159,701 per year for total health care costs (3). The model assumed cord blood testing was conducted only in those women whose fetus tested *RHD* negative. The study incorporated the following costs: test cost of $45, unit costs of Rh D immunoglobulin of $59 to $88, and packaging/transport costs of samples to be $15 to $40. The mean overall cost per pregnancy was estimated to be $7495 with universal prophylaxis compared with $7471 with targeted prophylaxis.	

Certainty of evidence of required resources

What is the certainty of the evidence of resource requirements (costs)?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Very low ○ Low ○ Moderate ○ High ● No included studies	Included costs are estimated. The Panel discussed higher costs than described in the Australian study (3), all of which are key drivers of the economic model. Cost of high-throughput NIPT: $55 per test Cost of Rh D IgG for routine/sensitizing event: unknown Cost/burden of donor boosting: $ unknown Cost production of Rh D IgG: $74 The Panel assumed transport costs would not substantially change as supply of Rh D immunoglobulin is bundled with other blood products.	

Cost effectiveness

Does the cost-effectiveness of the intervention favor the intervention or the comparison?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Favors the comparison ○ Probably favors the comparison ○ Does not favor either the intervention or the comparison ○ Probably favors the intervention ○ Favors the intervention ○ Varies ● No included studies	In the United Kingdom, target Rh D immunoprophylaxis is estimated to have a reduced cost (base case) of 485,000 pounds, but QALYs (quality of life years) were reduced by 0.5 per 100,000 pregnancies (4, 5). Other cost-effective studies varied in their assessment: Not cost saving: US (6) Uncertain: France (7) Cost-saving: Canada (8), Sweden (9).	

Equity

What would be the impact on health equity?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Reduced ○ Probably reduced ● Probably no impact ○ Probably increased ○ Increased ○ Varies ○ Don't know	The panel considered that the introduction of the test was probably not going to impact groups or settings that might be disadvantaged. Remoteness may increase the complexity of providing testing and ensuring results are available at relevant times during the pregnancy. The magnitude in change in practice should not dramatically improve or worsen current practice. The Panel assumes testing will not be at the cost of the patient, with funding paid via the Government.	

Acceptability

Is the intervention acceptable to key stakeholders?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ No ○ Probably no ○ Probably yes ● Yes ○ Varies ○ Don't know	Yes. It is expected that the intervention would be acceptable to key stakeholders. This is based on the assumption that there would be no additional episodes of phlebotomy (one additional blood test incorporated into current maternal care). Approximately one third of Rh D negative pregnant women would avoid unnecessary exposure to blood product. The NICE Adoption support resource (2017) (10) reports that NHS staff reported the following benefits of targeted antenatal prophylaxis (using NIPT to determine fetal RHD status): • preventing unnecessary administration of blood products (anti-D immunoglobulin) and their associated risk • avoiding unnecessary painful injections for women when the NIPT for fetal *RHD* genotype result is negative • reducing the number of antenatal anti-D prophylactic clinic appointments needed, and the amount of anti-D immunoglobulin used • increasing the availability of anti-D immunoglobulin for use after potentially sensitising events in pregnancy when the NIPT result for fetal *RHD* genotype is positive • reducing the anxiety associated with potentially sensitising events for D-negative women when the NIPT result for fetal *RHD* genotype is negative • providing information to allow D-negative women to make an informed decision about whether to have treatment with anti-D immunoglobulin.	There is a potential critical supply issue with an aging donor population providing less anti-D due to poorer immunological response. A reduced demand for antenatal Rh D immunoglobulin by around one-third would relieve some of the supply issue. It is assumed there will be less burden on donors for the need for primary immunisation or boosting (easing ethical concerns).

Feasibility Is the intervention feasible to implement?		
JUDGEMENT	**RESEARCH EVIDENCE**	**ADDITIONAL CONSIDERATIONS**
○ No ○ Probably no ○ Probably yes ○ Yes ○ Varies ○ Don't know	Targeted screening has been implemented in several countries and is being rolled out in the UK. The programme is feasible, but care will need to be taken as to timing of the screening, with current evidence predominantly in women at 24 to 28 weeks gestation.	It is likely the UK rollout will be a valuable resource for sharing learnings (see NICE website) for adoption of screening in various regional health centres.

SUMMARY OF JUDGEMENTS

	JUDGEMENT						
PROBLEM	No	Probably no	Probably yes	**Yes**		Varies	Don't know
DESIRABLE EFFECTS	Trivial	**Small**	Moderate	Large		Varies	Don't know
UNDESIRABLE EFFECTS	**Large**	Moderate	Small	Trivial		Varies	Don't know
CERTAINTY OF EVIDENCE	Very low	Low	Moderate	High			No included studies
VALUES	Important uncertainty or variability	Possibly important uncertainty or variability	**Probably no important uncertainty or variability**	No important uncertainty or variability			
BALANCE OF EFFECTS	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	Probably favors the intervention	**Favors the intervention**	Varies	Don't know
RESOURCES REQUIRED	Large costs	Moderate costs	Negligible costs and savings	Moderate savings	Large savings	Varies	**Don't know**
CERTAINTY OF EVIDENCE OF REQUIRED RESOURCES	Very low	Low	Moderate	High			**No included studies**

	JUDGEMENT						
COST EFFECTIVENESS	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	Probably favors the intervention	Favors the intervention	Varies	**No included studies**
EQUITY	Reduced	Probably reduced	**Probably no impact**	Probably increased	Increased	Varies	Don't know
ACCEPTABILITY	No	Probably no	Probably yes	**Yes**		Varies	Don't know
FEASIBILITY	No	Probably no	Probably yes	Yes		Varies	Don't know

TYPE OF RECOMMENDATION

Strong recommendation against the option	Conditional recommendation against the option	Conditional recommendation for either the option or the comparison	Conditional recommendation for the option	**Strong recommendation for the option**
○	○	○	○	●

CONCLUSIONS

Recommendation

The ERG **recommends** that antenatal Rh D immunoprophylaxis in Rh D negative pregnant women with no preformed anti-D antibodies be targeted to those predicted to be carrying an Rh D positive fetus, based on NIPT for fetal *RHD*. This applies to both routine and sensitising event immunoprophylaxis, if the result of fetal *RHD* genotyping is available (see EOP3 and EOP7) (*Strong recommendation, low certainty of evidence about the size of the effect*)

If fetal Rh D status is not available or is uncertain, the ERG **recommends** that antenatal Rh D immunoprophylaxis be offered to Rh D negative pregnant women with no preformed anti-D antibodies. (*Strong recommendation, low certainty of evidence about the size of the effect*)

The ERG currently **recommends** that postnatal Rh D immunoprophylaxis (Rh D immunoglobulin 625 IU) continue to be administered to all Rh D negative women with no preformed anti-D antibodies who have a baby who is predicted to be Rh D positive based on NIPT for fetal *RHD,* or cord blood or neonatal Rh D typing. The cord blood or neonatal testing should be performed regardless of the results NIPT for fetal *RHD*, but need not delay administration of Rh D immunoprophylaxis when the fetus has been shown to be *RHD* positive by NIPT testing. If the baby is Rh D positive, administer Rh D immunoglobulin even if the NIPT predicted an Rh D negative baby. (*Strong recommendation, high certainty of evidence)*

Related recommendation(s)

1. Should universal RAADP (1 or 2 doses) vs. placebo or no universal RAADP be used in Rh D negative pregnant women with no preformed anti-D?

The ERG **recommends** access to antenatal Rh D immunoglobulin for the prevention of Rh D alloimmunisation in Rh D negative pregnant women with no preformed anti-D antibodies (*strong recommendation, low to very low certainty of evidence about the size of effect*).

2. Should noninvasive prenatal testing be used to diagnose fetal Rh D status in Rh D negative pregnant women with no preformed anti-D (for routine or sensitising event prophylaxis)?

The ERG **recommends** the testing of maternal blood to determine fetal *RHD* genotype in all Rh D negative pregnant women to enable targeted antenatal Rh D immunoprophylaxis.[a] (*Strong recommendation, high certainty of evidence about the accuracy of the test*).

The ERG **recommends** test sensitivity be at least 99% in order to minimise the number of Rh D positive fetuses being missed by the test. (*Strong recommendation, high certainty of evidence about the accuracy of the test*).

The ERG **recommends** NIPT for fetal *RHD* from 11^{+0} weeks of pregnancy because of higher test accuracy than at earlier weeks. (*Strong recommendation, high certainty of evidence about the accuracy of the test*).

a The ERG's recommendation on the use of NIPT for fetal *RHD* is not a policy statement on funding and supply arrangements for the national provisions of NIPT for blood group genotyping to determine the Rh D status of the fetus.

Justification

Pregnant women and their clinicians highly value the avoidance of unnecessary blood product. Noninvasive prenatal testing offers the opportunity to avoid the unnecessary administration of Rh D immunoglobulin in nearly one-third of Rh D negative pregnant women.

Donors to the Rh D immunoglobulin programme bear an extremely heavy personal burden and significant risk so as to maintain a supply of Rh D immunoglobulin to meets Australia's needs. Noninvasive prenatal testing offers the opportunity to significantly reduce this burden.

Subgroup considerations

Evidence in women with multiple pregnancies is limited and caution should be taken in this population.

Inconclusive test results should lead to the administration of routine Rh D immunoprophylaxis.

Testing before 11 weeks' gestation is not recommended.

Implementation considerations

To implement this policy the following would be required:

* an additional blood test after the end of the first trimester in approximately 15% of pregnant women.

* an implementation programme to correctly target Rh D immunoprophylaxis in test positive women. (possibly similar to NICE tools and resources)

* To achieve the expected benefits, it would require funding for universal access and assumes cost of the test is subsidised through the government medical benefits scheme.

* Implementation of the test to be at least cost-neutral.

Monitoring and evaluation

Health care services should monitor targeted Rh D immunoprophylaxis to evaluate compliance, uptake and patient outcomes. This is to ensure appropriate administration of Rh D immunoglobulin.

Where possible, any proven false negative results should be reported through appropriate haemovigilance activities.

Research priorities

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10. National Institute of Health and Care Excellence, . Adoption support resource – insights from the NHS. High-throughput non-invasive prenatal testing for fetal RHD genotype.; 2017.

APPENDICES

Appendix 1

Outcomes	Impact	№ of participants (studies)	Certainty of the evidence (GRADE)
Incidence of Rh D alloimmunisation - not reported	No studies directly assessed effect of targeted routine antenatal or sensitising event prophylaxis on the incidence of RhD alloimmunisation. One study (Saramago 2018) conducted a simulation based on diagnostic accuracy of the test and expected management in patients with positive and negative test results. The report estimated targeted RAADP increased the risk of Rh D alloimmunisation from 281 per 100 000 pregnany women with universal RAADP to 284 (base case scenario) or 309 (worst case scenario) per 100 000 [1]	-	-
Utilisation of anti-D - not reported	No comparative studies directly assessed the effect of targeted routine antenatal or sensitising event prophylaxis on utilisation of anti-D. One study (Saramago 2018) conducted a simulation based on data from three non-comparative studies and estimated utilisation of anti-D would decrease by approximately 33.1% to 36.9%. [1,2,3,4]	-	-
Incidence of a positive test for feto-maternal haemorrhage - not reported	No studies directly assessed effect of targeted routine antenatal or sensitising event prophylaxis on the incidence of a positive test for feto-maternal haemorrhage.	-	-
Adverse neonatal events - not reported	No studies were identified that reported any data on adverse neonatal events relating to NIPT or antenatal anti-D administration.	-	-
Adverse maternal events attributed to anti-D (e.g. allergic response, infection) - not reported	No studies were identified that reported any data on adverse maternal events relating to NIPT or antenatal anti-D administration.	-	-

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F5 Question 3b

Should noninvasive prenatal testing be used to diagnose fetal Rh D status in Rh D negative pregnant women with no preformed anti-D (for routine or sensitising event prophylaxis)?	
POPULATION:	Rh D negative pregnant women with no preformed anti-D (for routine or sensitising event prophylaxis)
INTERVENTION:	noninvasive prenatal testing
PURPOSE OF THE TEST:	Noninvasive prenatal testing for fetal Rh D status is a test that predicts the Rh D genotype of a fetus from small fragments of fetal DNA that circulate in the maternal plasma.
ROLE OF THE TEST:	The aim of the test is to identify Rh D negative pregnant women who are carrying an Rh D positive fetus, to guide the decision to administer Rh D immunoglobulin to prevent sensitisation. Rh antibodies develop during pregnancy when an Rh negative woman carries an Rh positive fetus. It occurs when fetal red blood cells (RBCs) enter the maternal circulation and antibodies are produced towards the fetal Rh antigen. No apparent adverse health outcomes occur in the mother as a result of this sensitisation, but haemolytic disease of the fetus and newborn (HDFN) can arise in an Rh positive fetus (usually in subsequent pregnancies). HDFN occurs when maternal Rh antibodies cross the placenta into the baby's circulation and mediate destruction of the baby's RBCs. This destruction causes fetal anaemia (a shortage in RBCs, required to carry oxygen), and can lead to bilirubinemia (elevated levels of bilirubin, a waste product of the degraded RBCs) and jaundice (yellowing of the skin and whites of the eyes). In severe cases the HDFN causes kernicterus (a form of brain damage) or hydrops fetalis (gross oedema or accumulation of fluid leading to fetal death) (Bowman, 2003, McBain et al., 2015, Zwiers et al., 2018).
LINKED TREATMENTS:	Rh D immunoprophylaxis
ANTICIPATED OUTCOMES:	Universal Rh D immunoprophylaxis of Rh D negative pregnant women with no preformed anti-D antibodies is currently recommended to reduce the risk of Rh D sensitisation in the antenatal period. A significant proportion of women who receive Rh D immunoglobulin do not require it because they are carrying an Rh D negative child. Testing for fetal Rh D status would reduce the number of women who unnecessarily receive Rh D immunoglobulin.
SETTING:	Obstetrics and maternity, primary setting
PERSPECTIVE:	National health care perspective
BACKGROUND:	The National Health and Medical Research Council's (NHMRC) *1999 Guidelines on the Prophylactic Use of Rh D Immunoglobulin (Anti-D) in Obstetrics* were updated by the National Blood Authority (NBA) in 2003, with the aim of updating the guidance on antenatal prophylaxis. The updated 2003 Anti-D Guidelines aimed to inform clinicians, other health professionals, and policy makers of new recommendations for the staged implementation of full antenatal prophylaxis in Australia. The 2003 Anti-D Guidelines also included policy intent as a national program to address the issue of sufficiency of supply of product and were intended to be reviewed within five years, according to the availability of Rh D immunoglobulin. To ensure the Anti-D Guidelines continue to reflect current evidence and best clinical practice, the NBA undertook a scoping exercise in September 2016 to identify the extent of newly available evidence and areas of concerns (Health Research Consulting, 2017). Through a collaborative partnership with the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) and other relevant stakeholders, it was agreed that a new evidence-based guideline would be developed. A key area of concern identified in the scoping report included: *"To reduce unnecessary use of Rh D IgG, should noninvasive prenatal screening be used in the first trimester so that prophylaxis can be targeted?"* To answer this question, it is necessary to evaluate the accuracy of the test used to identify fetal Rh D status.
SUBGROUPS:	
CONFLICT OF INTERESTS:	

ASSESSMENT

Problem

Is the problem a priority?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ No ○ Probably no ○ Probably yes ● Yes ○ Varies ○ Don't know	Rh D immunoglobulin is produced from pooled plasma that is sourced and donated by Rh D negative people who have had been stimulated to produce D antibodies. It is a finite resource and needs to be used carefully maintained. No alternatives to the blood product (Rh D immunoglobulin) are currently available or anticipated within the near future. Boosting donors to maintain the supply of Rh D immunoglobulin poses potential clinical risks and places a considerable burden on those donors. Current protocols recommend antenatal immunoprophylaxis for all Rh D negative women, whereas this could be avoided in about one-third of women who do not delivery an Rh D positive child. This mean unnecessary exposure to blood product to around 5% of pregnant women (about 15,000 women per year), which raises important ethical issues (1, 2).	

Test accuracy

How accurate is the test?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Very inaccurate ○ Inaccurate ○ Accurate ● Very accurate ○ Varies ○ Don't know	**TEST ACCURACY** Data from 48 studies involving more than 75000 RhD negative pregnancy women gives the following range: Sensitivity: 0.93 to 1.00 this means that, potentially, up to 7% of women with an Rh D positive fetus would be incorrectly identified. Specificity: 0.92 to 1.00 this mean that, potentially, up to 8.4% of women with an Rh D negative fetus would be incorrectly identified.	Each of the included studies varied with regards to inclusion criteria (e.g. exclusion of multiple pregnancies), how inconclusive test results were handled (e.g. counted as test positive or investigated further), gestational age at sampling, and the conduct of the test (e.g. number and location of exons used, type of platform, source of fetal DNA sample); all of which would have implications for diagnostic performance.

Desirable Effects

How substantial are the desirable anticipated effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Trivial ○ Small	**Desirable**	

○ Moderate ● Large ○ Varies ○ Don't know	Estimated around 33-38% of women will avoid unnecessary exposure to blood product and receive one less injection during pregnancy. Reduced cost associated with implementation of programme to produce and supply Rh D immunoglobulin. Reduced burden on donors and conserve supply of Rh D immunoglobulin. **Undesirable** Increase in Rh D alloimmunisations among false negatives leading to theoretical increase in incidence of HDFN and associated complications. Increased costs associated with management of a sensitised women.	

Undesirable Effects

How substantial are the undesirable anticipated effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Large ○ Moderate ● Small ○ Trivial ○ Varies ○ Don't know	**Desirable** Estimated around 33-38% of women will avoid unnecessary exposure to blood product and receive one less injection during pregnancy. Reduced cost associated with implementation of programme to produce and supply Rh D immunoglobulin. Reduced burden on donors and conserve supply of Rh D immunoglobulin. **Undesirable** Increase in Rh D alloimmunisations among false negatives leading to theoretical increase in incidence of HDFN and associated complications. Increased costs associated with management of a sensitised women.	

Certainty of the evidence of test accuracy

What is the overall certainty of the evidence of test accuracy?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Very low ○ Low ○ Moderate ● High ○ No included studies	A bivariate meta-analysis of included studies revealed a sensitivity of 0.997 (95% CI 0.994, 0.999) and specificity of 0.983 (95% CI 0.974, 0.989) (random effects correlation 0.412) *See Appendix 1* *See Appendix 3*	High throughput testing may need to be specified, setting minimum standards with a threshold of sensitivity/specificity. A process of test validation that is consistent with international standards will be required. Lower certainty of evidence in multiple pregnancies. Possible issue in different ethnic populations.

Certainty of the evidence of test's effects

What is the overall certainty of the evidence for any critical or important direct benefits, adverse effects or burden of the test?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Very low ○ Low ○ Moderate ● High ○ No included studies	Noninvasive testing for fetal Rh D status is considered highly accurate with no apparent adverse effects.	Burden of testing not an issue. There are examples of countries ceasing post-natal cord blood testing after two years introduction of NIPT (3).

Certainty of the evidence of management's effects

What is the overall certainty of the evidence of effects of the management that is guided by the test results?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Very low ○ Low ○ Moderate ● High ○ No included studies	Although the comparative evidence is of low to very low certainty, large studies on the incidence of Rh D alloimmunisation show a reduction in risk since the introduction of antenatal immunoprophylaxis. (see question 1) In the absence of Rh D immunoprophylaxis, it is estimated that Rh D alloimmunisation occurred in 14% to 17% of Rh D negative women who delivered a Rh D positive baby (4). After the introduction of routine postnatal prophylaxis, the rate of Rh D alloimmunisation dropped to around 1% to 2% (5, 6). The addition	

	of antenatal Rh D immunoprophylaxis further reduced the risk of Rh D alloimmunisation to approximately 0.1% to 0.2% (5, 7).	

Certainty of the evidence of test result/management

How certain is the link between test results and management decisions?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Very low ○ Low ○ Moderate ● High ○ No included studies	A reduction in Rh D immunoglobulin use has been recorded in countries that have implemented targeted NIPT for fetal Rh D status (eg Finland, Denmark) (8, 3). There is also evidence that pragmatic application on a population-wide basis is possible (9).	Geography and remoteness in Australia may make test application more challenging. There is a need for robust systems to be put in place to notify women of test results.

Certainty of effects

What is the overall certainty of the evidence of effects of the test?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Very low ○ Low ○ Moderate ● High ○ No included studies	Please refer to Question 3a (main question) (Saramago et al., 2018). The model by Saramago 2018 estimated targeted RAADP increased the risk of Rh D alloimmunisation from 281 per 100 000 pregnant women with universal RAADP to 284 (base case scenario) or 309 (worst case scenario) per 100 000. That is, the use of NIPT to determine if women would receive Rh D IgG prophylaxis would increase the number of Rh D sensitisations by between 3 and 15 in 100,000 pregnancies if postpartum cord blood testing is continued, or between 15 to 28 per 100,000 women if postpartum cord blood testing is withdrawn (and anti-D is given on the basis of the NIPT result). The range in numbers is due to different assumptions as to whether women who do not receive NIPT would still be offered RAADP.	It is estimated that roughly between 1% and 4.5% of Rh D negative pregnant would be potentially sensitised. (assuming postnatal RhD Ig remains after cord serology) Certain reduction in use.

Values

Is there important uncertainty about or variability in how much people value the main outcomes?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Important uncertainty or variability ○ Possibly important uncertainty or variability ○ Probably no important uncertainty or	No uncertainty for the main outcome (diagnostic accuracy) No uncertainty regarding supply	

variability ● No important uncertainty or variability	No uncertainty regarding donor burden	

Balance of effects

Does the balance between desirable and undesirable effects favor the intervention or the comparison?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Favors the comparison ○ Probably favors the comparison ○ Does not favor either the intervention or the comparison ○ Probably favors the intervention ● Favors the intervention ○ Varies ○ Don't know	Certain reduction in unnecessary use of Rh D immunoglobulin.	

Resources required

How large are the resource requirements (costs)?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Large costs ○ Moderate costs ● Negligible costs and savings ○ Moderate savings ○ Large savings ○ Varies ○ Don't know	One Australian study estimated net cost savings of AUD$159,701 per year for total health care costs (10). The model assumed cord blood testing was conducted only in those women whose fetus tested *RHD* negative. The study incorporated the following costs: test cost of $45, unit costs of Rh D immunoglobulin of $59 to $88, and packaging/transport costs of samples to be $15 to $40. The mean overall cost per pregnancy was estimated to be $7495 with universal prophylaxis compared with $7471 with targeted prophylaxis.	

Certainty of evidence of required resources

What is the certainty of the evidence of resource requirements (costs)?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Very low ● Low ○ Moderate ○ High ○ No included studies	Included costs are estimated. The Panel discussed higher costs than described in the Australian study (10), all of which are key drivers of the economic model. Cost of high-throughput NIPT: $55 per test Cost of Rh D IgG for routine/sensitizing event: unknown Cost/burden of donor boosting: $ unknown Cost production of Rh D IgG: $74 The Panel assumed transport costs would not substantially change as supply of Rh D immunoglobulin is bundled with other blood products.	

Cost effectiveness

Does the cost-effectiveness of the intervention favor the intervention or the comparison?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Favors the comparison ○ Probably favors the comparison ○ Does not favor either the intervention or the comparison ○ Probably favors the intervention ● Favors the intervention ○ Varies ○ No included studies	In the United Kingdom, target Rh D immunoprophylaxis is estimated to have a reduced cost (base case) of 485,000 pounds, but QALYs (quality of life years) were reduced by 0.5 per 100,000 pregnancies (11, 12). Other cost-effective studies varied in their assessment: Not cost-saving: US (13) Uncertain: France (14) Cost-saving: Canada (15), Sweden (16).	

Equity

What would be the impact on health equity?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Reduced ○ Probably reduced ● Probably no impact ○ Probably increased ○ Increased ○ Varies ○ Don't know	The panel considered that the introduction of the test was probably not going to impact groups or settings that might be disadvantaged. In the Australian Indigenous population Rh D negativity is rare (< 1%) compared with those of European descent (16%). Women of African descent are more likely to have the RHD pseudogene which is more likely to generate an inconclusive or false-positive NIPT result. These patients would be offered antenatal anti-D prophylaxis (as they would have in current practice), therefore they would not be worse off than in current practice. It is not known if other ethnic groups represented in Australia would be worse off due to possible differences in RHD phenotype, but this was considered not likely to significantly impact health equity. One study (3) reported high sensitivity among various ethnic groups, as illustrated below. Higher heterogeneity for specificity would suggest a higher rate of false positives. As noted above, these patients would be offered Rh D immunoglobulin (as is current practice), therefore would not be worse off. *See Appendix 2*	Remoteness may increase the complexity of providing testing and ensuring results are available at relevant times during the pregnancy. The magnitude in change in practice should not dramatically improve or worsen current practice. The Panel assumes testing will not be at the cost of the patient, with funding paid via the Government.

Acceptability

Is the intervention acceptable to key stakeholders?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ No ○ Probably no ● Probably yes ○ Yes ○ Varies ○ Don't know	Yes. It is expected that the intervention would be acceptable to key stakeholders. This is based on the assumption that there would be no additional episodes of phlebotomy (one additional blood test incorporated into current maternal care). Approximately one third of Rh D negative pregnant women would avoid unnecessary exposure to blood product. The NICE Adoption support resource (2017) (9) reports that NHS staff reported the following benefits of targeted antenatal prophylaxis (using NIPT to determine fetal RHD status): • preventing unnecessary administration of blood products (anti-D immunoglobulin) and their associated risk • avoiding unnecessary painful injections for women when the NIPT for fetal *RHD* genotype result is negative	There is a potential critical supply issue with an aging donor population providing less anti-D due to poorer immunological response. A reduced demand for antenatal Rh D immunoglobulin by around one-third would relieve some of the supply issue. It is assumed there will be less burden on donors for the need for primary immunisation or boosting (easing ethical concerns).

	• reducing the number of antenatal anti-D prophylactic clinic appointments needed, and the amount of anti-D immunoglobulin used • increasing the availability of anti-D immunoglobulin for use after potentially sensitising events in pregnancy when the NIPT result for fetal *RHD* genotype is positive • reducing the anxiety associated with potentially sensitising events for D-negative women when the NIPT result for fetal *RHD* genotype is negative • providing information to allow D-negative women to make an informed decision about whether to have treatment with anti-D immunoglobulin.	

Feasibility

Is the intervention feasible to implement?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ No ○ Probably no ○ Probably yes ● Yes ○ Varies ○ Don't know	Targeted screening has been implemented in several countries and is being rolled out in the UK. The programme is feasible, but care will need to be taken as to timing of the screening, with current evidence predominantly in women at 24 to 28 weeks gestation.	It is likely the UK rollout will be a valuable resource for sharing learnings (see NICE website) for adoption of screening in various regional health centres.

SUMMARY OF JUDGEMENTS

	JUDGEMENT						
PROBLEM	No	Probably no	Probably yes	**Yes**		Varies	Don't know
TEST ACCURACY	Very inaccurate	Inaccurate	Accurate	**Very accurate**		Varies	Don't know
DESIRABLE EFFECTS	Trivial	Small	Moderate	**Large**		Varies	Don't know
UNDESIRABLE EFFECTS	Large	Moderate	**Small**	Trivial		Varies	Don't know
CERTAINTY OF THE EVIDENCE OF TEST ACCURACY	Very low	Low	Moderate	**High**			No included studies

	JUDGEMENT						
CERTAINTY OF THE EVIDENCE OF TEST'S EFFECTS	Very low	Low	Moderate	**High**			No included studies
CERTAINTY OF THE EVIDENCE OF MANAGEMENT'S EFFECTS	Very low	Low	Moderate	**High**			No included studies
CERTAINTY OF THE EVIDENCE OF TEST RESULT/MANAGEMENT	Very low	Low	Moderate	**High**			No included studies
CERTAINTY OF EFFECTS	Very low	Low	Moderate	**High**			No included studies
VALUES	Important uncertainty or variability	Possibly important uncertainty or variability	Probably no important uncertainty or variability	**No important uncertainty or variability**			
BALANCE OF EFFECTS	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	Probably favors the intervention	**Favors the intervention**	Varies	Don't know
RESOURCES REQUIRED	Large costs	Moderate costs	**Negligible costs and savings**	Moderate savings	Large savings	Varies	Don't know
CERTAINTY OF EVIDENCE OF REQUIRED RESOURCES	Very low	**Low**	Moderate	High			No included studies
COST EFFECTIVENESS	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	Probably favors the intervention	**Favors the intervention**	Varies	No included studies
EQUITY	Reduced	Probably reduced	**Probably no impact**	Probably increased	Increased	Varies	Don't know
ACCEPTABILITY	No	Probably no	**Probably yes**	Yes		Varies	Don't know
FEASIBILITY	No	Probably no	Probably yes	**Yes**		Varies	Don't know

TYPE OF RECOMMENDATION

Do not cover	Cover with evidence development	Cover with price negotiation	Restricted coverage	**Cover**
○	○	○	○	●

CONCLUSIONS

Decision

The ERG **recommends** the testing of maternal blood to determine fetal *RHD* genotype in all Rh D negative pregnant women to enable targeted antenatal Rh D immunoprophylaxis.[a] (*strong recommendation, high certainty of evidence about the accuracy of the test*).

The ERG **recommends** test sensitivity be at least 99% in order to minimise the number of Rh D positive fetuses being missed by the test. (*strong recommendation, high certainty of evidence about the accuracy of the test*).

The ERG **recommends** NIPT for fetal *RHD* from 11_{+0} weeks of pregnancy because of higher test accuracy than at earlier weeks. (*strong recommendation, high certainty of evidence about the accuracy of the test*).

[a] The ERG's recommendation on the use of NIPT for fetal *RHD* is not a policy statement on funding and supply arrangements for the national provisions of NIPT for blood group genotyping to determine the Rh D status of the fetus.

Justification

Overall justification

Pregnant women and their clinicians highly value the avoidance of unnecessary blood product. Noninvasive prenatal testing offers the opportunity to avoid the unnecessary administration of Rh D immunoglobulin in nearly one-third of Rh D negative pregnant women.

Donors to the Rh D immunoglobulin programme bear an extremely heavy personal burden and significant risk so as to maintain a supply of Rh D immunoglobulin to meets Australia's needs. Noninvasive prenatal testing offers the opportunity to significantly reduce this burden.

Detailed justification

Desirable Effects

Based on diagnostic performance around 57.5% to 62.0% of Rh D negative women would receive Rh D immunoglobulin (true positives); and around 34.8% to 38.0% of Rh D negative women would avoid unnecessary Rh D immunoglobulin (true negatives).

Undesirable Effects

Based on diagnostic performance around 0% to 4.5% of Rh D negative women with an Rh D positive fetus would not receive Rh D immunoglobulin (false negatives); and around 0% to 3.2% of women would continue to receive Rh D immunoglobulin unnecessarily (false positives).

Subgroup considerations

Evidence in women with multiple pregnancies is limited and caution should be taken in this population.

Inconclusive test results should lead to the administration of routine Rh D immunoprophylaxis.

Testing before 11 weeks gestation is not recommended.

Implementation considerations

To implement this policy the following would be required:

* an additional blood test after the end of the first trimester in approximately 15% of pregnant women.

* an implementation programme to correctly target Rh D immunoprophylaxis in test positive women. (possibly similar to NICE tools and resources)

* To achieve the expected benefits, it would require funding for universal access and assumes cost of the test is subsidised through the government medical benefits scheme.

* Implementation of the test to be at least cost neutral.

Monitoring and evaluation

Health care services should monitor targeted Rh D immunoprophylaxis to evaluate compliance, uptake and patient outcomes. This is to ensure appropriate administration of Rh D immunoglobulin.

Where possible, any proven false negative results should be reported through appropriate haemovigilance activities.

Research priorities

What is the accuracy of noninvasive prenatal tests for fetal Rh D status in Rh D negative women with multiple pregnancies?

Further research on acceptability of the service among users is needed.

There is a paucity of data on the prevalence of RHD genotype as it relates to pregnant women or the current ethnic populations in Australia.

Are there alternative tests for postnatal cord serology?

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APPENDICES

Appendix 1

Test result	Number of results per 1000 patients tested (95% CI)			№ of participants (studies)	Certainty of the evidence (GRADE)
	Prevalence 55%	Prevalence 62%	Prevalence 75%		
True positives patients with fetal Rh D status	548 (547 to 549)	618 (616 to 619)	748 (746 to 749)	76349 (48)	⨁⨁⨁⨁ HIGH[a,b,c,d,e]
False negatives patients incorrectly classified as not having fetal Rh D status	2 (1 to 3)	2 (1 to 4)	2 (1 to 4)		
True negatives patients without fetal Rh D status	442 (438 to 445)	374 (370 to 376)	246 (244 to 247)	76349 (48)	

Test result	Number of results per 1000 patients tested (95% CI)			№ of participants (studies)	Certainty of the evidence (GRADE)
	Prevalence 55%	Prevalence 62%	Prevalence 75%		
False positives patients incorrectly classified as having fetal Rh D status	8 (5 to 12)	6 (4 to 10)	4 (3 to 6)		⨁⨁⨁⨁ HIGH[a,b,c,d,e]

a. The evidence was considered applicable to the Australian healthcare context with some caveats. Much of the evidence is in Northern European countries with predominantly Caucasian majority. This was considered comparable to the Australian context in which the prevalence of RhD negative phenotype among donors is around 15%. The prevalence of RhD negative babies born to RhD negative women is estimated to be 38%, however the prevalence of specific RhD genotypes is not known. The meta-analyses by Zhu 2012 and Geifman-Holtzman 2006 were not included as changes and improvements in conduct of the test have occurred. It is expected that the screening test would, at a minimum, include primers for 2 exons (either 4, 5, 7, or 10), involve RT-qPCR, and be conducted in duplicate.

b. Diagnostic performance may by overestimated if only high throughput studies are considered (as reported in Saramago 2018), therefore the inclusion of Mackie (2016) and smaller studies was considered appropriate for the Australian context. Care should be taken when interpreting test results in women with multiple pregnancies as this subgroup was excluded from the meta-analysis by Mackie 2016 and other studies.

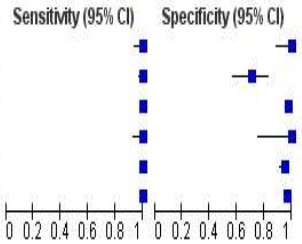
c. Almost all studies consistent, and inconsistencies could be explained. Samples taken prior to GW12 would reduce confidence in specificity of the test. Some studies did not report inconclusive results, which would favour the index test, however this was not considered to substantially reduced the confidence in the overall quality of evidence.

d. A large number of studies included. Smaller confidence intervals observed in the large studies with central reference laboratories and those that used thresholds to maintain an acceptable level of sensitivity. Here, confidence in the evidence is high. In small, single centre studies, wider confidence interval would suggest a lower certainty of evidence.

e. Despite some gaps in reporting, the majority of included studies were judged to be at low risk of bias. Concerns relating to patient selection bias (e.g., exclusion of multiple pregnancies, exclusion of sensitised women) or conduct of the index test (e.g., number of exons amplified, controls used) were small, and are not considered to substantially alter the test results. Cord blood serology was the reference standard in all studies and was usually conducted independent of the index test.

Appendix 2

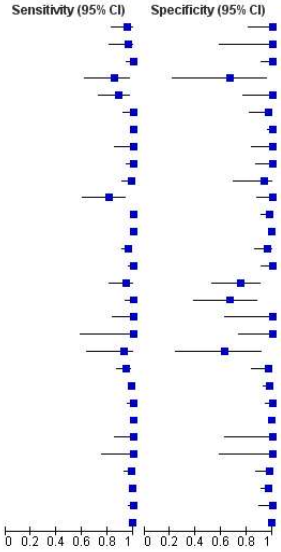
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
De Haas 2016 Asian	54	0	0	31	1.00 [0.93, 1.00]	1.00 [0.89, 1.00]
De Haas 2016 Creole	116	15	0	37	1.00 [0.97, 1.00]	0.71 [0.57, 0.83]
De Haas 2016 European	11839	147	6	7486	1.00 [1.00, 1.00]	0.98 [0.98, 0.98]
De Haas 2016 Hindustani	53	0	0	13	1.00 [0.93, 1.00]	1.00 [0.75, 1.00]
De Haas 2016 Mediterranean	602	14	0	263	1.00 [0.99, 1.00]	0.95 [0.92, 0.97]
De Haas 2016 Other	540	10	1	309	1.00 [0.99, 1.00]	0.97 [0.94, 0.98]



Appendix 3

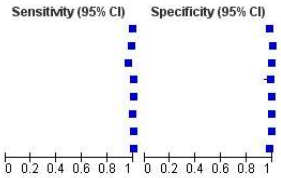
Mackie 2016

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Lo 1998	37	0	2	18	0.95 [0.83, 0.99]	1.00 [0.81, 1.00]
Zhong 2001	26	0	1	7	0.96 [0.81, 1.00]	1.00 [0.59, 1.00]
Costa 2002	62	0	0	40	1.00 [0.94, 1.00]	1.00 [0.91, 1.00]
Siva 2003	17	2	3	4	0.85 [0.62, 0.97]	0.67 [0.22, 0.96]
Turner 2003	30	0	4	14	0.88 [0.73, 0.97]	1.00 [0.77, 1.00]
Rijnders 2004	43	1	0	28	1.00 [0.92, 1.00]	0.97 [0.82, 1.00]
Gautier 2005	170	0	0	102	1.00 [0.98, 1.00]	1.00 [0.96, 1.00]
Hromadnikova 2005	24	0	0	21	1.00 [0.86, 1.00]	1.00 [0.84, 1.00]
Zhou 2005	68	0	0	26	1.00 [0.95, 1.00]	1.00 [0.87, 1.00]
Machado 2006	58	1	1	15	0.98 [0.91, 1.00]	0.94 [0.70, 1.00]
Al-Yatama 2007	21	0	5	28	0.81 [0.61, 0.93]	1.00 [0.88, 1.00]
Rouillac-Le 2007	229	2	0	77	1.00 [0.98, 1.00]	0.97 [0.91, 1.00]
Minon 2008	359	1	0	185	1.00 [0.99, 1.00]	0.99 [0.97, 1.00]
Grill 2009	122	2	5	49	0.96 [0.91, 0.99]	0.96 [0.87, 1.00]
Hyland 2009	95	0	0	40	1.00 [0.96, 1.00]	1.00 [0.91, 1.00]
Sesarini 2009	34	6	2	18	0.94 [0.81, 0.99]	0.75 [0.53, 0.90]
Wang 2009	60	5	0	10	1.00 [0.94, 1.00]	0.67 [0.38, 0.88]
Aykut 2010	21	0	0	8	1.00 [0.84, 1.00]	1.00 [0.63, 1.00]
Gunel 2010	7	0	0	12	1.00 [0.59, 1.00]	1.00 [0.74, 1.00]
Mohammed 2010	12	3	1	5	0.92 [0.64, 1.00]	0.63 [0.24, 0.91]
Achargul 2011	83	1	5	31	0.94 [0.87, 0.98]	0.97 [0.84, 1.00]
Bombard 2011	278	3	4	121	0.99 [0.96, 1.00]	0.98 [0.93, 0.99]
Tynan 2011	86	0	0	62	1.00 [0.96, 1.00]	1.00 [0.94, 1.00]
Clausen 2012	1368	6	2	862	1.00 [0.99, 1.00]	0.99 [0.99, 1.00]
Han 2012	24	0	0	8	1.00 [0.86, 1.00]	1.00 [0.63, 1.00]
Sbarsi 2012	13	0	0	7	1.00 [0.75, 1.00]	1.00 [0.59, 1.00]
Manzanares 2013	73	1	1	40	0.99 [0.93, 1.00]	0.98 [0.87, 1.00]
Moise 2013	220	3	1	96	1.00 [0.98, 1.00]	0.97 [0.91, 0.99]
Polin 2013	88	0	0	34	1.00 [0.96, 1.00]	1.00 [0.90, 1.00]
Chitty 2014	2563	18	19	1920	0.99 [0.99, 1.00]	0.99 [0.99, 0.99]



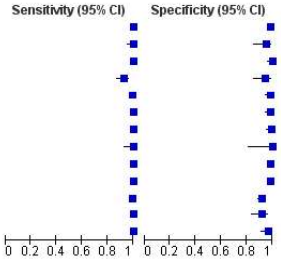
Saramago 2018

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Finning 2008	1118	14	3	670	1.00 [0.99, 1.00]	0.98 [0.97, 0.99]
Akolekar 2011	332	0	6	164	0.98 [0.96, 0.99]	1.00 [0.98, 1.00]
Wikman 2012	1340	15	55	2242	0.96 [0.95, 0.97]	0.99 [0.99, 1.00]
Grande 2013	183	1	0	91	1.00 [0.98, 1.00]	0.99 [0.94, 1.00]
Banch Clausen 2014	7636	41	11	4706	1.00 [1.00, 1.00]	0.99 [0.99, 0.99]
Chitty 2014	2563	18	19	1920	0.99 [0.99, 1.00]	0.99 [0.99, 0.99]
Soothill 2015	267	1	0	170	1.00 [0.99, 1.00]	0.99 [0.97, 1.00]
Thurik 2015	15816	225	9	9739	1.00 [1.00, 1.00]	0.98 [0.97, 0.98]



Additional studies identified

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Macher 2012 Multiplex PCR	619	7	0	386	1.00 [0.99, 1.00]	0.98 [0.96, 0.99]
Macher 2012 Single PCR	76	3	0	55	1.00 [0.95, 1.00]	0.95 [0.86, 0.99]
Orzinska 2015	308	0	0	99	1.00 [0.99, 1.00]	1.00 [0.96, 1.00]
Picchiassi 2015	116	4	9	64	0.93 [0.87, 0.97]	0.94 [0.86, 0.98]
Moise 2016 1st trimester	310	2	1	128	1.00 [0.98, 1.00]	0.98 [0.95, 1.00]
Moise 2016 2nd trimester	301	2	0	129	1.00 [0.99, 1.00]	0.98 [0.95, 1.00]
Moise 2016 3rd trimester	277	1	0	121	1.00 [0.99, 1.00]	0.99 [0.96, 1.00]
Papasawa 2016	53	0	0	18	1.00 [0.93, 1.00]	1.00 [0.81, 1.00]
Haimila 2017	7133	39	1	3640	1.00 [1.00, 1.00]	0.99 [0.99, 0.99]
Hyland 2017 BCT tubes	370	3	0	226	1.00 [0.99, 1.00]	0.99 [0.96, 1.00]
Jakobsen 2018	983	53	4	578	1.00 [0.99, 1.00]	0.92 [0.89, 0.94]
Manfroi 2018	200	7	0	77	1.00 [0.98, 1.00]	0.92 [0.84, 0.97]
Sorenson 2018	183	3	0	95	1.00 [0.98, 1.00]	0.97 [0.91, 0.99]



F6 Question 4

Does increasing BMI increase the risk of failure of anti-D administration in Rh D negative pregnant or postpartum women with no preformed anti-D?	
PROBLEM:	In December 2014, CSL Behring updated the Rh(D) Immunoglobulin-VF Product Information (PI) to include a recommendation that the clearance of fetal cells and the presence of Rh(D) antibody be confirmed post-administration in patients with a BMI ≥ 30. The Rhophylac PI was also updated to recommend intravenous administration of this product in patients with a BMI >30. These data were based on post-marketing adverse events that showed the proportion of all reports consistent with reduced effect was approximately 4-fold higher in patients with BMI ≥ 30 compared to non-obese patients (defined as BMI <30 or weight ≤90kg). (Expert Panel Consensus, 2015).
OPTION:	increased dose of RAADP
COMPARISON:	
MAIN OUTCOMES:	Incidence of Rh D alloimmunisation (any timepoint); Anti-D serum levels after administration of Rh D IgG (2 doses, 28 and 34 weeks gestation); Anti-D serum levels after administration of Rh D IgG (single dose, 28 weeks gestation); Anti-D serum levels after delivery of an Rh D positive child; Incidence of a positive test for feto-maternal haemorrhage; Adverse neonatal events (e.g., jaundice); Adverse maternal events;
SETTING:	Obstetrics and maternity
PERSPECTIVE:	National healthcare perspective
BACKGROUND:	The National Health and Medical Research Council's (NHMRC) *1999 Guidelines on the Prophylactic Use of Rh D Immunoglobulin (Anti-D) in Obstetrics* were updated by the National Blood Authority (NBA) in 2003, with the aim of updating the guidance on antenatal prophylaxis. The updated 2003 Anti-D Guidelines aimed to inform clinicians, other health professionals, and policy makers of new recommendations for the staged implementation of full antenatal prophylaxis in Australia. The 2003 Anti-D Guidelines also included policy intent as a national program to address the issue of sufficiency of supply of product and were intended to be reviewed within five years, according to the availability of Rh D immunoglobulin. To ensure the Anti-D Guidelines continue to reflect current evidence and best clinical practice, the NBA undertook a scoping exercise in September 2016 to identify the extent of newly available evidence and areas of concerns (Health Research Consulting, 2017). Through a collaborative partnership with the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) and other relevant stakeholders, it was agreed that a new evidence-based guideline would be developed. A key area of concern identified in the scoping report included: *"Does higher body mass index (BMI) impact on the efficacy of Rh D immunoglobulin?"*
CONFLICT OF INTERESTS:	

ASSESSMENT

Problem

Is the problem a priority?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ No ○ Probably no ○ Probably yes ● Yes ○ Varies ○ Don't know	CSL postmarketing event data suggests there is a potential 4-fold increased risk of lack of effect among Rh D negative pregnant women with a BMI ≥ 30 compared with those who are not obese. In Australia, the proportion of pregnant women with a BMI ≥ 30 is substantial. An Expert Panel convened by the Australian Red Cross Blood Service and the National Blood Authority on 22 May 2015 discussed the issue of a potentially reduced effectiveness of Rh D immunoglobulin in Rh D negative women with BMI ≥ 30. A draft Consensus Statement indicated there is still uncertainty around this issue and that further research is needed.	

Desirable Effects

How substantial are the desirable anticipated effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Trivial ● Small ○ Moderate ○ Large ○ Varies ○ Don't know	The absolute number of reports of lack of effect (ie incidence of alloimmunisation, incidence of baby developing signs consistent with haemolysis) is small relative to the number of doses of Rh D immunoglobulin used. However, if preventable factors contributing to the incidence of alloimmunisations can be identified, then further decreases in the incidence of haemolytic disease of the fetus and newborn and associated morbidities can be attained.	

Undesirable Effects

How substantial are the undesirable anticipated effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Large ○ Moderate ● Small ○ Trivial	The absolute number of reports of lack of effect (ie incidence of alloimmunisation, incidence of baby developing signs consistent with haemolysis) is small relative to the number of doses of Rh D immunoglobulin used. However, if preventable factors contributing to the incidence of alloimmunisations can be identified, then further decreases in the incidence of haemolytic disease of the fetus and newborn and associated morbidities can be attained.	

○ Varies ○ Don't know		

Certainty of evidence

What is the overall certainty of the evidence of effects?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
● Very low ○ Low ○ Moderate ○ High ○ No included studies	Increasing BMI does not appear to have any effect on the incidence of Rh D alloimmunisation in Rh D negative women, but the evidence is sparse and very uncertain. Increasing BMI may affect peak serum levels of Rh D immunoglobulin however there is little to no evidence that increasing BMI affects the persistence of anti-D. *See Appendix 1*	A correlation between low serum anti-D levels and incidence of Rh D alloimmunisation is not known.

Values

Is there important uncertainty about or variability in how much people value the main outcomes?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Important uncertainty or variability ○ Possibly important uncertainty or variability ○ Probably no important uncertainty or variability ● No important uncertainty or variability		

Balance of effects

Does the balance between desirable and undesirable effects favor the intervention or the comparison?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Favors the comparison ○ Probably favors the comparison ○ Does not favor either the intervention or the comparison ○ Probably favors the intervention ○ Favors the intervention ○ Varies ● Don't know	Theoretical improved efficacy balanced by the potential for confusion with different dosage or route of administration in women with BMI > 30.	

Resources required

How large are the resource requirements (costs)?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Large costs ● Moderate costs ○ Negligible costs and savings ○ Moderate savings ○ Large savings ○ Varies ○ Don't know	There is insufficient evidence to support changes to current recommendations. Larger doses or different route of administration would be required in Rh D women with increased BMI, which would increase costs associated with RAADP.	

Certainty of evidence of required resources

What is the certainty of the evidence of resource requirements (costs)?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Very low ○ Low ○ Moderate ○ High ● No included studies		

Cost effectiveness

Does the cost-effectiveness of the intervention favor the intervention or the comparison?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Favors the comparison ○ Probably favors the comparison ○ Does not favor either the intervention or the comparison ○ Probably favors the intervention ○ Favors the intervention ○ Varies ● No included studies		

Equity

What would be the impact on health equity?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ Reduced ○ Probably reduced ● Probably no impact ○ Probably increased ○ Increased ○ Varies ○ Don't know	No changes to current recommendations are made, therefore no impact on health equity is expected.	

Acceptability

Is the intervention acceptable to key stakeholders?

JUDGEMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
○ No ○ Probably no ○ Probably yes ● Yes ○ Varies ○ Don't know	RAADP is currently recommended for all Rh D negative women (regardless of weight) and is considered acceptable to key stakeholders.	

Feasibility Is the intervention feasible to implement?		
JUDGEMENT	**RESEARCH EVIDENCE**	**ADDITIONAL CONSIDERATIONS**
○ No ○ Probably no ○ Probably yes ● Yes ○ Varies ○ Don't know	RAADP is currently recommended for all Rh D negative women (regardless of weight) and is considered feasible to implement.	

SUMMARY OF JUDGEMENTS

	JUDGEMENT						
PROBLEM	No	Probably no	Probably yes	**Yes**		Varies	Don't know
DESIRABLE EFFECTS	Trivial	**Small**	Moderate	Large		Varies	Don't know
UNDESIRABLE EFFECTS	Large	Moderate	**Small**	Trivial		Varies	Don't know
CERTAINTY OF EVIDENCE	**Very low**	Low	Moderate	High			No included studies
VALUES	Important uncertainty or variability	Possibly important uncertainty or variability	Probably no important uncertainty or variability	**No important uncertainty or variability**			
BALANCE OF EFFECTS	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	Probably favors the intervention	Favors the intervention	Varies	**Don't know**
RESOURCES REQUIRED	Large costs	**Moderate costs**	Negligible costs and savings	Moderate savings	Large savings	Varies	Don't know
CERTAINTY OF EVIDENCE OF REQUIRED RESOURCES	Very low	Low	Moderate	High			**No included studies**

	JUDGEMENT						
COST EFFECTIVENESS	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	Probably favors the intervention	Favors the intervention	Varies	**No included studies**
EQUITY	Reduced	Probably reduced	**Probably no impact**	Probably increased	Increased	Varies	Don't know
ACCEPTABILITY	No	Probably no	Probably yes	**Yes**		Varies	Don't know
FEASIBILITY	No	Probably no	Probably yes	**Yes**		Varies	Don't know

TYPE OF RECOMMENDATION

Strong recommendation against the option	**Conditional recommendation against the option**	Conditional recommendation for either the option or the comparison	Conditional recommendation for the option	Strong recommendation for the option
○	●	○	○	○

CONCLUSIONS

Recommendation

The ERG **does not currently support** an increased dose of Rh D immunoglobulin or changes in laboratory testing on the basis of high BMI in Rh D negative women. (*Weak recommendation, very low certainty of evidence about the size of effect*)

Expert opinion point:

Rh D immunoglobulin must be given by deep intramuscular injection. For women with a BMI or more than 30, particular consideration should be given to factors that may affect the adequacy of the injection (eg the site of administration and the length of the needle used).

Related recommendation(s)

1. Should universal RAADP (1 or 2 doses) vs. placebo or no universal RAADP be used in Rh D negative pregnant women with no preformed anti-D?

The ERG **recommends** access to antenatal Rh D immunoglobulin for the prevention of Rh D alloimmunisation in Rh D negative pregnant women with no preformed anti-D antibodies (*Strong recommendation, low to very low certainty of evidence about the size of effect*).

2. Should targeted RAADP (based on noninvasive prenatal screening) vs. universal RAADP be used in Rh D negative pregnant women with no preformed anti-D?

The ERG **recommends** that antenatal Rh D immunoprophylaxis in Rh D negative pregnant women with no preformed anti-D antibodies be targeted to those predicted to be carrying an Rh D positive fetus, based on NIPT for fetal *RHD*. This applies to both routine and sensitising event immunoprophylaxis, if the result of fetal *RHD* genotyping is available (see EOP3 and EOP7). (*Strong recommendation, low certainty of evidence about the size of the effect*)

If fetal Rh D status is not available or is uncertain, the ERG **recommends** that antenatal Rh D immunoprophylaxis be offered to Rh D negative pregnant women with no preformed anti-D antibodies. (*Strong recommendation, low certainty of evidence about the size of the effect*)

Justification

There is no established relationship between lower post-administration serum anti-D levels and alloimmunisation rates or poor clinical outcomes.

All serious outcomes for Rh D alloimmunisation are very uncommon. This is despite the proportion of women with a BMI >30 progressively increasing, now comprising almost one third of all women giving birth in Australia.

Subgroup considerations

No subgroups considered.

Implementation considerations

Monitoring and evaluation

Research priorities

Neonatal exchange transfusion and intrauterine transfusion may not be the most appropriate measure for assessing the number of fetuses with severe HDFN. Clinical practise and thresholds for implementation have changed.

Evidence relating to the incidence of Rh D alloimmunisation as it relates to BMI in the Australian population is needed.

Further research should be undertaken in this area, specifically examination of Rh(D) immunoglobulin in patients with a BMI >30 and studies looking at the incidence and causes of Rh(D) alloimmunisation during pregnancy.

APPENDICES

Appendix 1

Outcomes	Impact		№ of participants (studies)	Certainty of the evidence (GRADE)	
Incidence of Rh D alloimmunisation (any timepoint)	No significant association between body mass index, mean body weight, weight >75 kg or weight >100 kg on the incidence of Rh alloimmunisation reported in a small case-control study (Koelewijn 2009).	-	42 cases 146 controls (1 observational study)[1]	⨁◯◯◯ VERY LOW[a,b,c,d]	Increasing BMI does not appear to have any effect on the incidence of Rh D alloimmunisation in Rh D negative women, but the evidence is very uncertain.
Anti-D serum levels after administration of Rh D IgG (2 doses, 28- and 34-weeks' gestation)	One small study reported a correlation between peak anti-D serum levels and maternal body surface area and weight measured at 7 days after the first dose but found no significant difference relating to persistence measured at 12 weeks after the first dose.	-	45 (1 observational study)[2]	⨁◯◯◯ VERY LOW[c,e,f,g,h]	Increasing BSA appears to have little to no effect on persistence of anti-D serum levels after administration of Rh D IgG (two doses, 28- and 34-weeks' gestation) but the evidence is very uncertain.
Anti-D serum levels after administration of Rh D IgG (single dose, 28 weeks gestation)	In a single arm of an RCT, women with body weight >80 kg (n=2) had lower peak serum levels than women who weighed <80 kg (n = 6); however anti D IgG remained quantifiable in these women at last scheduled follow-up (week 9 and 11).	-	(1 RCT)[3]	⨁◯◯◯ VERY LOW[c,h,i,j]	Increased body weight appears to have little to no effect on persistence of anti-D serum levels after administration of Rh D IgG (single dose, 28 weeks gestation) but the evidence is very uncertain.
Anti-D serum levels after delivery of an Rh D positive child	Based on the general linear model over time, the study authors found each kg/m2 BMI higher than 27 kg/m2 reduced the Rh D Ig G serum concentration by the calculated value.	-	26 (1 observational study)[4]	⨁◯◯◯ VERY LOW[c,h,k,l]	Increasing BMI may result in reduced anti-D serum concentration after delivery of an Rh D positive child but the evidence is very uncertain. The link between lower anti-D levels and incidence of Rh D alloimmunisation is unknown.
Incidence of a positive test for feto-maternal haemorrhage - not reported	No studies identified reported this outcome.	-	-	-	
Adverse neonatal events (e.g., jaundice) - not reported	No studies identified reported this outcome.	-	-	-	

Outcomes	Impact		№ of participants (studies)	Certainty of the evidence (GRADE)	
Adverse maternal events	A total of 7 adverse events reported among 5 women, none of which were considered related to study drug.	-	(1 RCT)	⨁◯◯◯ VERY LOW[c,d,i,m]	

1. Koelewijn, J. M., de Haas, M., Vrijkotte, T. G., van der Schoot, C. E., Bonsel, G. J. Risk factors for RhD immunisation despite antenatal and postnatal anti-D prophylaxis. BJOG: An International Journal of Obstetrics & Gynaecology; 2009.
2. MacKenzie, I. Z., Roseman, F., Findlay, J., Thompson, K., Jackson, E., Scott, J., Reed, M. The kinetics of routine antenatal prophylactic intramuscular injections of polyclonal anti-D immunoglobulin. BJOG: An International Journal of Obstetrics & Gynaecology; 2006.
3. Bichler J, Schondorfer G. Pabst G. Andresen I. Pharmacokinetics of anti-D IgG in pregnant RhD-negative women. 2003.
4. Woelfer, B., Schuchter, K., Janisiw, M., Hafner, E., Philipp, K., Panzer, S. Postdelivery levels of anti-D IgG prophylaxis in D-- mothers depend on maternal body weight. Transfusion; 2004.

a. One case-control study that appears to provide sound evidence for a non-randomised study but cannot be considered comparable to a well-performed RCT. There was an over-representation of women from the primary versus obstetric setting (3:1) in the control group compared with cases, resulting in the use of weighted data in the analysis. This was not thought to seriously affect the overall direction of effect.
b. The study is not statistically powered to inform decision making. A very small number of women with a high BMI included.
c. Single study. Heterogeneity not assessed. Certainty of evidence not downgraded.
d. Evidence is directly generalisable to the target population and applicable to the Australian healthcare system with some caveats. The study was conducted in The Netherlands in Rh D negative women who received 1000 IU of anti-D at GW 30 and within 48 hours of delivery of an Rh D positive child. This is different to the recommended dose in Australia of 625 IU at GW 28 and GW 34 and within 72 hours of delivery of an Rh D positive child.
e. One study with some important problems that seriously weaken the confidence in the results.
f. Small cohort with some concerns with reporting bias and missing data.
g. Evidence is directly generalisable to the target population and applicable to the Australian healthcare system with some caveats. The study was conducted in the UK in Rh D negative pregnant women. Anti-D (500 IU) was administered at GW 28 and 34 but the dose was lower than recommended in Australia (625 IU)
h. Small cohort with insufficient longer-term data to provide meaningful information relating to BMI and incidence of Rh D alloimmunisation in a subsequent pregnancy.
i. The study is too problematic to provide any useful evidence on the outcome of interest.
j. Evidence is probably generalisable to the target population but difficult to judge if sensible to apply to the Australian healthcare system. The study was conducted in Germany in Rh D negative women. Anti-D (1500 IU) was administered at GW28, which is different to that recommended in Australia (625 IU at GW 28 and GW 34). The correlation between body weight and BMI is poor, with the BMI of patient 12 = 26.79 and patient 9 = 32.29.
k. One cohort study that appears to provide sound evidence for a non-randomised study but cannot be considered comparable to a well-performed RCT.
l. Evidence is directly generalisable to the target population and is applicable to the Australian healthcare system with some caveats. The study was conducted in Austria in Rh D negative women who had delivered an Rh D positive child. Anti-D was administered with 72 hours after birth, but at a dose higher than that recommended in Australia (1500 IU vs 625 IU).
m. Small study unlikely to be sufficiently powered to detect a statistically significant difference.