Why the future of foetal/neonate Rhesus Testing is looking so good

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The Introduction

- 20/10/2013 FP presented to Alice Springs Hospital in labour
- Group O negative
- Up to date with Anti-D immunoglobulin

- The next day, FP gave birth to a baby girl
- Baby of FP’s cord blood was sent to the lab for a routine group and DAT
  - Northern Territory were still performing group and DAT’s on all neonates from rhesus negative mothers
The Group

- Biovue ABO cassette
  - A = 4
  - B = 0
  - D = 2
  - Control = 1

- Ortho Clinical Diagnostics information leaflet states if any positive reactivity is observed in the control column, valid interpretation of the blood group can not be determined
  - Further investigation required
    - DAT, elution and eventually sending the specimen to Melbourne ARCBS
The Direct Antiglobulin Test

- Important test for the diagnosis of haemolytic disease of the newborn (HDN)
- Determines whether red blood cells have been coated in vivo with immunoglobulin, complement or both
- Positive DAT reported in 1:1,000 to 1:14,000 and 1–15% of hospital patients
- Most donors with a positive DAT are healthy
- Clinical significance of DAT relies on history, diagnosis and other laboratory results
  - All by itself not diagnostic of HDN
- Biovue DAT cassette:
  \[ \text{IgG} = 4 \]
  \[ \text{C3C4} = 1 \]
  \[ \text{Control} = 1 \]
• Mothers antibodies can be transported across the placenta
• Necessary as newborns have primitive immune system
• Helps ensure neonates survive while immune system matures
  • Downside maternal antibodies can cause haemolytic disease of the newborn (HDN)
• Major cause of HDN Rh blood group
  • ABO less severe
    • Foetal RBC express less ABO antigens compared to adults
• Acid Glycine elution used
  • Result = Anti–A specificity => ?ABO incompatibility
The Tube Group

- ANZBT guidelines ABO and Rh(D) confirmed by second test
- Newborn RhD typing may be difficult
  - Wharton’s Jelly
  - Anti–D immunoglobulin IgG
- Use of monoclonal reagents
  - Monoclonal CSL Epicolone Anti–A/Anti–B/Anti–D used
- Result:  Anti–A = 4
  Anti–B = 0
  Anti–D = 0
The Australian Red Cross Blood Service Report

- Group A
- Rh group tested
  - Negative when tested with IgM anti–D that doesn’t detect cells of RhD category VI
  - Negative when tested with IgM anti–D that does detect cells of RhD category IV
  - Weak D testing unsucessful due to positive DAT
  - Phenotype C–c+E–e+

- Comments
  - Negative result from IgM testing and phenotype suggest Baby of FP is likely to be Rh(D) negative
  - Not able to test weak D
  - Baby can only be correctly determined when cells are DAT negative or by DNA typing
The future of Rhesus Testing
HDN and the history of Rhesus testing

- Beneficial to know foetal RhD status
  - If negative, not at risk of haemolytic disease of the foetus or newborn (HDFN)
  - If positive appropriate measures taken
- HDFN causes haemolytic anaemia
  - Leading to hydrops fetalis, jaundice and intrauterine or neonatal death
- Foetal typing available since mid 1990’s
  - Source; aminocytes or chorionic villi
  - Expensive, invasive and present a risk to the foetus
- Free foetal DNA preferred source of DNA
Circulating cell – free foetal DNA

• 1997 discovery of cell–free foetal DNA (cffDNA) in maternal circulation
• 1998 non–invasive detection of foetal RhD gene (RHD)
• cffDNA highly fragmented, function unknown
• Levels vary throughout pregnancy
• Detection unreliable before 7 weeks
• Potential applications – single gene disorders
Free DNA foetal kit RhD

- Currently used in many parts of Europe
- Detects foetal RHD DNA in maternal blood
- Very early detection of RhD status
- Detection via PCR amplification
  - Using EXONS 5, 7, and 10
  - Greatest coverage of RhD variants
Method

- Real-Time PCR
- Centrifugation to remove plasma then second centrifugation at high speed or filtration
- Sex determination region gene (SRY) used during amplification
- Highly polymorphic markers used for differences between maternal and foetal DNA
- Introduced nationally in Denmark, The Nederlands and Finland to guide prophylaxis
Pros vs Cons

Pros:

- Women with a Rh negative foetus avoid unnecessary immunoprophylaxis
- No more shortages
- Isoimmunised patients avoid intensive care for Rh negative babies
- Decrease in administration discomfort, inconvenience, and risk of infection
- Robotic techniques making it cost effective
- Reduction in hospital costs
- Ethical issues
Pros vs Cons... continued

Cons:

- False negatives
- False positives
- Caucasian populations validated
- No ‘gold’ standard
- Manual testing increases risk of human error
- Low concentrations
- Terminating cord blood typing no proficiency markers
- Samples can be affected by preanalytical conditions
The conclusion

- Doctor in charge was informed to keep an eye on Baby of FP.
- 40% of the Caucasian population receives unnecessary administration of anti–D.
- Understanding molecular bases of D phenotypes increases predicted accuracy
- Screening reduces the amount of blood products given routinely
- Increasing accuracies and sensitivities
  - Making invasive RhD testing almost obsolete and newborn RhD testing also obsolete in some countries
References:

- Clausen F, Damkjaer M, Dziegiel M; Review – Noninvasive fetal RhD genotyping. Transfusion and Apheresis Science 2014: 50; 154–162.
- Epiclone–2 Anti–D (IgM/IgG) – Human Monoclonal IgM/IgG Blood Grouping Reagents leaflet, CSL Limited.

And many thanks to Jenny Condon from ARCBS in Melbourne for answering all my questions.
Thanks for listening