Validation of the Fronine Kleihauer test kit

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Rebecca Crockett
Validation of the Fronine Kleihauer Test kit

Why change?

- St Vincent’s Pathology Melbourne have been using an in-house test method based on a modified acid-elution for nearly 30 years (Dacie and Lewis, 6th ed, 1984)
- The stain is reliable and easy to use with no external quality control issues
- Change to a commercial kit as part of a laboratory wide push to move away from in-house reagents with the changing regulations regarding IVDs from 2014
Validation of the Fronine Kleihauer Test kit

Comparison of Kleihauer guidelines and laboratory practice

<table>
<thead>
<tr>
<th></th>
<th>St V’s</th>
<th>Lab 1</th>
<th>Lab 2</th>
<th>Lab 3</th>
<th>ANZSBT (2002)</th>
<th>BCSH (2009)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos Control</td>
<td>10% fetal cells</td>
<td>0.25%</td>
<td>10%</td>
<td>10%</td>
<td>&gt;0.25% Run calculated controls</td>
<td>1%</td>
</tr>
<tr>
<td>QC Material</td>
<td>Fresh</td>
<td>Fresh</td>
<td>Fixed slides kept max. 1 week</td>
<td>Washed &amp; suspended 1 week. Fresh slides</td>
<td>Store max. 4 days Fresh slides</td>
<td></td>
</tr>
<tr>
<td>Minimum adult cell count</td>
<td>2000</td>
<td>6000</td>
<td>1000</td>
<td>6000</td>
<td>Not stated</td>
<td>10,000</td>
</tr>
<tr>
<td>Refer to flow</td>
<td>&gt;2.5mL</td>
<td>&gt;1 vial</td>
<td>&gt; 1 vial</td>
<td>&gt;4.4mL</td>
<td>N/S</td>
<td>&gt;2mL</td>
</tr>
</tbody>
</table>
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Quality control

• Little scientific guidance from the literature as a basis for the 1 in 10 positive control used
• Suggestion by ANZSBT (FMH Guidelines 2002) to run calculated controls with each batch (<20 samples week or once a week if >20 samples)
  – No other laboratories reviewed currently doing this
  – ? Value of staff time, acceptable limits

No positive control is really an “accurate” positive control unless you count it!

• Decision to leave positive control at 10% with review after 12 months
• Decision to continue using fresh QC material each test due to low test sample numbers
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Counting adult cells

- Labs reviewed counted between 1000-6000 adult cells in a manual KT
- BCSH archived guidelines (1999) suggested counting 6000, current BCSH guidelines (2009) suggest 10,000
- Recommendation to change from 6000 to 10,000 was due to the need to reduce the co-efficient of variation (CV) of a manual count

White JL, Milkins CE, Rowley MR, Lubenko A. Transfusion Medicine 2006; 16(S1); 35-36
Gathering the evidence for BCSH FMH Guidelines, counting controversies in FMH.
Issues of manual KT counting

• Questionable value counting 10,000 (BCSH 2009) cells vs 6000 cells (BCSH 1999)
  - in a laboratory where flow cytometry counting large numbers of “events” are utilised for confirmation of FMH
  - in a laboratory with a low volume limit before the sample is referred to flow cytometry
  - Follow-up KTs are used to monitor cell clearance after Rh(D) Ig is issued

• Decision made to change from counting 2000 cells to 6000
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When to refer to flow cytometry

- Currently referring if >2.5mL
- Inter-laboratory CV% averages 38% for bleeds to 2-6mL in UK NEQAS exercises
- So for a target count of
  - 2.5mL: 0.6mL-4.4mL (95% CI = 2SD)
  - 3mL: 0.72 – 5.28mL (95% CI = 2SD) which is approaching the limit of 1 vial of Rh(D) Ig

- Decision to leave current limit to refer if >2.5mL FMH
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Stain validation

- Package insert recommended counterstaining for 10-60 seconds
- Slides sets were counterstained for 10, 20, 30, 40, 50, 60 seconds. No visible difference in staining quality of foetal cells & no increase in background staining of adult cells
- Decision made to counterstain for 30 seconds
- Issues with precipitate forming from eluting solution. Stain works better if slides are on a staining rack as opposed to staining jars and washed at least 30 seconds after the eluting stage

- Stain stability– slides fixed and stained with same eluting solution & counterstain each day for 7 days. Some increase in precipitate background, no change in quality of adult or foetal cells
- Decision: make up eluting solution fresh for each batch of tests. Eosin and 80% ethanol for fixing stable for 7 days
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Stain validation

- Patient KT slides run in parallel using the Fronine Kit and the in-house method for period of four weeks
- 21 patient samples showed the sample result using both methods during the trial (all <1mL or no foetal cells seen)
- 1 patient sample eluted poorly with both test methods and was proven by HPLC to have an increased HbF commonly associated with pregnancy
- 1 patient sample showed a heterogenous distribution of HbF by both methods and was shown by HPLC to have delta-beta thalassemia
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Stain validation

- 9 samples, with target ranging from 0.125% to 1.6% foetal cells were stained via both methods and each slide was counted at least five times (once by different scientists) to account for variation in manual counting. Scientists did not know which staining technique they were counting nor were aware of the target value.
- Samples were made in the laboratory using the method in the ANZSBT Assessment of FMH Guidelines (2002) and stained using both methods.
- Of the 55 manual counts performed, all but 2 fell within RCPA limits (1 Fronine, 1 in-house) and both of these counts were performed by the same scientist.
- <0.18% +/- 0.06%
- >0.18% +/- 40% from the target value
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MULTIPLE REGRESSION PLOT

Test result
Foetal cell%

Fronline Kit
In house method

<table>
<thead>
<tr>
<th></th>
<th>Test result</th>
<th>Target foetal cell %</th>
<th>Passing and Bablok regression.</th>
<th>S.E. of estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In house</td>
<td>Target</td>
<td>Deming regression: 1.115, Intercept: -0.05796, S.E. of estimate: 0.207</td>
<td>Compare +ve slope</td>
</tr>
<tr>
<td></td>
<td>Fronine</td>
<td>C.Coeff: 0.9373</td>
<td>Passing &amp; Bablok: 1.056, Intercept: 0.0104</td>
<td></td>
</tr>
<tr>
<td></td>
<td>In house</td>
<td>Target</td>
<td>Deming regression: 0.9853, Intercept: 0.09249, S.E. of estimate: 0.2302</td>
<td>Compare +ve slope</td>
</tr>
<tr>
<td></td>
<td>Fronine</td>
<td>C.Coeff: 0.8903</td>
<td>Passing &amp; Bablok: 1.034, Intercept: 0.04823</td>
<td></td>
</tr>
</tbody>
</table>
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**Precision test**

- Target value 0.4%
- Two slides made and stained with each method
- Counted 9 times, each time by different scientist

<table>
<thead>
<tr>
<th></th>
<th><strong>Fronline</strong></th>
<th><strong>In house method</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>9.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Low value</td>
<td>0.37</td>
<td>0.38</td>
</tr>
<tr>
<td>High value</td>
<td>0.53</td>
<td>0.65</td>
</tr>
<tr>
<td>Sum</td>
<td>3.9</td>
<td>4.02</td>
</tr>
<tr>
<td>Mean</td>
<td>0.4333</td>
<td>0.4467</td>
</tr>
<tr>
<td>Median</td>
<td>0.43</td>
<td>0.43</td>
</tr>
<tr>
<td>Variance</td>
<td>0.003225</td>
<td>0.00715</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.05679</td>
<td>0.08456</td>
</tr>
<tr>
<td>Coefficient of Variation%</td>
<td>13.11</td>
<td>18.93</td>
</tr>
</tbody>
</table>
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Conclusion

• I am never staining or counting another Kleihauer ever again
• Introduction of the Fronine kit presents an opportunity to decrease staff exposure to hazardous chemicals, save staff time and save administrative time after the changes to IVD are introduced
• The Fronine Kleihauer test kit performs in an equivalent manner to the existing in-house method and was implemented at St Vincent’s Pathology after staff training
Acknowledgements

Colin Horner, Senior Scientist (and statistics king) Biochemistry, St Vincent’s Health

Friend Maviza, RCPA
Questions?