Screening for Alpha Thalassaemia:
Classical HbH Staining vs \textit{i-LAB}* Immunochromatographic Method

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- Alpha thalassaemia syndromes result from the defective synthesis of alpha chains.
- As a result in adults there is a relative excess of beta chains, which are able to form tetramers (Beta4). This tetramer is called Haemoglobin H (HbH).
- To detect HbH, red cells are incubated with a supravital redox dye. Oxidation and precipitation of the HbH takes place within the red cell.
- The diagnosis of any type of alpha thalassaemia syndrome is important to identify couples who both have an alpha thal trait (Alpha 1 or Alpha 2) to have a child with either HbH disease (3 gene deletions) or Barts Hydrops Foetalis (4 gene deletions) which can lead to moderate to severe anaemia or death in utero.
- Alpha Thal Trait can be provisionally screened by its microcytic red cells and by exclusion of iron deficiency and excluding Beta-Thalassaemia by HbA2 and HbF analysis.
- A positive HbH stain will usually confirm the diagnosis of an Alpha Thalassaemia trait.
- In cases where HbH cannot be detected, PCR analysis, though expensive and not routinely available, is the definitive diagnostic method, but generally not for screening.
PROBLEMS WITH H-BODIES STAINING PROCEDURE

- Blood film must be made fresh (within 24 hours).
- Incubation with stain takes 2 hours (at 37 deg C)
- Two slides must be made and each reviewed for at least 10 minutes (total 20 minutes)
- Staff issues to do with posture and maintaining concentration with microscopic review with numerous slides.
- The number of cells containing inclusions depends on the type of alpha thalassaemia syndrome.
- In HbH disease the detection of HbH in almost all red cells should be apparent.

In an alpha thalassaemia trait with 2 genes (Alpha 1) deleted HbH inclusions may only be found in 1:1000 to 1:3000 red cells. In alpha thalassaemia trait with single gene deletion (Alpha 2), HbH Bodies become very difficult to detect and are often missed or just not present.

Our laboratory was keen to trial an immunochromatographic strip method in order to improve productivity in this area, without loss in our ability to diagnose Alpha-Thalassaemia
Rapid i-LAB Immuno-Chromatographic Test (ICT) Method for Screening for Alpha Thalassaemia

- The i-LAB IC test is a rapid strip chromatographic immunoassay for the qualitative determination of Hb Barts in a red cell haemolysate.
- It uses an antibody to Hb Barts, which is present in most cases of Alpha Thalassaemia to some extent.
- The ICT method can identify the presence of Alpha thal trait 1 (2 gene), homozygous Alpha trait 2, HbH disease, HbH-H-Constance Springs.

**Results:**
- **POSITIVE**
- **WEAK POSITIVE**
- **NEGATIVE**

**CONTROL BAND**
Comparative Study: HbH Stain Method vs i-LAB ITC Method

- 133 samples for routine Thalassaemia studies were tested for HbH cells using our Brilliant Cresyl Blue staining method
- The same samples were setup for the ITC method and results compared.
- A cohort of 20 samples were also setup with a basic PCR method (7 determinants)
- There was > 95% agreement for positives between both methods
- There were a number of false positives (14%) with respect to the i-LAB ITC method compared to the HbH stain
- This resulted in a lower “specificity” value of 86%
- However 4 of these “False Positives”, were actually found to be positive by PCR.
- Thus the ITC method may better reflect the PCR method

### RESULTS

<table>
<thead>
<tr>
<th></th>
<th>HbH Bodies POS</th>
<th>HbH Bodies Negative</th>
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</thead>
<tbody>
<tr>
<td>ICT POS</td>
<td>44 (TP)</td>
<td>12 (FP)**</td>
</tr>
<tr>
<td>ICT NEG</td>
<td>2 (FN)</td>
<td>75 (TN)</td>
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</table>

**TP=True positive, TN=True negative, FP=False positive, FN=False negative**

**4/12FP were shown to be positive by PCR**
CONCLUSION

- This data shows that the i-LAB ITC kit method is capable of standing on its own as a part of a screen for most cases of Alpha Thalassaemia in the routine laboratory.

- It is probably more sensitive than the HbH Stain method as we observed better compliance with it to our PCR method.

- In saying the above, it is much easier to set up, and takes much less time to perform than the HbH stain which will result in better productivity and staff morale as the HbH stain test can be tedious.

- This study was carried out in the Haematology Department, Douglass Hanly Moir Pathology, Macquarie Park, Sydney, Australia.

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