Standardising Leucocyte PNH Clone Detection: An International Study

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INTRODUCTION

Paroxysmal Nocturnal Haemoglobinuria (PNH) is a haematopoietic stem cell disorder, characterised by somatic mutations within the PIG-A gene that leads to absence or reduced expression of GPI linked antigens by all blood cells derived from this mutated stem cell. PNH patients have underlying bone marrow failure often accompanied by haemolytic anaemia and an increased risk of thrombosis. Clinical management can be challenging therefore accurate and rapid diagnosis of PNH is essential. Flow cytometry is now recognised as the method of choice to detect/quantify PNH RBC and WBC clone size (1). However, the UK NEQAS proficiency testing (PT) programme has identified large variance in testing protocols and reagent selection in PNH leucocyte clone detection (2). Thus, in an attempt to achieve standardisation, UK NEQAS LI studied the use of FLAER as a single colour reagent with a standardised protocol and found it produced comparable results to those obtained using multi colour techniques (3). This follow up study further examines the use of FLAER by including it in a standardised reagent cocktail, integrating this into an internationally standardised PNH testing protocol (4) and comparing this to in-house methods.

METHOD

UK NEQAS LI issued 3 stabilised samples (2) to 19 international centres. These were selected based on their UK NEQAS LI PNH PT programme performance and associated laboratory experience.

- Samples were manufactured to contain 0%, 0.1% and 8% PNH leucocyte clone populations.
- Centres were issued with standardised, platform specific protocols and pre-titrated antibody cocktails that had been optimised for use on their flow cytometer. Reagent combinations and details of fluorochromes and clones used are shown in table 1.
- Centres tested all samples using both the centrally supplied standardised reagents/protocol (figure 1) and their in-house methods and local reagents.
- All results were returned to UK NEQAS LI for data analysis.

Table 1: Standardised panels for each flow cytometer type with details of fluorochrome and each of reagent shown in brackets.

<table>
<thead>
<tr>
<th>Standardised Reagent Panels</th>
<th>0% PNH</th>
<th>0.1% PNH</th>
<th>8% PNH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beckman Coulter Cytometers</td>
<td>Neutrophils</td>
<td>Monocytes</td>
<td>FLAER ALEXA488</td>
</tr>
<tr>
<td>Becton Dickinson Cytometers</td>
<td>Neutrophils</td>
<td>Monocytes</td>
<td>FLAER ALEXA488</td>
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<tr>
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RESULTS

- Both approaches were in consensus when reporting the presence or absence of PNH clones.
- Average leucocyte PNH clone size was similar for both approaches.
- For both the PNH sample with 8% clone and the High Resolution PNH sample with 0.1% clone there was lower variation around the median for the standardised approach compared to ‘in-house’ methods for both the neutrophil and monocyte populations (see table 2).

DISCUSSION

Whilst the overall medians between the two approaches for leucocyte PNH clone detection (neutrophils and monocytes) were similar, the standardised approach had lower variation around the median compared to ‘in-house’ methods for these populations.

Our results highlight the importance of reagent choice and a standardised approach in performing PNH analysis, even amongst experienced laboratories.

Whilst this study used highly experienced laboratories additional studies and panel optimization are planned to evaluate if greater concordance can be achieved among less experienced laboratories and therefore further underline the benefits of standardisation.

ACKNOWLEDGEMENTS

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REFERENCES


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