Step 4 – Report*

Standardised terminology for paroxysmal nocturnal haemoglobinuria (PNH) clones

A glycosylphosphatidylinositol (GPI)-deficient population of cells is the terminology used to describe the absence of GPI-linked proteins on red blood cells (RBCs), granulocytes and monocytes. PNH clone size is determined by the size of the GPI-deficient population in the largest of the white blood cell lineages tested (i.e. granulocytes or monocytes). The sensitivity of the assay should be determined by performing a ‘spiking’ assay (see “Spiking assay”), which describes the lower limit of detection.

<table>
<thead>
<tr>
<th>GPI-deficient population</th>
<th>Standardised terminology</th>
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<tbody>
<tr>
<td>&gt;1%</td>
<td>Population of GPI-deficient cells (granulocytes or monocytes) or ‘PNH clone’</td>
</tr>
<tr>
<td>0.1–1%</td>
<td>Minor population of GPI-deficient cells (granulocytes or monocytes) or ‘minor PNH clone’</td>
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<tr>
<td>&lt;0.1%</td>
<td>Rare GPI-deficient cells or ‘rare cells with PNH phenotype’</td>
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GPI, glycosylphosphatidylinositol

Reporting PNH test results

- Report the proportions (%) of GPI-deficient cells in each lineage tested:
  - Type III RBCs (GPI-deficient; CD235a+, CD59-)
  - Type II RBCs (partially GPI-deficient; CD235a+, CD59-intermediate)
  - Total proportion of Type III plus Type II RBCs
  - GPI-deficient granulocytes/neutrophils (CD15+, fluorescent aerolysin negative (FLAER-), CD157-)
  - GPI-deficient monocytes (CD64+, FLAER-, CD157-)
- To avoid confusion, do not report Type I cells with normal CD59 expression.
- Avoid the use of ambiguous language, such as ‘positive’ or ‘negative’, when reporting test results (eg ‘the PNH test was negative’ may be interpreted as that the test was negative for a marker and, therefore, positive for PNH).
  - Clear language such as “deficiency of GPI-linked proteins” should be used.1 Laboratory results can then be combined with clinical findings to confirm or determine the clinical diagnosis.
- Include details of which reagents were used, to confirm that high-sensitivity flow cytometry was appropriately performed.
- Report the level of assay sensitivity separately for RBCs and white blood cells (WBCs) to report the lower limit of detection in each lineage (ie how many PNH cells need to be present for them to be detected, compared with background levels in normal samples?).
- Include results from current and previous assessments to allow clinicians to easily observe any trends in PNH clone size over time.
Interpretation of PNH test results

- In the case of minor or significant GPI-deficient populations/PNH clones, further testing relating to haemolysis may be indicated to determine the next steps in patient management.

- If supporting clinical evidence is provided indicating aplastic anaemia or myelodysplastic syndrome, a recommendation for further testing should be included on the report.

Reference


*These protocols were developed in close collaboration with Mrs Andrea Illingworth of Dahl-Chase Diagnostic Services in Bangor, ME, USA, Drs Thomas Matthes and Mathieu Hauwel of the Swiss Flow Cytometry School at the University Hospital of Geneva, Switzerland, and Dr Iuri Marinov of Hematology and Blood Transfusion in Prague, Czech Republic.