UK NEQAS

UNITED KINGDOM NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEMES FOR LEUCOCYTE IMMUNOPHENOTYPING

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Assessment Of Fluorescently Labelled Aerolysin And A Standard Protocol For The Detection Of Paroxysmal Nocturnal Haemaglobinuria (PNH) Cell Populations

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INTRODUCTION

PNH is a rare acquired haematopoietic stem cell disorder. It is caused by a genetic mutation of *PIG-A* within haematopoietic stem cells and therefore all lineages of blood cell are subsequently affected.

Whilst there is consensus as to the best approach for testing erythrocytes, there is no agreement on best practice for testing granulocyte populations.

UK NEQAS LI produced a standardised protocol for PNH testing and issued it in combination with fluorescently labelled aerolysin (FLAER) as a standardised reagent to all participants in the PNH External Quality Assessment (EQA) programme. The study then assessed the performance of the standard reagent/protocol combination against methods currently in use in the EQA programmes.

METHOD

- UK NEQAS LI issued stabilised samples to 104 laboratories worldwide for PNH population detection[1].
- Participants were issued with 2 x 1ml aliquots of stabilised whole blood (one normal, one PNH sample), a standardised testing protocol and 40ul of liquid FLAER (Figure 1).
- Participants tested samples using their usual technique and the standardised protocol and reagents (Figure 2).
- Results for both analyses were returned to UK NEQAS LI for analysis.

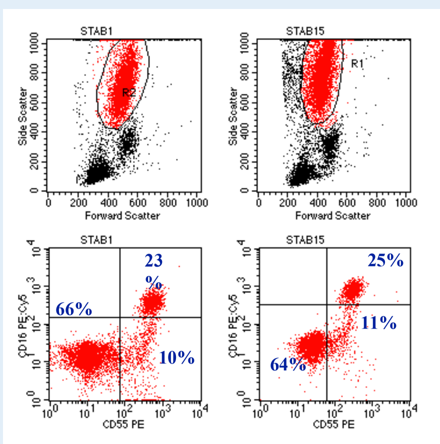


Figure 1: Flow cytometric analysis of stabilised granulocytes at time points day 1 (left) and day 113 (right) demonstrating sample stability

Antigen	n	PNH population %	CV%
Aerolysin	40	98.1	3.8
CD16	50	97.4	10.8
CD24	43	98.2	4.4
CD55	44	88.5	47.6
CD59	49	88.1	50.5
CD66b	34	98.0	4.1
FLAER (Study)	84	96.6	7.2

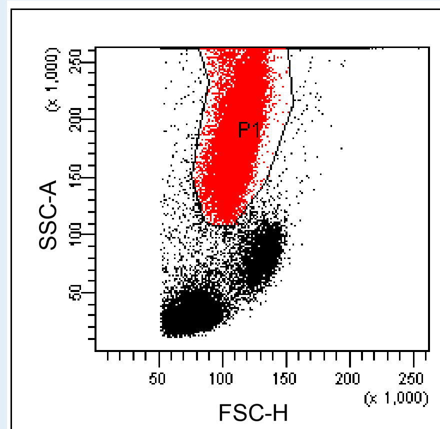
Table 1: Comparison of CVs for different antigens when used for granulocyte PNH detection

RESULTS

- Using a level of sensitivity of 1%[2] the data was examined for accuracy of testing.
- Routine analysis gave 16.8% incorrectly reporting a PNH clone on the normal sample.
- Standardised protocol gave a 25.3% incorrectly reporting a PNH clone on the normal sample.
- Neither technique failed to report the clone on the PNH sample.
- Precision of analysis was examined using the co-efficient of variation (CV) of the reported PNH population (See Table 1).

CONCLUSION

- Standardised protocol produced CVs comparable to multi colour techniques.
- Incorrect identification rates of PNH clones on the normal sample were higher with the standardised protocol.
- First time users of FLAER were twice as likely to give an incorrect result.
- These finding suggest a lack of familiarity with the use of FLAER reagent and adoption of the standardised protocol.
- FLAER in the study was used in a single colour technique, and so a subsequent multi colour follow on study is planned to further evaluate the use of FLAER with a standardised protocol.



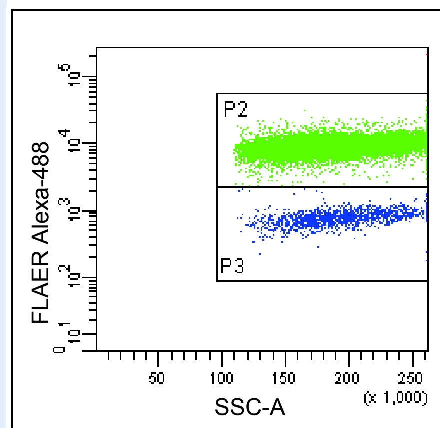


Figure 2 : Gating strategy used for identification of granulocyte populations and FLAER staining patterns in the standardised protocol

References

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2. Borowitz MJ, Craig FE, DiGiuseppe JA, Illingworth AJ, Rosse W, Sutherland DR, Wittwer CT and Richards SJ. Guidelines for the diagnosis and monitoring of Paroxysmal Nocturnal Hemoglobinuria and related disorders by flow cytometry. Clinical Cytometry 2010; 78B: 211-230.

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