UK NEQAS

United Kingdom National External Quality Assessment Service For Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
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Current international Flow Cytometric Practices for the Detection and Monitoring of Paroxysmal Nocturnal Hemoglobinuria (PNH) Clones

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INTRODUCTION

UK NEQAS for Leucocyte Immunophenotyping is an international External Quality Assessment (EQA) provider covering most areas of flow cytometry. In order to ascertain current flow cytometric practices amongst participating centres and help ensure that the programmes meet the needs of participants as defined by their clinical practices, a dedicated questionnaire was issued to all participating centres (n=1587) to survey current and planned changes in flow cytometric techniques. The PNH section of the questionnaire was to assess concordance with international guidelines.

METHOD

- UK NEQAS LI issued an internet based questionnaire designed using a commercial software package
- Questionnaire featured several sections, each related to different aspects of flow cytometry
- The PNH section focussed on adherence to recent international guidelines
- Returned results (n=105) for the PNH section were downloaded into Microsoft Access and analysed

RESULTS

- Only 14 laboratories (13%) were identified using a method that followed the international consensus guidelines[1]
- No two laboratories were using identical methods
- 5 Laboratories only tested 1 population of cells (either Red Blood Cells (RBCs) or granulocytes)
- Of these 3 tested only (RBCs) despite 48% of PNH cases not having a RBCs clone of >3%[2]
- Large variation in gating and testing protocols employed by laboratories, with 784 possible combinations for gating and 1014 for testing (see table 1).
- 44% of laboratories testing RBCs did not perform the required two washing steps of samples before acquisition, potentially leading to missed RBCs clones[1]
- GPI linked antibodies incorrectly being used in gating protocols e.g. CD14, CD16, CD24 (see table 2)
- No consensus on optimal GPI linked antibody selections for PNH testing (see table 3)
- Use of CD55 and CD59 for granulocyte PNH testing persists despite weaker separation of populations[1] and evidence of higher result variation[3]
- Post analytical variables also showed lack of concordance, e.g. Levels of sensitivity for RBCs, granulocytes and monocytes ranging from <0.01%-5%
- Lack of consensus was also seen in the reporting of results; with 12% recording clone present/clone absent only, 44% recording percentage clone size only and 29% using a combination of both.

Cell Population Targeted (number of laboratories providing full details of method)	Number of Different Strategies Used	Minimum Number of Antibodies Used (method and number of users)	Maximum Number of Antibodies Used (method and number of users)	Most Popular Method (number of users)
Gating Protocols				
Granulocytes (n=91)	14	0 (Forward scatter/ Side scatter n=2)	5 (CD15/CD33/CD45/CD64/ CD11b n=1)	CD15/CD45 (n=13)
Monocytes (n=75)	14	1 (CD45 n=4; CD33 n=23; CD64 n=7; CD14 n=4)	3 (CD15/CD33/CD45 n=7; CD33/CD45/CD64 n=1; CD15/CD33/CD64 n=1)	CD33 (n=23)
RBCs (n=72)	4	0 (Forward scatter/ Side scatter n=9)	2 (CD45/CD235a n=8)	CD235a (n=51)
Testing Protocols				
Granulocytes (n=92)	26	1 (FLAER n=8; CD24 n=6; CD66b n=2; CD14 n=1)	5 (CD24/CD55/CD59/ CD66b/FLAER n=2)	FLAER CD24 (n=36)
Monocytes (n=79)	13	1 (FLAER n=8; CD1 4 n=24)	4 (CD14/CD55/CD59/ FLAER n=1)	FLAER CD14 (n=33)
RBCs (n=80)	3	1 (CD55 n=1; CD59 n=61)	2 (CD55/CD59 n=18)	CD59 (n=61)

Table 1: Variation in PNH Target Population Gating and Testing Protocols

Antibody/ Method	Gating of Target Cells (% of users)		
	Granulocytes (n=91)	Monocytes (n=75)	RBCs (n=72)
CD11b	1 (1%)	1 (1%)	
CD14		6 (8%)	
CD15	56 (62%)	13 (17%)	
CD16	1 (1%)		
CD24	1 (1%)		
CD33	42 (46%)	55 (76%)	
CD45	44 (48%)	33 (44%)	12 (17%)
CD64	4 (4%)	14 (17%)	
CD235a			59 (82%)
Forward scatter/ Side scatter	2 (2%)		9 (13%)

Table 2: Method and Antibody usage rates in laboratory panels for PNH gating

Antibody	Testing of Target Cells (% of users)		
	Granulocytes (n=92)	Monocytes (n=79)	RBCs (n=80)
CD14	2 (2%)	64 (81%)	
CD16	20 (22%)		
CD24	65 (71%)	2 (3%)	
CD55	11 (12%)	9 (11%)	19 (24%)
CD59	10 (11%)	5 (6%)	79 (99%)
CD66b	20 (22%)		
FLAER	79 (86%)	50 (63%)	

Table 3: Antibody usage rates in laboratory panels for PNH testing

CONCLUSION

- No evidence of testing consensus despite availability of numerous publications and guidelines.
- Use of incorrect practices that could lead to PNH clones being missed; e.g. insufficient washing of RBCs and testing only one population of cells.
- No consensus seen in overall panel design with regards to any antibody.
- Findings of the survey appear to show the majority of laboratories lack an understanding of PNH and the required testing methods
- Results suggest there is an urgent need for better adoption of PNH guidelines and for better education of testing centres.

- Further information may be obtained by visiting www.ukneqasli.co.uk or by emailing matthew.fletcher@ukneqasli.co.uk

References

1. 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.
2. Sutherland R, Kuek N, Azcona-Olivera J, Anderson T, Acton E, Barth D, Keeney M. Use of a FLAER-Based WBC Assay in the Primary Screening of PNH Clones AJCP 2009 132:564-572
3. Richards SJ, Whitby L, Cullen MJ, Dickinson AJ, Granger V, Reilly JT, Hillmen P, and Barnett D. Development and evaluation of a stabilized whole-blood preparation as a process control material for screening of paroxysmal nocturnal hemoglobinuria by flow cytometry. Cytometry Part B 2009; 76B: 47–55.