UKNEQAS United Kingdom National External Quality Assessment Service For Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust
Excellence as standard

Current international Flow Cytometric Practices for the testing of Leukaemia/Lymphoproliferative Disorders

A Whitby L Whitby M Fletcher JT Reilly D Barnett

United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping (UK NEQAS LI), Sheffield, UK

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.

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
- 60% of participants enrolled in the leukaemia programme (164 out of 273) returned results for the Leukaemia/ Lymphoproliferative section
- Returned results were downloaded into Microsoft Access and analysed

RESULTS

- No two centres used identical techniques
- Returned data was compared to the Euroflow[1] ALOT and LST screening tubes (see table 1 and table 2) to ascertain (i) if the Euroflow approach could be implemented and (ii) how much concordance there was in panel use

Euroflow ALOT Tube	CD3	CD45	Myeloperoxidase	CD79a	CD34	CD19	CD7
All Laboratories (n=164)	162 (99%)	160 (98%)	148 (90%)	127 (77%)	162 (99%)	163 (99%)	160 (98%)
Laboratories with ≥8 colour capability (n=95)	93 (98%)	91 (96%)	87 (92%)	79 (83%)	92 (97%)	92 (97%)	92 (97%)

Table 1: Comparison of antibody usage rates for Euroflow ALOT tube combination

* Euroflow LST Tube	CD20	CD4	CD45	CD8	Lambda	CD56	Kappa	CD5	CD19	CD3	CD38
All Laboratories (n=164)	160 (98%)	162 (99%)	160 (98%)	160 (99%)	156 (95%)	152 (93%)	157 (96%)	163 (99%)	163 (99%)	162 (99%)	153 (93%)
Laboratories with ≥8 colour capability (n=95)	91 (96%)	93 (98%)	91 (96%)	93 (98%)	86 (91%)	90 (95%)	87 (92%)	93 (98%)	92 (97%)	93 (98%)	89 (94%)

Table 2: Comparison of antibody usage rates for Euroflow LST tube combination * Data not surveyed forTCRγδ

- Majority of laboratories have the necessary antibody combinations (TCRγδ not surveyed) to implement the Euroflow ALOT and LST screening tubes (See tables 1 and 2)
  - 119/164 (73%) of all laboratories could implement the Euroflow ALOT screening tube
  - 136/164 (83%) of all laboratories could implement the Euroflow LST screening tube
  - 108/164 (66%) of all laboratories could implement both Euroflow screening tubes
- Of the 95/164 (58%) laboratories with ≥8 colour capability (8 colours being the minimum requirement to implement Euroflow protocols)
  - 74/95 (78%) could implement the Euroflow ALOT screening tube
  - 79/95 (83%) could implement the Euroflow LST screening tube
  - 67/95 (71%) could implement both Euroflow screening tubes
- **Despite availability of antibodies, only 11/164 (7%) of centres use Euroflow screening tubes routinely**
- When asked if Euroflow panels had been implemented partially or fully, 70/164 (43%) stated they had implemented the panels partially and 7/164 (4%)had implemented them fully. However, 23/164 (14%) stated they were unaware of Euroflow – of these 14/23 (61%) were European
- There is no evidence of 100% concordance in antigen selection or availability for any antigen across all centres
  - Highest levels of agreement for antigen selection observed was 99% usage for CD3, CD4, CD5, CD8, CD19 and CD34
- CD79a was the most underused antibody of those recommended by Euroflow, utilised by 77% of all centres surveyed and 83% using ≥8 colours
- Data was also analysed with respect to the analysis software used by laboratories
  - 92% laboratories routinely using analysis software supplied with their flow cytometer – 35% Beckman Coulter, 57% Becton Dickinson
  - Only 8% laboratories routinely using Cytognos InfinicytTM – a pivotal part of Euroflow panel analysis

CONCLUSION

- No evidence of harmonisation despite availability of guidelines and antibodies
- 67/95 (71%) of labs in our survey have the capability to implement the Euroflow ALOT and LST screening tubes but failed to do so effectively
- Of all labs surveyed, 7 stated they used the Euroflow panel. However, none of these centres stated that they used all the required antibodies
- Despite the acceptance by laboratories that Euroflow approach has been demonstrated to be scientifically sound, and 71% of laboratories with ≥8 colour capability can implement both tubes, no single laboratory in our survey is either using Euroflow, or where they state they do, it is not implemented correctly.
- Results suggest a fragmented/partial approach to the adoption of Euroflow guidelines
- Urgent need for full implementation of testing techniques that provide standardisation and harmonisation
- For further information visit www.ukneqasli.co.uk or email alison.whitby@ukneqasli.co.uk

References

1. van Dongen JJ, Lhermitte L, Böttcher S, Almeida J, van der Velden VH, Flores-Montero J, et al.EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia. 2012;26(9):1908-75.