The ISHAGE Protocol: Are We Doing It Correctly?

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Introduction

Flow cytometric CD34+ Stem cell enumeration is a routine pre-stem cell harvest procedure with a variety of gating protocols having been described, the most popular of which is the ISHAGE protocol (Sutherland et al (1996), J. Hematotherapy 5:213-226). UK NEQAS CD34+Stem Cell Enumeration programme has 258 international participants with 201 (80%) routinely reporting that they use the ISHAGE protocol. Recently, 2 laboratories were identified as poor performers, the cause of which was attributed to incorrect ISHAGE protocol usage/setup. This prompted us to question if others were also making similar errors and how these would impact on individual EQA performance. Consensus target values were calculated from single platform ISHAGE protocol data (defined as the predicate method) and laboratories that used ISHAGE (both correct and incorrect usage) compared directly to those values.
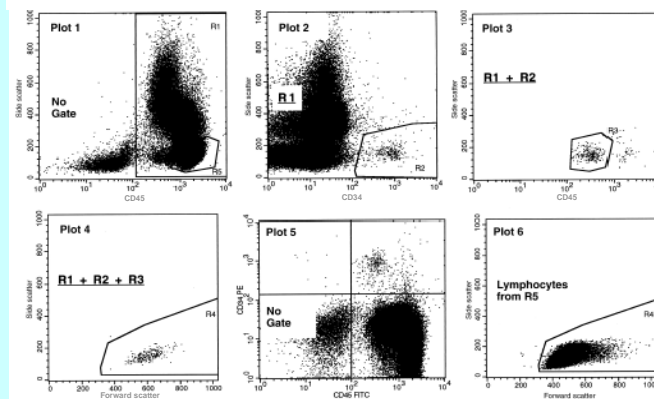
Methods and Materials

Two stabilised blood samples were dispatched to all participants in the UK NEQAS CD34+ Stem cell enumeration programme for the 0801 trial (03/03/2008 – 21/03/2008). Following analysis, the CD34+ stem cell count for each sample (cells/µl) was returned to UK NEQAS where data analysis and performance criteria was undertaken. Satisfactory performance is based upon how close a participants result is to the median for the sample. Dot plots and gating strategies were requested from participants and these were compared to the stated ISHAGE protocol. The data was then analysed and average scores, CVs, and running scores were calculated to ascertain if the incorrect use of a gating strategy would adversely affect performance.

Results

- Of all the gating strategies returned (Table 1) 81/101 were using the ISHAGE gating strategy (Figure 1). 36/81 were subsequently identified to be using it incorrectly.

- The primary error (Graph 1) was the absence of the R5 gate in R1 to identify lymphocyte position, essential to pinpoint true CD34+ stem cells.

- Another common error was the absence of the correct gate defining CD45 dim cells on the CD45/SSC plot.

- One participant was found to be using CD3 positive cells to define lymphocyte position.

- 2 participants believed that they were using ISHAGE. They were actually using a different gating strategy (Bender).

- Assessing the individual performance by comparing the average scores showed a 20% increased chance of failing an EQA exercise if the ISHAGE gating strategy was incorrect.

- Target values calculated from single platform ISHAGE data (Keeney et al (1998), Cytometry 34:61-70), had a higher overall coefficient of variation (CV) when using ISHAGE incorrectly compared to those using ISHAGE correctly

Table 1- Breakdown of Gating strategies used

Gating Strategy	Gating strategies returned for study	Gating strategies from trial
ISHAGE	81%	78%
STEMKIT	7%	8%
PROCOUNT	5%	4%
MILAN	1%	6%
BENDER	7%	0.52%

Figure 1 : ISHAGE Gating Strategy – derived from ISHAGE Gating Protocol (Sutherland et al (1996))

Graph 1: Breakdown of Application of ISHAGE Gating Strategy

57%
32%
2%
1%
1%
5%
1%
1%

- Correct
- Missing gate
- Gating on wrong parameter
- Using wrong antibody
- Missing plot and gate
- Wrong gate and parameter
- Wrong gate and parameter and missing gate
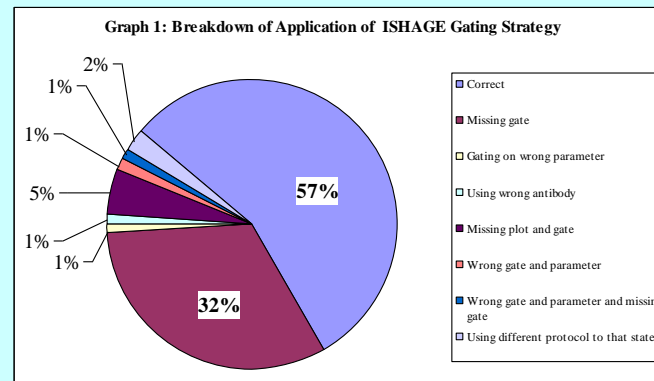- Using different protocol to that stated

Discussion

- The evidence gathered from this study shows that 43% of ISHAGE gating strategy users are actually using the strategy incorrectly.

- 39% of users failed to show the R5 lymph gate.

- Target values calculated from ISHAGE protocols users showed the overall CV for incorrect single platform ISHAGE users was more than 10% higher than that seen for correct single platform ISHAGE users (the predicate method).

- Assessing individual performance showed that there was a 20% increased chance of failing an EQA exercise if the ISHAGE gating strategy was incorrect when compared to those using it correctly

- The high number of incorrect laboratories is skewing data and hiding genuine issues

- For trial 0802, 31 participants fell into unsatisfactory performance. Using revised running scores derived from correct ISHAGE user data, 53 participants fell into unsatisfactory performance – an increase of 70%.

Conclusion

From our evidence it would appear that the ISHAGE protocol has been poorly understood by at least 43% of ISHAGE Gating Strategy users. It would be prudent for any centres currently performing this assay to check that their gating strategy matches the recommended method.

References

1. Sutherland D. R, Anderson L., Keeney M., Nayar R., Chin-Yee I. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. Journal of Hematotherapy 1996; 5: 213-226

2. Michael Keeney, Ian Chin-Yee, Karin Weir, Jan Popma, Rakash Nayar, D. Robert Sutherland. Single Platform Flow Cytometris Absolute CD34+ Cell Counts Based on the ISHAGE Guidelines. Cytometry 1998; 34: 61-70

Further Information

For further information visit www.ukneqasli.org or contact alison.whitby@btconnect.com