Evaluation of Sysmex Kappa and Lambda Alexa Fluor™488 Conjugated Monoclonal Antibodies for the Determination of B-Cell Surface Light Chains

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INTRODUCTION

Flow cytometric analysis of kappa and lambda surface light chains is an important requirement for assessing clonality in the investigation of B-lymphoproliferative disorders (BLPD)[1].

As such the choice of antibody and fluorochrome conjugate are important when designing an antibody panel. Factors that should be considered include antibody specificity, clone effectiveness in haematological neoplasm investigations, and strength of antigen expression in the populations of interest[2].

Historically, the Haemato Oncology Diagnostic Service at Sheffield Teaching Hospitals NHS Foundation Trust has utilised polyclonal antibodies for the detection of light chains in BLPD investigations. More recently monoclonal kappa and lambda antibodies have become available.

Here we have compared the performance of new monoclonal kappa and lambda Alexa Fluor™488 (AF488) conjugated antibodies with polyclonal kappa and lambda FITC antibodies, examining the median fluorescence intensity of each, and using the stain index as a measure of antibody quality.

METHOD(S)

20 normal and 20 BLPD samples were selected following completion of routine testing. All samples were anonymised and randomised.

The testing of the samples was performed using the following steps:

- Samples were washed twice in warm phosphate buffered saline prior to incubation with the appropriate antibodies
- Four combinations of antibodies were performed for each sample (Table 1). Blood and antibody volumes were as recommended by each manufacturer according to the package insert

Tube	Panel Combination		
1	CD45 Pacific Orange (Sysmex, clone: 2D1)	CD19 APC (Sysmex, clone: LT19)	Kappa AF488 (Sysmex, clone: TB28-2)
2			Kappa FITC (Dako, polyclonal)
3			Lambda AF488 (Sysmex, clone: 1-155-2)
4			Lambda FITC(Dako, polyclonal)

Table 1: Panel combination used for comparison of monoclonal and polyclonal antibodies.

- Following a 10 minute incubation, samples were lysed for 10 minutes using ammonium chloride, then washed twice in PBS
- Samples were acquired on a BD FACSCanto™ II flow cytometer and analysed using Beckman Coulter Kaluza software
- B-lymphocytes were identified on the basis of their CD45/CD19/side scatter expression
- The median fluorescence intensity (MFI) and standard deviation (SD) were determined for the kappa/lambda positive and negative populations as appropriate, and the stain index (SI) calculated using this data:

$$SI = \frac{MFI_{pos} - MFI_{neg}}{2 \times SD_{neg}}$$

For the 20 normal samples tested, there were 80 data sets where a SI could be calculated.

For the 20 BLPD samples there were 76 data sets where a SI could be calculated as 1 sample failed to provide a monoclonal population.

RESULT(S)

Kappa antibodies

- In the BLPD group, the Sysmex monoclonal AF488 antibody gave a higher SI than the Dako polyclonal FITC antibody in 93.3% of cases. Figure 1 is an example illustrating the differences in kappa light chain expression on B-lymphocytes for the two antibodies compared
- Discrimination between kappa positive and kappa negative populations was enhanced using the Sysmex monoclonal AF488 antibody when compared with the Dako polyclonal FITC antibody. An example of this is shown in figure 2
- The Sysmex monoclonal AF488 antibody produced the greatest SI in the BLPD group, as can be seen in figure 3. It can also be seen that more than 25% of the SIs achieved by the Sysmex monoclonal AF488 antibody were greater than the highest SI achieved by the Dako polyclonal FITC antibody
- In the normal group, the Sysmex monoclonal AF488 antibody gave a higher SI than the Dako polyclonal FITC antibody in 60% of cases
- The increase in the SI achieved by the Sysmex monoclonal AF488 antibody over the Dako FITC polyclonal antibody was subjected to a paired t-test and found to be statistically significant (p=0.019)

Lambda antibodies

- In the BLPD group, the Sysmex monoclonal AF488 antibody gave a higher SI than the Dako polyclonal FITC antibody in 50% of cases
- In the normal group, the Sysmex monoclonal AF488 antibody gave a higher SI than the Dako poyclonal FITC antibody in 74% of cases
- Due to the limited number of available BLPD cases expressing lambda, there were insufficient patients with a lambda monoclone for separate statistical analysis

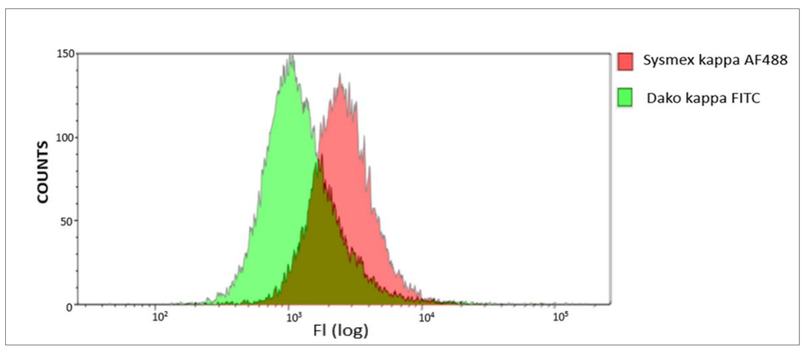

Figure 1: Overlay histogram illustrating kappa expression on B-lymphocytes using Sysmex kappa AF488 (red peak) compared with Dako FITC (green peak)

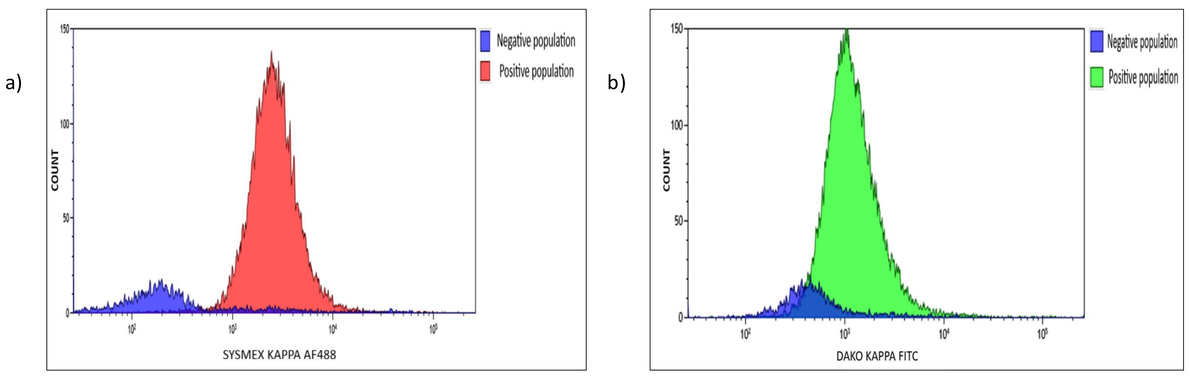

Figure 2: a) Histogram demonstrating kappa expression on CD19 positive (red) and CD19 negative (blue) lymphocytes using Sysmex kappa AF488. b) Histogram demonstrating kappa expression on CD19 positive (green) and CD19 negative (blue) lymphocytes using Dako FITC

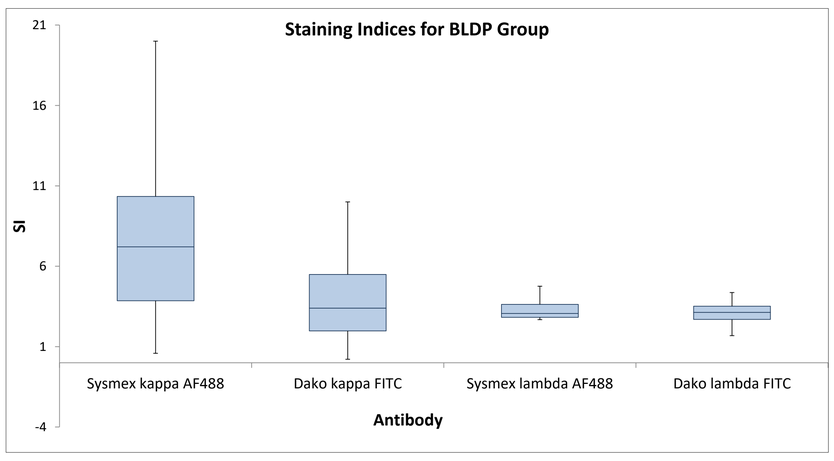

Figure 3: Box and Whisker plot illustrating range of staining indices for the different antibodies in the BLPD group

REFERENCES

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CONCLUSION(S)

The ability to distinguish between B-lymphocyte populations that express kappa or lambda light chains, and those that do not, is a key aspect in the BLPD diagnostic process. This is particularly important in cases where expression is weak, as the positive and negative populations may not be well separated.

Increased discrimination, through greater separation of populations, improved population distribution, or both of these factors, leads to a higher SI. The use of antibodies with a higher SI will aid in efficiently interpreting data in BLPD investigations (Figure 2).

In this study the use of the Sysmex AF488 monoclonal kappa antibody gave higher SIs compared to the Dako polyclonal FITC antibody (Figure 3), and may be a suitable alternative to FITC polyclonal antibodies. More data is required to draw conclusions regarding the lambda antibody comparison.

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