Evaluation of Sysmex Kappa and Lambda PE Conjugated Monoclonal Antibodies for the Determination of B-Cell Surface Light Chains

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INTRODUCTION

The presence or absence of a monoclonal B lymphocyte population is routinely demonstrated using antibodies directed against kappa and lambda surface light chain receptors.

Demonstration of clonality is a vital requirement in the investigation and diagnosis of B-lymphoproliferative disorders (BLPD)[1].It is also important in cases of low level B lymphocytosis to differentiate between reactive causes and early malignancy.

When designing an antibody panel, the choice of reagent and fluorochrome conjugates is important to ensure accurate and precise results. External Quality Assessment data has shown that many laboratories currently use polyclonal kappa and lambda reagents in the assessment of clonality. (UK NEQAS LI Personal communication)

To assess the feasibility of introducing monoclonal reagents into local panel design a study was undertaken to compare Sysmex monoclonal kappa and lambda reagents conjugated to Phycoerythrin **(**PE) with currently utilised polyclonal PE-conjugated kappa and lambda.

METHOD(S)

- 20 normal and 20 BLPD samples were identified as suitable for testing and were anonymised and randomised.
- Cells were washed twice in excess warm phosphate buffered saline prior to incubation with antibodies.
- Each sample was tested using the panel combination as shown in Table One.

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

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

- Following incubation, samples were lysed using ammonium chloride (Sysmex) and then washed twice.
- Samples were acquired on a BD FACSCanto™ II and analysed using BD FACSDiva software using a gating strategy as shown in Figure One.
- Lymphocytes were identified using a CD45/side scatter gate and the B-lymphocyte population identified based on CD19 expression and side scatter characteristics.
- Median fluorescence intensity (MFI) and standard deviation were obtained for the kappa/lambda positive and negative populations.
- The stain index (SI) was then calculated for each of the kappa and lambda reagents.
- For the 20 normal samples tested, there were 80 data sets where a SI could be calculated.
- For the 20 BLPD samples there were only 60 data sets where a SI could be calculated. This was due to no demonstrable expression of kappa in 4 cases and no demonstratable expression of lambda in 6 cases.

RESULT(S)

- Results were split into 2 sections for analysis purposes, normal and BLPD cases.

Normal Cases

- The results obtained for the normal group showed that for both kappa and lambda reagents, there were no observable differences between manufacturers or in terms of the average SI obtained.

BLPD Cases

- Overall the SIs obtained when testing BLPD samples with each reagent were higher when using Sysmex monoclonal PE reagents compared to when testing the identical samples using the equivalent Dako polyclonal reagent. See Figure Two.

- Analysis of the results showed that the Sysmex monoclonal PE kappa and lambda reagents gave a higher SI than the corresponding Dako polyclonal PE reagents in >81% and >61% of BLPD cases respectively.

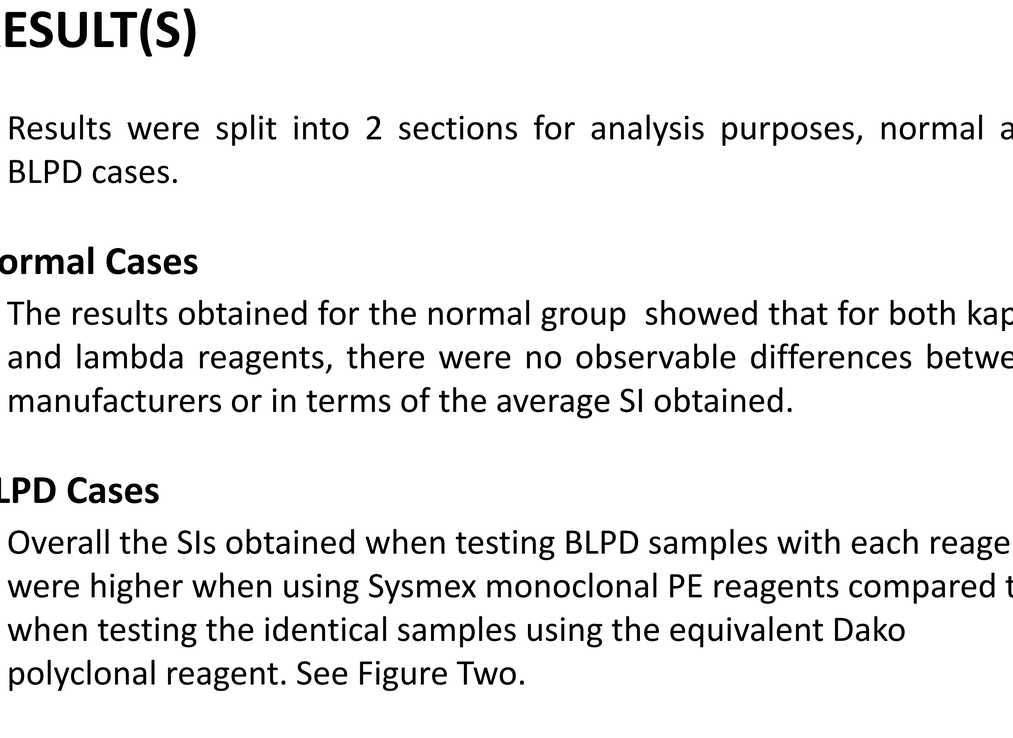

Figure Two: Box and Whiskers Plots of Staining Indices per Reagent

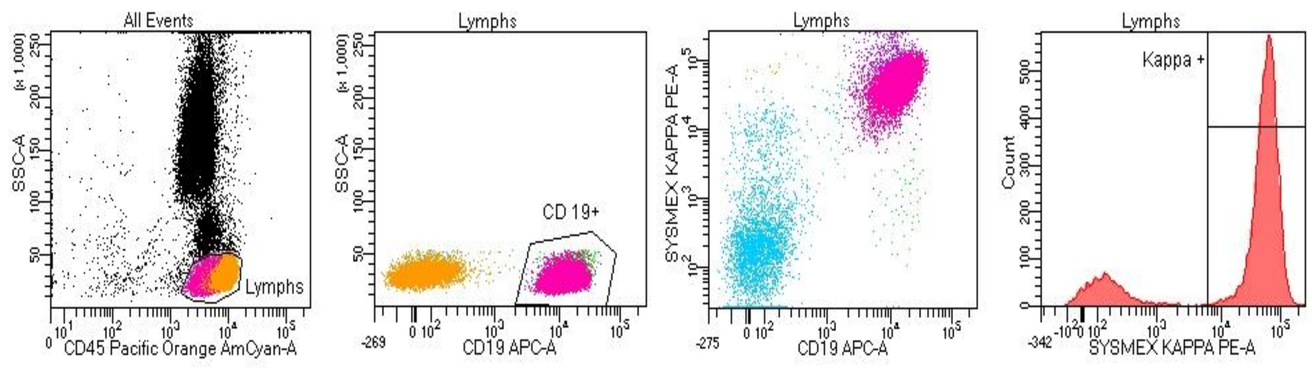

Figure One: Illustration of dot plots and gating strategy employed

CONCLUSION(S)

- When assessing the normal and BLPD cases, Sysmex monoclonal reagents gave comparable expression patterns in terms of flow cytometric analysis to those seen with the Dako polyclonal reagent.
- The results demonstrated that Sysmex monoclonal reagents gave a higher SI in the majority of all samples tested. An increased SI represents clearer separation between positive and negative populations and as such will result in easier identification and gating.
- For BLPD cases, the primary group for Kappa/Lambda clonality assessment, it was found that Sysmex monoclonal reagents gave a higher SI in >81% of Kappa and >61% of Lambda cases with monoclonal B lymphocyte populations.
- The ability to detect the presence of kappa and lambda light chains is a key requirement in the investigation of BLPD to confirm monoclonality. Reagents producing a higher SI allow optimal data interpretation and this was supported in this study with regards to the Sysmex monoclonal reagents.

REFERENCES

1. Swerdlow SH, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues Revised 4th ed. Lyon: International Agency for Research on Cancer; 2017

CONTACT INFORMATION

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