WHITE PAPER

Comparison of Dako's and Beckman Coulter's anti-Kappa and Lambda light chains antibodies with protocols including 1, 2 or 3 washing steps of the sample

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IN THIS PAPER YOU WILL

Discover performances of new goat polyclonal anti kappa and lambda light chains antibodies against Dako's gold standard

Visualize the importance of washing steps when assessing Kappa and Lambda light chains expression

Introduction

Each mature B lymphocyte expresses surface immunoglobulins bearing only one class of light chains, either Kappa or Lambda. Assessment of immunoglobulins light chains expression on B-cells by flow cytometry is the hallmark of B-cell malignancies studies. Indeed, while in healthy individuals the ratio of kappa+ to lambda+ B-cells is roughly 3:2, this ratio is skewed by the expansion of clonal malignant cells.

Kappa/Lambda flow cytometry assays can sometimes be challenging for laboratories, as results interpretation may be difficult. It is especially the case when non-specific staining occurs using non-optimized protocols or reagents. Dako's rabbit polyclonal anti-Kappa and Lambda antibodies are widely known as the gold standard, delivering best-in-class performances. In this study, we compared Dako's antibodies to Beckman Coulter's new goat polyclonal anti-Kappa and Lambda antibodies, conjugated to FITC and PE, respectively.

Methods

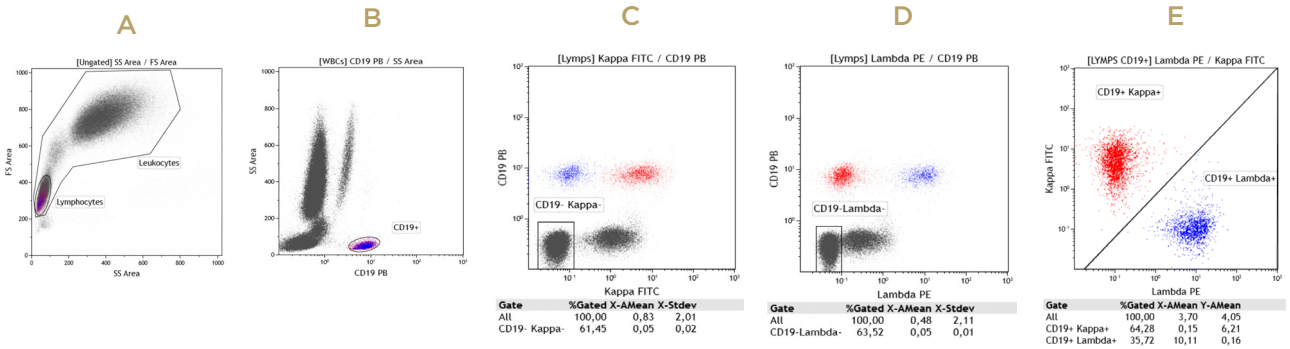
Both suppliers' reagents were compared 15 times in various conditions. The Stain/Wash/Lyse protocol* recommended by the International Clinical Cytometry Society (ICCS) was used in three variants, incorporating 1, 2 or 3 sample washes. Five random whole blood samples from healthy volunteers were processed in parallel with the three variants. Comparison between conjugated antibodies was based primarily on the assessment of the Staining Index, calculated as (MFI positive population – MFI negative population)/(2xSD of negative population). The immunostained samples were acquired on a Gallios flow cytometer and analyzed with Kaluza software.

*Flow Cytometric Testing for Kappa and Lambda light chains. ICCS 2018. https://www.cytometry.org/web/modules/Module%206.pdf

Staining protocol:

- Wash 1 mL of specimen with: 2 mL PBS
- 1 wash = add PBS, re-suspend, spin for 3 minutes at 400g, discard supernatant by aspiration. Repeat 1 or 2 times for 2 and 3 washes, respectively.
- Add antibodies in tubes: CD19-PB, Kappa-FITC and Lambda-PE
- Add 100 µL of washed specimen
- 30 minutes incubation (RT, protected from light)
- 1 mL of "Fix-and-Lyse" mixture
- 10 minutes incubation (RT, protected from light)
- Centrifugation for 3 minutes at 400g, discard supernatant
- Mix and wash 2x using 2 mL of PBS, centrifuge for 3 minutes at 400g
- Discard supernatant and re-suspend cell pellet with 500 µL PBS and mix well
- Acquisition within 2 hours and at least 100 000 white blood cells

Gating strategy



The Mean Fluorescence Intensity (MFI) and the Standard Deviation (SD) of the negative population CD19- Kappa- or Lambda- (MFI or SD $_{CD19\text{-}K/L\text{-}}$) are collected from the dot plots.

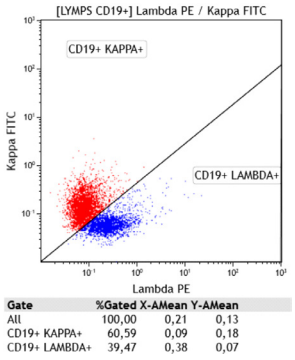
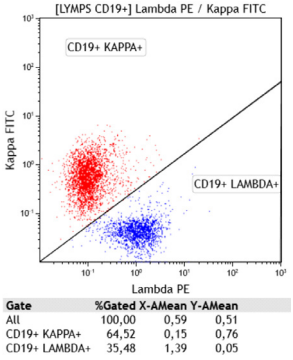
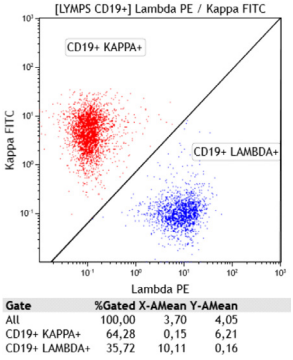
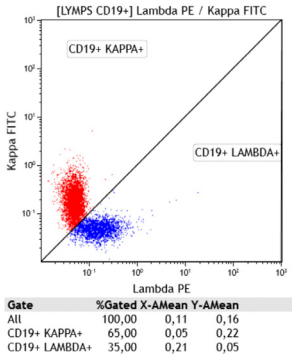
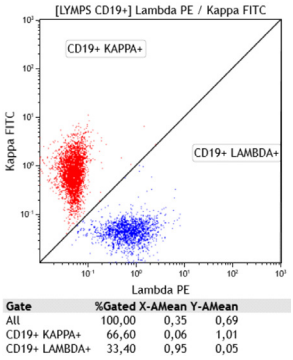
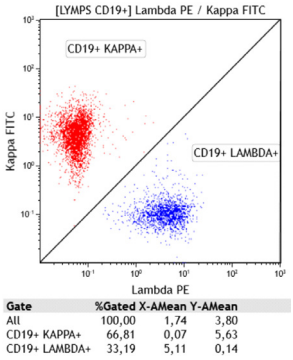
CD19-PB vs Kappa-FITC or Lambda-PE (C or D) gated on the whole lymphocyte population (A).

The MFI from the positive population CD19+Kappa+ or CD19+Lambda+ (MFI $_{CD19+K/L+}$) are obtained from the dual color dot plot Lambda-PE vs Kappa-FITC (E) gated on the CD19+ lymphocytes.

Results

The Staining Indexes (SI) are calculated by applying the following formula:

$$SI = [\ (MFI_{CD19+K/L+} - MFI_{CD19-K/L-}) / 2 \times SD_{CD19-K/L-}\ ]$$

	1 wash	2 washes	3 washes
Beckman Coulter	 Gate %Gated X-AMean Y-AMean All 100,00 0,21 0,13 CD19+ KAPPA+ 60,59 0,09 0,18 CD19+ LAMBDA+ 39,47 0,38 0,07	 Gate %Gated X-AMean Y-AMean All 100,00 0,59 0,51 CD19+ KAPPA+ 64,52 0,15 0,76 CD19+ LAMBDA+ 35,48 1,39 0,05	 Gate %Gated X-AMean Y-AMean All 100,00 3,70 4,05 CD19+ KAPPA+ 64,28 0,15 6,21 CD19+ LAMBDA+ 35,72 10,11 0,16
Dako	 Gate %Gated X-AMean Y-AMean All 100,00 0,11 0,16 CD19+ KAPPA+ 65,00 0,05 0,22 CD19+ LAMBDA+ 35,00 0,21 0,05	 Gate %Gated X-AMean Y-AMean All 100,00 0,35 0,69 CD19+ KAPPA+ 66,60 0,06 1,01 CD19+ LAMBDA+ 33,40 0,95 0,05	 Gate %Gated X-AMean Y-AMean All 100,00 1,74 3,80 CD19+ KAPPA+ 66,81 0,07 5,63 CD19+ LAMBDA+ 33,19 5,11 0,14

Statistical analysis: comparison of Beckman Coulter's versus Dako's Staining Indexes

Antibody	Condition	Staining Index Beckman Coulter	Staining Index Dako	Difference	SE	p-value	Lower	Upper	Conclusion
Anti-Kappa-FITC	2 washes	22.0	**43.8**	-21.8	5.2	0.0135	36.2	-7.5	Statistically different
	3 washes	**148.7**	132.0	16.7	12.8	0.2634	-19.0	52.4	Non-statistically different
Anti-Lambda-PE	2 washes	**53.0**	39.6	13.4	3.1	0.0131	4.7	22.1	Statistically different
	3 washes	**361.7**	192.9	168.8	26.6	0.0032	94.9	242.7	Statistically different

Performances of the reagents from both suppliers are similar.

Performing a single washing step of the samples did not allow to gate and discriminate positive and negative subsets. In contrast, two washes greatly facilitated the distinction and three washes offered a clear separation of Kappa+ and Lambda+ populations, no matter the supplier.

Conclusion

Each laboratory must perform their own protocol optimization and validation, as there are no validated and standardized consensus protocol for Kappa and Lambda light chains assessment. It is particularly important to determine the number of washing steps required to deliver accurate and trusted results. At least 2 washing steps are required to get data consistency, no matter the supplier. Lambda-PE from Beckman Coulter Life Sciences provided better staining index with either two or three washing steps. Kappa-FITC from Dako delivered better staining index with two washes, while both Kappa-FITC were equivalent when the protocol included three washing steps.

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For Beckman Coulter's worldwide office locations and phone numbers, please visit "Contact Us" at beckman.com

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