Evaluation of Commercial Kappa/Lambda Reagents for Flow Cytometric Analysis of Normal and Abnormal Specimens

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Abstract: The evaluation of cell surface Kappa/Lambda expression can identify clonally restricted B lymphocyte populations and thus can aide in the diagnosis of hematologic malignancy. Several B cell disorders are associated with decreased levels of Kappa/Lambda at the cell surface. In these cases, it is important to utilize Kappa/Lambda reagents that can optimally identify Kappa/Lambda cell surface expression even when decreased levels are present. In this study, flow cytometric analysis was used to evaluate the performance of Kappa/Lambda reagents from two different commercial vendors (Becton Dickinson, and Dako). Normal specimens and abnormal specimens with decreased levels of Kappa/Lambda cell surface expression were interrogated in the study. Mean channel fluorescence (MCF) and Signal to noise ratios (S:N) of Kappa and Lambda expression for all reagents were collected and compared. The Dako reagents demonstrated an increased MCF and S:N of both Kappa and Lambda expression compared with the Becton Dickinson reagents when normal peripheral blood specimens were analyzed ($P < 0.05$). Abnormal specimens with decreased levels of Kappa at the cell surface were also analyzed. Again, The Dako reagents demonstrated an increased MCF and S:N compared with the Becton Dickinson reagents ($P < 0.05$). Dako Kappa and Lambda reagents are superior to Becton-Dickinson in detecting cell surface expressed Kappa and Lambda especially when decreased levels are present.

Introduction:

Historically, the diagnosis and monitoring of patients with leukemia/lymphoma was largely accomplished using morphologic examination. The advent of flow cytometric analysis provided the pathologist with a valuable diagnostic adjunct. With the increase in the number and quality of commercially available antibodies, improved gating strategies, and significantly improved instrumentation, flow cytometric analysis has become a critical tool for the diagnosis and monitoring of hematologic malignancies. Three, four and even five color multi-parameter flow cytometric analysis is routinely utilized in the clinical laboratory to evaluate specific cell surface and cytoplasmic markers of interest. Expression patterns of these markers can be used to identify specific cell lineages, classify hematologic malignancies, provide prognostic information, and monitor response to therapy.

The number and combination of specific antibodies utilized to diagnosis and monitor hematologic malignancies is not standardized among clinical diagnostic laboratories. It is however universally accepted that Kappa/Lambda expression on B lymphocytes be evaluated. The evaluation of cell surface Kappa/Lambda expression can identify clonally restricted B lymphocyte populations and thus can aide in the diagnosis of hematologic malignancy. Several B cell lymphoproliferative disorders and plasma cell dycrasias are associated with decreased levels of Kappa/Lambda at the cell surface. In these cases, it is important to utilize Kappa/Lambda reagents that can optimally identify Kappa/Lambda cell surface expression even when decreased levels are present.

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In this Study we compared the performance of Kappa-FITC and Lambda-PE flow cytometry reagents from two different commercial vendors (Becton Dickinson and Dako). Normal specimens and abnormal specimens with decreased levels of Kappa/Lambda cell surface expression were evaluated.

Materials and Methods:

Specimens:

This study was reviewed by the ConVerge institutional review board (IRB). The IRB determined that formal IRB approval was not necessary as the study will only be conducted utilizing leftover specimens which in accordance with FDA guidance document "*Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable*", issued April 2006 does not require informed consent if specific conditions are met.

All of the specimens included in the study were originally obtained for clinical testing purposes and were made available for the study only after all requested clinical testing had been completed. No specimens were specifically collected to support any particular part of the study and no information about any patient was provided to any patient's physician or was made part of any patient's medical record.

Each selected specimen was assigned a random identifier. Specimen information was collected, correlated with the assigned random identifier, and recorded in a spread-sheet. Each specimen was then de-identified so that the randomly assigned identifier could not be linked back to the original specimen identity directly or through original identifiers linked to the specimen by anyone.

As part of the de-identification process, all specimens were removed from their original collection container and aliquoted to a tube compatible with flow cytometric analysis and affixed with the randomly generated identifier. Also as part of the de-identification process, all specimens will be stripped of all unique identifiers associated with them. Identifiable information encompassed all entities included in the HIPPA Privacy Rule.

Specimen Selection Criteria:

Normal Specimen Set:

Five (5) peripheral blood and five (5) bone marrow specimens were included in the study. For peripheral blood specimens "normal" was defined by a concomitant CBC result that was within standard reference intervals as defined by the clinical diagnostic laboratory. For bone marrow specimens "normal" was defined by a concomitant morphologic examination that renders unremarkable results.

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Abnormal Specimen Set:

Twenty (20) abnormal specimens were included in the study. The abnormal specimen set consisted of peripheral blood and bone marrow specimens. Abnormal specimens included specimens with hematologic malignancies associated with markedly decreased or moderately decreased levels of Kappa/Lambda cell surface expression. For peripheral blood and bone marrow specimens "abnormal" was defined by a concomitant clinical flow cytometry result that identified a monoclonal B lymphocyte population.

General Specimen Requirements:

All specimens included in the study were less than seventy-two (72) hours old, had been stored at room temperature, had at least 1.0 mls of residual volume, were not clotted or hemolyzed, and had been collected in EDTA, ACD, or Heparin anticoagulants.

Testing Procedures:

The study was performed in CLIA and CAP-certified clinical diagnostic laboratory. All normal and abnormal peripheral blood and bone marrow specimens were interrogated using Kappa/Lambda reagents from two (2) different commercial manufacturers (Becton Dickinson and Dako).

Specimens were prepared using standard red-blood cell lysing procedures and analyzed using flow cytometric analysis. All specimens will be interrogated using the various antibodies assigned to an eight (8) tube panel listed below. Mean channel fluorescence of Kappa-FITC and Lambda-PE expression for each commercial product were collected and recorded.

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Tube#	FL-1 FITC	FL-2 PE	Fl-3 ECD	FL-4 PC5	FL-5 PC7
1	Isotype (340755) Becton Dickinson				
2	Kappa (643774) Becton Dickinson		CD19 (IM2708) Beckman Coulter		CD45 (IM3548U) Beckman Coulter
3		Isotype (340761) Becton Dickinson			
4		Lambda (642924) Becton Dickinson	CD19 (IM2708) Beckman Coulter		CD45 (IM3548U) Beckman Coulter
5	Isotype (X0929) Dako				
6	Kappa (F0434) Dako		CD19 (IM2708) Beckman Coulter		CD45 (IM3548U) Beckman Coulter
7		Isotype (X0930) Dako			
8		Lambda (R0437) Dako	CD19 (IM2708) Beckman Coulter		CD45 (IM3548U) Beckman Coulter

Statistical Analysis:

The significance of the differences between each commercial product were determined utilizing one-tailed T-tests for independent samples. The alpha level was set at 0.05 before the study began.

Results:

Kappa-FITC and Lambda-PE products from each commercial vendor were tittered using a fresh peripheral blood specimen from a normal donor. Signal to noise (S:N) ratios for

each antibody amount were calculated. Signal was defined as mean channel florescence (MCF) of either Kappa-FITC or Lambda-PE on CD19 positive cells. Noise was defined as the MCF of either Kappa-FITC or Lambda-PE on CD19 negative cells (ie T cells).

Figure 1. demonstrates that the Kappa-FITC antibody concentration yielding the optimal S:N was 14ul (S:N of 18.5) and 13ul (S:N of 45.6) for the BD and Dako products respectively. Figure 1. also demonstrates that the Dako product consistently yielded a higher S:N across all Kappa-FITC antibody concentrations tested.

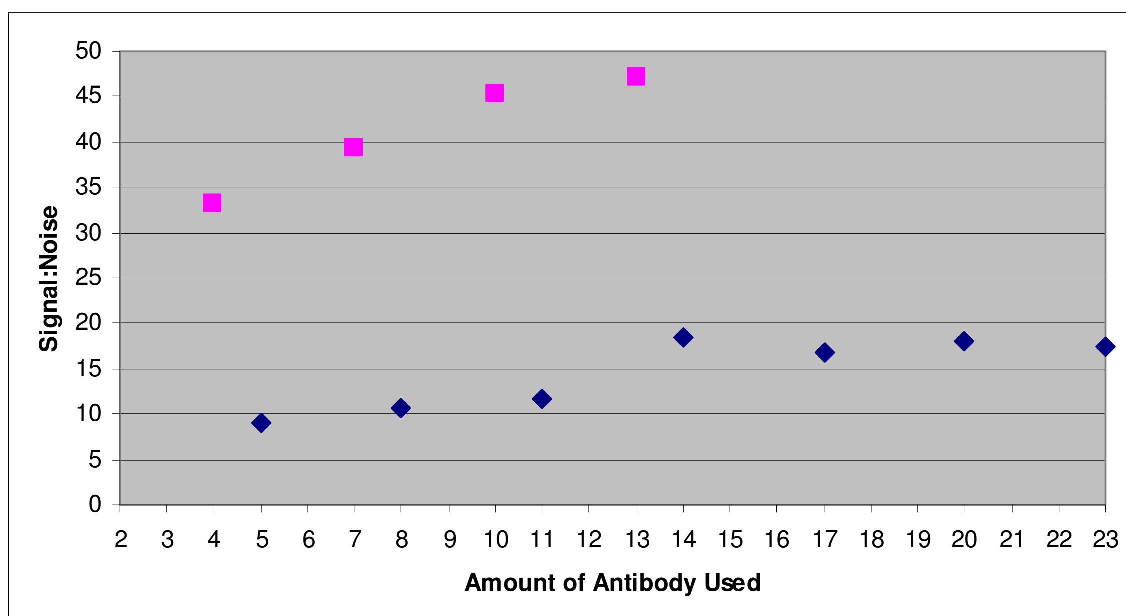

Figure 1. S:N determinations for various Kappa-FITC antibody concentrations tested utilizing a fresh peripheral blood specimen from a normal donor and either BD (γ) or Dako ( ) Kappa-FITC antibody.

Figure 2. demonstrates that the Lambda-PE antibody concentration yielding the optimal S:N was 17ul (S:N of 30.8) and 13ul (S:N of 62.3) for the BD and Dako products respectively. Figure 2. also demonstrates that the Dako product consistently yielded a higher S:N across all Lambda-PE antibody concentrations tested.

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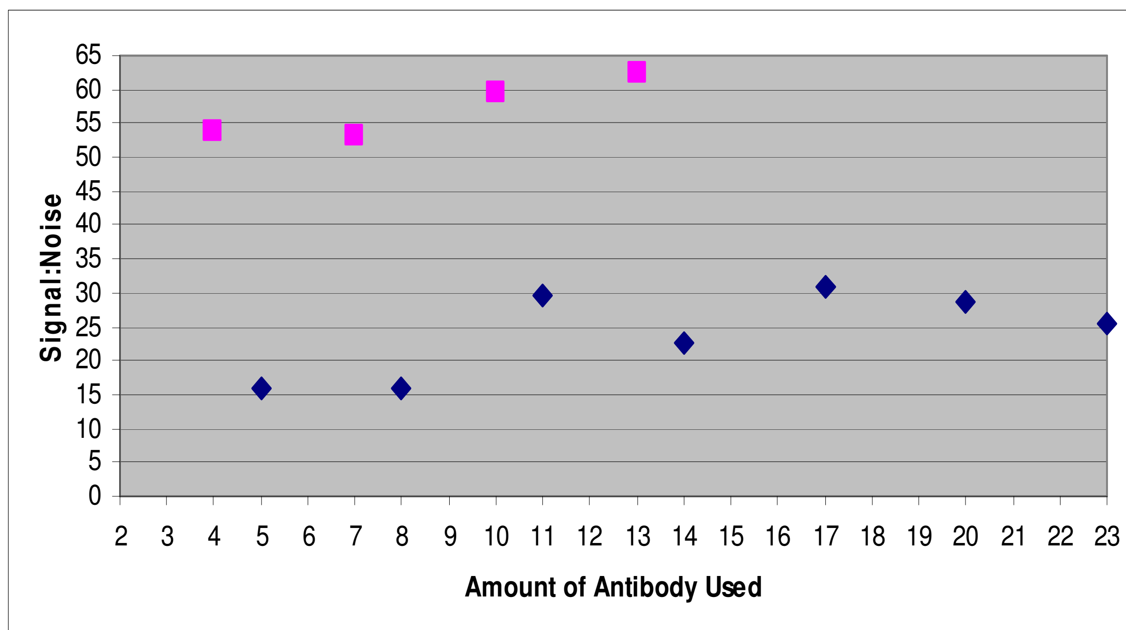

Figure 2. S:N determinations for various Lambda-PE antibody concentrations tested utilizing a fresh peripheral blood specimen from a normal donor and either BD (γ) or Dako ( ) Kappa-FITC antibody.

To further evaluate the performance of each commercial product, peripheral blood and bone marrow specimens from normal and abnormal donors were analyzed using the BD and Dako reagents. For the BD reagents, all specimens were labeled using antibody concentrations that yielded the optimal S:N as determined in the titration experiments (14ul for Kappa-FITC and 17ul for Lambda-PE). For the Dako reagents, all specimens were labeled using antibody concentrations that yielded near optimal S:N as determined in the titration experiments (10ul for Kappa-FITC and 10ul for Lambda-PE).

The same five (5) normal specimens were evaluated using Kappa-FITC and Lambda-PE reagents from BD and Dako. Figure 3. demonstrates that the MCF yielded by the Dako Kappa-FITC (B) and Lambda-PE (D) reagents was significantly (P< 0.05) greater than the MCF yielded by the BD Kappa-FITC (A) and Lambda-PE (C) reagents for all five (5) specimens evaluated.

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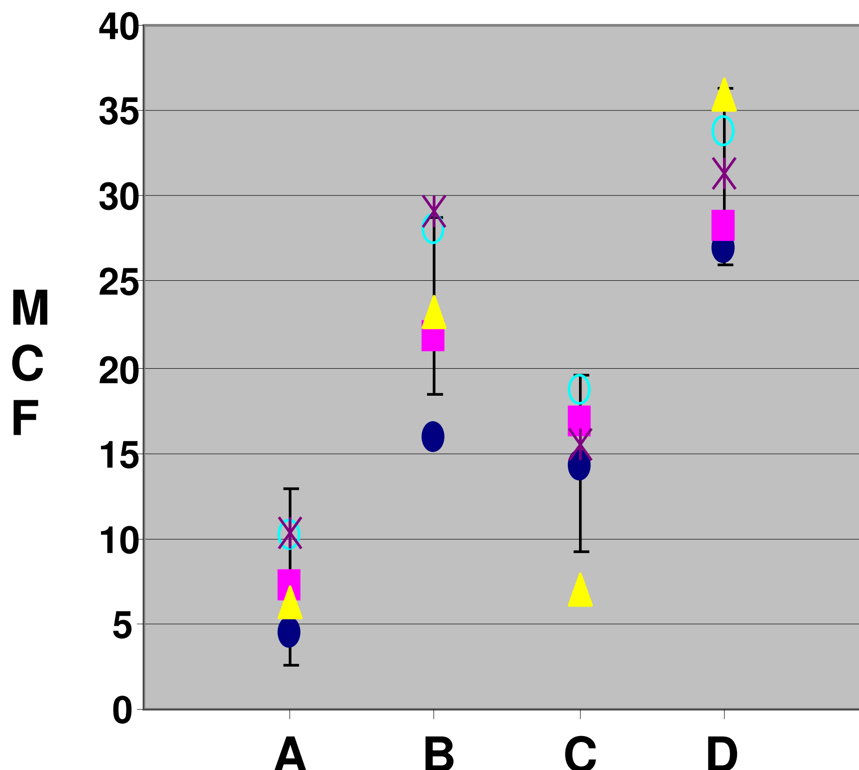

Figure 3. MCF determinations for 5 normal peripheral blood specimens evaluated with Kappa-FITC BD (A) or Dako (B) reagents and Lambda-PE BD (C) or Dako (D) reagents. Error bars represent standard error of the mean. Differences between populations A and B and C and D were statistically significant (P<0.05).

To evaluate if the increased MCF demonstrated by the Dako reagents compromised the reagents specificity, S:N's were determined and compared (Figure 4.). Signal was defined as MCF of either Kappa-FITC or Lambda-PE on CD19 positive cells. Noise was defined as the MCF of either Isotype-FITC or Isotype-PE matched controls from both vendors. Antibody concentrations for each of the isotype controls were matched to their specific antibody counterpart.

Figure 4. demonstrates that the S:N's yielded by the Dako Kappa-FITC (B) and Lambda-PE (D) reagents was significantly (P< 0.05) greater than the S:N's yielded by the BD Kappa-FITC (A) and Lambda-PE (C) reagents for all five (5) specimens evaluated.

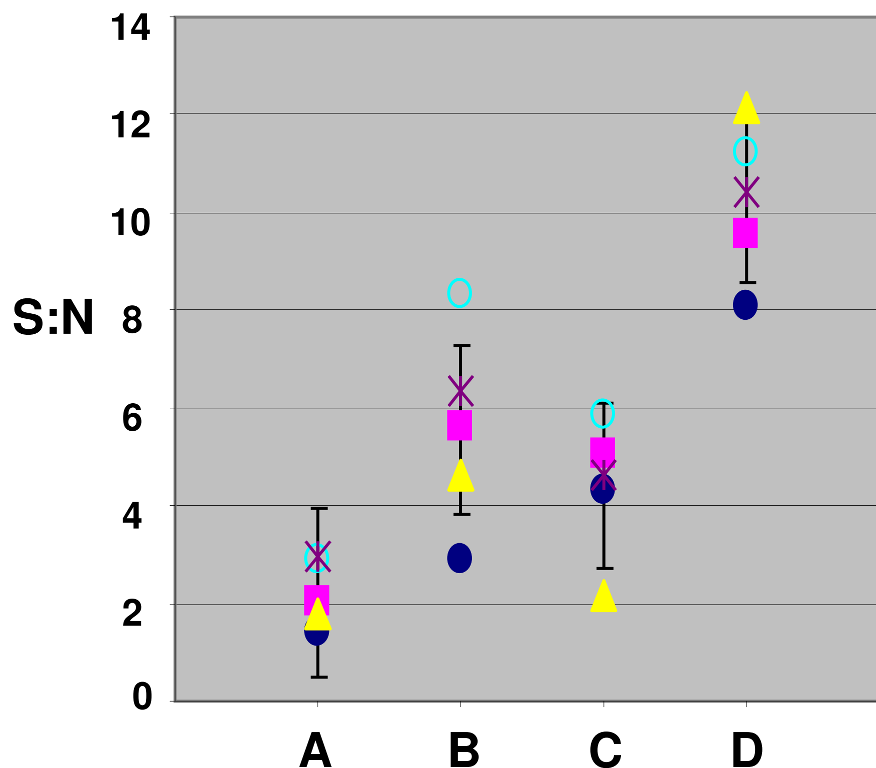

Figure 4. S:N's determinations for 5 normal peripheral blood specimens evaluated with Kappa-FITC BD (A) or Dako (B) reagents and Lambda-PE BD (C) or Dako (D) reagents. Error bars represent standard error of the mean. Differences between populations A and B and C and D were statistically significant ($P<0.05$).

We further evaluated the performance of the commercial products using abnormal specimens. Figure 5. shows two representative abnormal specimens containing Kappa-restricted B cell populations evaluated with Kappa-FITC reagents from either BD (A and C) or Dako (B and D). In both cases, the Dako reagent more clearly delineates the presence of the Kappa-restricted B cell population present in each specimen.

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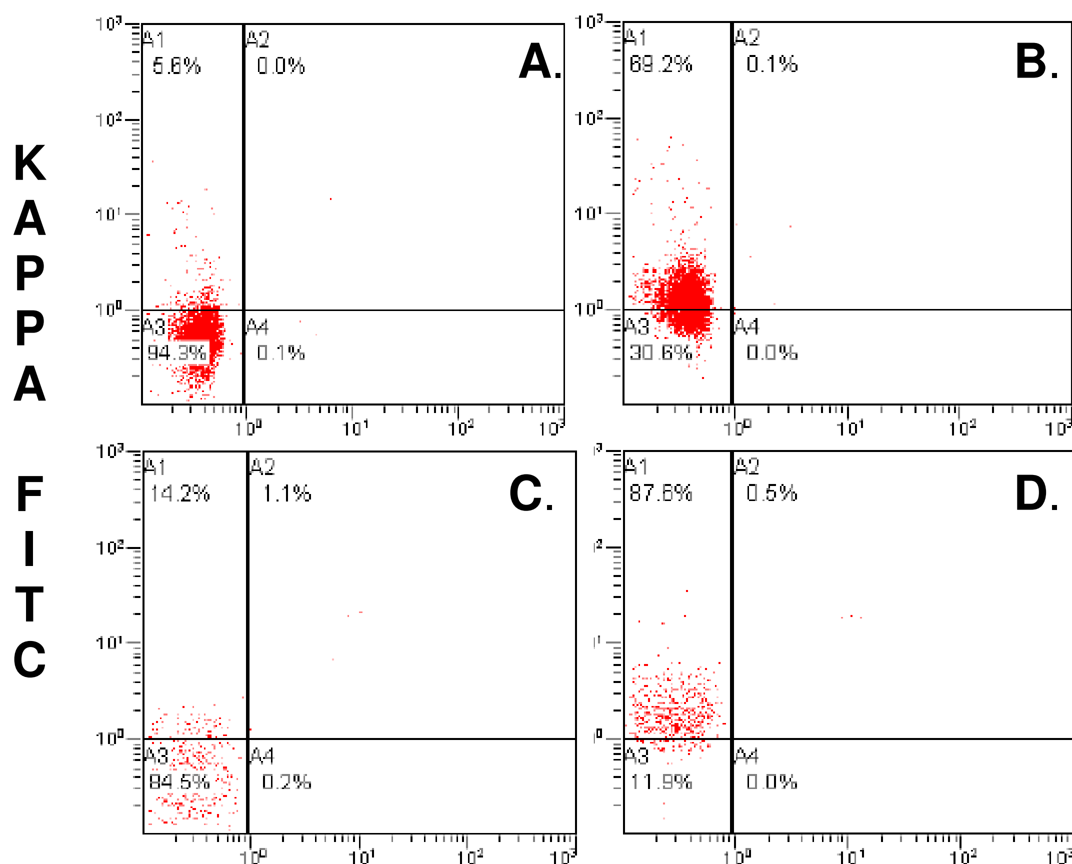

Figure 5. Representative abnormal specimens containing Kappa-restricted B cell populations evaluated with Kappa-FITC reagents from either BD (A and C) or Dako (B and D).

The Dako Kappa-FITC reagent yielded significantly ($P<0.05$) greater MCF than the BD Kappa-FITC reagent on abnormal specimens expressing either markedly decreased (Figure 6.) or moderately decreased (Figure 7.) levels of surface Kappa.

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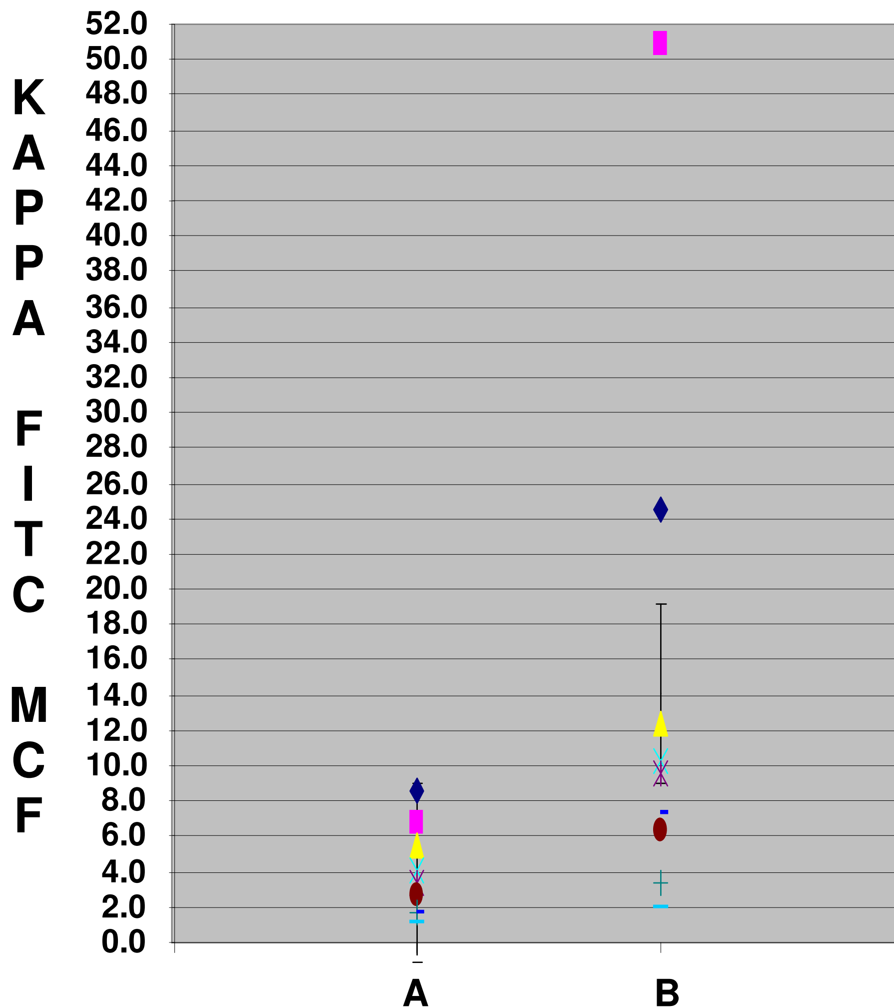

Figure 6. MCF determinations for 9 abnormal specimens with known Kappa monoclonal B cell populations expressing moderately decreased levels of surface Kappa evaluated with Kappa-FITC BD (A) or Dako (B) reagents. Error bars represent standard error of the mean. Differences between populations A and B were statistically significant (P<0.05).

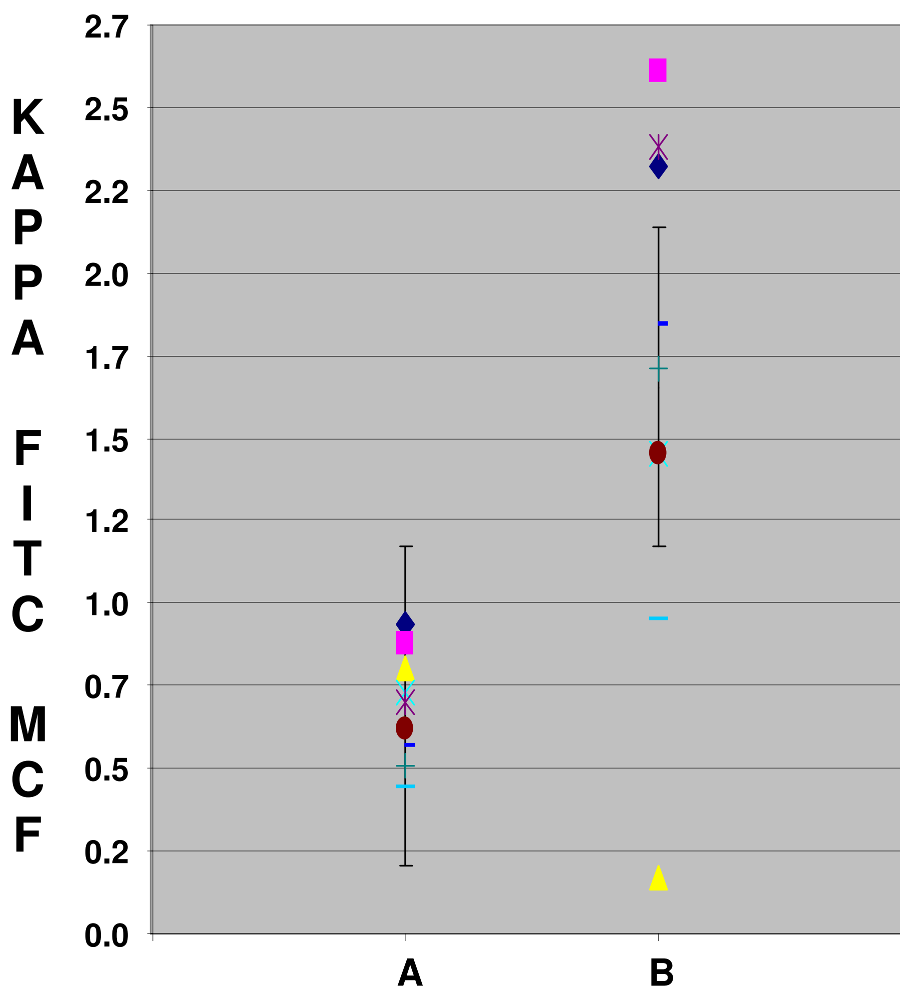

Figure 7. MCF determinations for 9 abnormal specimens with known Kappa monoclonal B cell populations expressing markedly decreased levels of surface Kappa evaluated with Kappa-FITC BD (A) or Dako (B) reagents. Error bars represent standard error of the mean. Differences between populations A and B were statistically significant (P<0.05).

To evaluate if the increased MCF demonstrated by the Dako reagents compromised the reagents specificity when abnormal specimens were evaluated, S:N's were determined and compared (Figure 8.). Signal was defined as MCF of Kappa-FITC on CD19 positive cells. Noise was defined as the MCF of Isotype-FITC matched controls from both vendors. Antibody concentrations for each of the isotype controls were matched to their specific antibody counterpart.

Figure 8. demonstrates that the S:N's yielded by the Dako Kappa-FITC (B) reagent was significantly ($P < 0.05$) greater than the S:N's yielded by the BD Kappa-FITC reagent (A).

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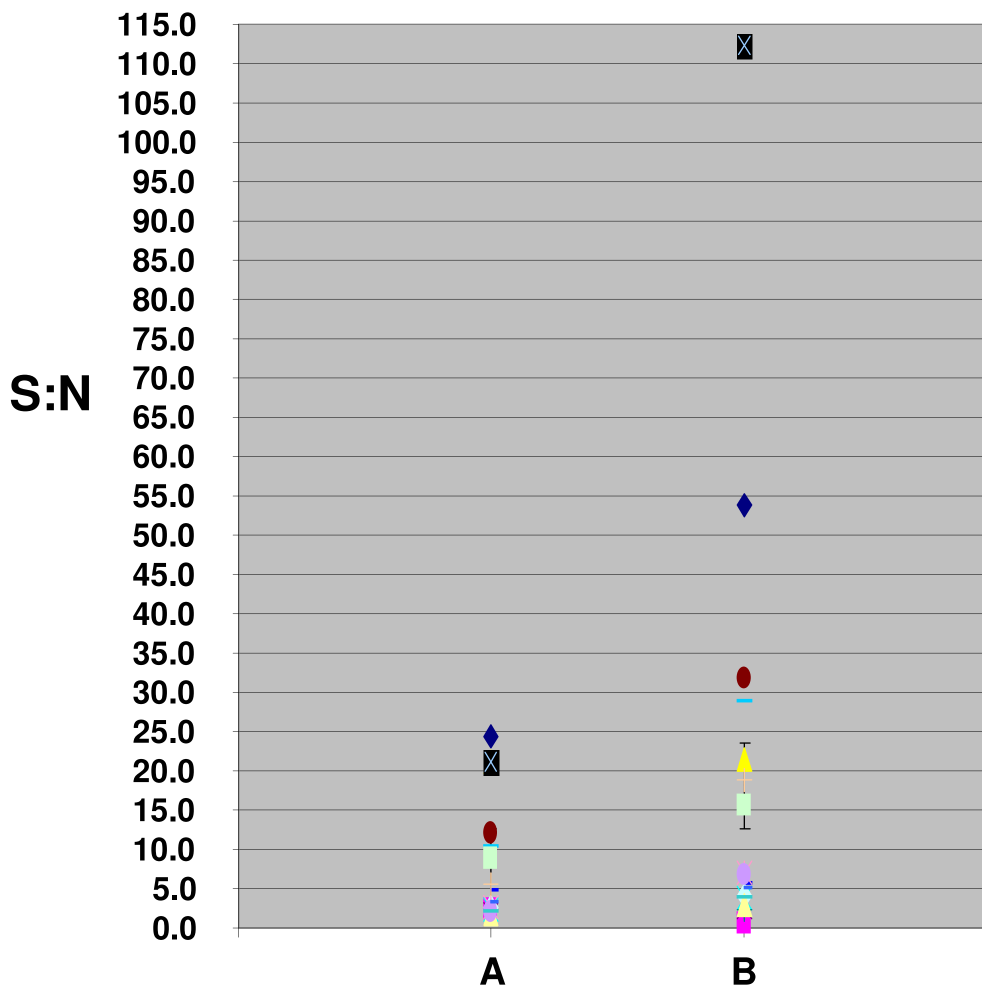

Figure 7. S:N's determinations for 18 abnormal specimens with known Kappa monoclonal B cell populations expressing decreased levels of surface Kappa evaluated with Kappa-FITC BD (A) or Dako (B) reagents. Error bars represent standard error of the mean. Differences between populations A and B were statistically significant (P<0.05).

Conclusions:

Dako Kappa-FITC and Lambda-PE reagents are superior to Becton-Dickinson Kappa-FITC and Lambda-PE reagents in detecting cell surface expressed Kappa and Lambda especially when decreased levels are present. The use of a superior Kappa and Lambda reagents may mitigate the chance of missing a clonally restricted B lymphocyte population and may also reduce the need to perform the more labor intensive intracellular Ig Kappa and Lambda analysis.