The therapeutic antibody rituximab (Rituxan®) is used to treat patients with a broad range of conditions including autoimmune diseases such as rheumatoid arthritis and Antineutrophil Cytoplasmic Autoantibodies (ANCA) vasculitis. It is also useful in the therapy of B-cell malignancies such as chronic lymphocytic leukemia and non-Hodgkin’s lymphoma. Rituximab works by targeting and instigating the rapid depletion of circulating CD20 expressing B-cells. The immune monitoring of patients that receive rituximab is useful in assessing the therapeutic efficacy and dosing of the drug. It is useful to include CD20 in addition to CD19 in rituximab monitoring evaluation to assess for the presence of CD19+CD20- cells which could potentially be coated with rituximab (prior to clearance by the immune system) or immature circulating plasma cells. This study evaluated the performance of a flow cytometric based Lab Developed Test (LDT) compared to a commercially available reagent (Becton Dickinson (BD) Multitest™ 6-color TBNK plus CD20) for measuring T and B cells in peripheral blood.

Methods:

One hundred and four (104) serial, blood samples submitted for rituximab monitoring at Massachusetts General Hospital (Boston MA) between March and May 2017 were tested using the LDT (CD3, CD4, CD5, CD8, CD19, CD20 and CD45) and a BD FACSCanto™ 10-color flow cytometer. Remnant patient samples were subsequently tested using the TBNK (CD3, CD4, CD8, CD19, CD16, CD56, CD45+) plus CD20 as the test method (TBNK+CD20) on a BD Canto™ II flow cytometer. Linear regression was performed for lymphocyte subset absolute counts and percentages. Range (minimum and maximum), the coefficient of determination ($R^2$) and intercepts, slopes and their corresponding 95% confidence intervals were calculated. An additional 15 samples were tested comparing the LDT to a newly launched dried version of the TBNK + CD20 reagent (BD Horizon Dri TBNK+CD20) and the coefficient of determination ($R^2$) calculated.

Results:

The results demonstrated that the LDT method was equivalent to the commercial test method (TBNK+CD20). The regression slopes ranged from 0.9 to 1.05 with 95% confidence intervals from 0.8 to 1.2 when the LDT and test method TBNK+CD20 reagent were compared. The $R^2$ values were above 0.88 for the test method (TBNK+CD20) compared to the LDT. In the TBNK+CD20 results, the slopes were 1.02 (0.99 to 1.05) and 1.03 (0.99 to 1.06) for CD19 absolute counts and CD19+CD20 absolute counts, respectively. The slopes with 95% confidence were within a 10% bias interval of 0.9 to 1.1 and the $R^2$ values were all greater than 0.88. When comparing the LDT to the BD Horizon Dri TBNK+CD20 reagent, our data indicate good correlation with an $R^2$> 0.9 for all specificities (range $R^2 = 0.92-0.99$).

Conclusion:

The results of this study demonstrates good performance for the BD Multitest 6-color TBNK+CD20 reagent compared to the LDT. In our opinion, either assay may be confidently used to monitor patients receiving rituximab therapy. Additionally our data supports good correlation between the LDT and the newly launched dried version of the TBNK +CD20 (BD Horizon Dri TBNK +CD20).