Introduction

A multi-center study was conducted to establish the performance of the AQUIOS TL flow cytometer with AQUIOS Tetra Panel reagents for lymphocyte subset analysis in patients suspected of immunodeficiency, post-transplant patients and apparently healthy individuals. Performance equivalence was evaluated compared to the BD FACSCalibur™ with Multitest™ IMK kit and TruCount tubes.

Objectives and Methods

Precision performance of the AQUIOS Tetra system was evaluated based on CLSI EP5-A using control materials as samples.

To assess substantial equivalency, the AQUIOS CL with AQUIOS Tetra reagents was compared against the BD FACSCalibur with BD Multitest reagents using a single platform approach for absolute counts. A multi-center study was conducted to target the intended use population following CLSI EP09-A3. The AQUIOS Tetra gating method was compared to the FACSCalibur with Multitest™ software in ≥400 samples covering the CD4+ medical decision levels. Fully automated sample preparation was performed by the AQUIOS CL and manually for analysis on the FACSCalibur.

Hematologically normal adult donors between 18 – 65 years old with a targeted ratio of 50:50 males to females from three (3) geographical locations were combined to establish the reference interval for AQUIOS Tetra-1 Panel and Tetra-2+ Panel. The adult reference interval was determined following CLSI EP26 A2.

Results

The AQUIOS TL flow cytometer showed excellent reproducibility and reproducibility with AQUIOS Tetra-1 and AQUIOS Tetra-2+ reagents for all sites combined. Two levels of control material were evaluated at three (3) sites. Tables 1 and 2 summarize the combined sites for AQUIOS Tetra-1 and AQUIOS Tetra-2+ for CD3+ and CD3- reagents as well as the corresponding ±/CD56+/CD16+ levels.

Substantial equivalency of the AQUIOS CL with AQUIOS Tetra reagents was demonstrated against the BD FACSCalibur with BD Multitest reagents (CD3-FL/T/Tetra-PC5/CD16+/CD19-APC) using a single platform approach for absolute counts (TruCount). A total of 443 samples combined from four (4) clinical sites covering the CD4 analytical measuring range with emphasis on the medical decision ranges were tested on both platforms.

Percent marker recovery comparisons had clinically insignificant to no bias. A negative bias for the comparison of AQUIOS CL with BD FACSCalibur was observed in the absolute count marker recovery for all four (4) sites combined. This negative trend observed for all markers was not clinically significant. Importantly, smaller differences were observed for CD3+CD4+ counts compared to other markers as summarized in Tables 3 and 4. The bias was clinically insignificant at the medical decision points for CD3+CD4+ as noted in Table 5. The additional parameters that AQUIOS provides (CD45+ and CD45+ Low SS) are not provided.

Figures 1 and 2 illustrate regression and Bland-Altman plots, respectively for CD3+CD4+ count confirming comparability of AQUIOS Tetra against the BD FACSCalibur.

A total of 161 hematological normal adult donors were combined to establish reference Interval. Normal reference intervals for lymphocyte subsets (data not shown) were consistent with published values.

Conclusion

The AQUIOS TL flow cytometer demonstrated excellent performance in lymphocyte subset analysis with AQUIOS Tetra Panel Reagents.