CHAPTER 5

Conclusion

FLT3 protein is one of the important biological markers for severity evaluation of acute myelogenous leukemia (AML). This study focused on the investigation of a reliable flow cytometric technique to monitor the FLT3 protein expression levels on leukemic cell surface. Until now, there is no specific regulation on bioanalytical flow cytometric method validation. This study has developed the flow cytometric method for FLT3 protein level detection by using a leukemic cell line model. Screening of six leukemic cell lines including K562, U937, Molt4, HL60, EoL1, and MV4-11cells demonstrated that EoL1 cells expressed the highest FLT3 protein level comparing to other cell lines. Consequently, EoL1 cells were used as a positive control cell line. Antibody concentrations, a number of cells, and incubation times were titrated in appropriate sample treatment conditions. Antibody concentration was reduced for analytical cost saving and related with a decrease in required cell number. Incubation time was also concerned for rapid routine analysis and complete antigen-antibody interaction. Optimal condition for cell sample treatment was consisted of $5x10^5$ cells reacted with 1.0 µg of anti-FLT3 antibody and incubated for 45 min. Then the developed sample treatment with flow cytometric determination was validated in the aspects of linearity and range, precision, accuracy, lower limit of quantification (LLOQ) and stability. The calibration curve was constructed with six FLT3 concentrations performed by a serial dilution of EoL1 as positive cell control for FLT3 expression and adjusted to $5x10^5$ cells (optimal cell number) by adding K562 as negative cell control. For more reliable evaluation, quality control (QC) samples with three levels of positive cell control (EoL1) at 25, 50, and 75% were included in each analytical run. Calibration curves demonstrated a good linearity ($r^2 > 0.995$) of the assay. The percentage coefficient of variation (%CV) determined for analytical method precision were less

than 20% for both intra-day and inter-day precision evaluations. The accuracy of method represented by the percent recoveries of intra-day at 25, 50, and 75% of QC levels were 97.74±3.27, 106.39±3.58, and 104.54±5.83%, respectively. The percent recoveries of inter-day assay were 99.85±7.76, 103.36±5.99, and 98.64±5.88%, respectively. In addition, the percentage relative errors (%RE) of the inter-day precision evaluation were less than 15% in all cases. LLOQ was found to be 10% of a positive cell concentration with acceptable precision and accuracy. The stability after cell staining was monitored by %CV of QC samples on day 0, 1, 2, 7, and 14 under temperature of 2-8°C in the assay matrix. The %CVs were less than 10% in all cases so the refrigerated samples were suitable for analysis within two weeks. Validation of the developed flow cytometric method for FLT3 protein determination was done with acceptable criteria according to guidance documents from a variety of scientific article literature review. The developed flow cytometric method was applied for FLT3 protein levels evaluation in leukemic cells obtained from patient's bone marrow samples, and the calibration curve and QC levels were included in every analysis. FLT3 protein expression on patient bone marrow samples were in the range of analysis, and demonstrated the same tendency by comparing to the Western blotting and RT-PCR.

In summary, this study reported the optimization and validation of flow cytometric method which showed the effective, reliable, rapid, and economical analytical technique for FLT3 protein levels detection. Application of this method validation protocols will provide a model for analytical method development for biomarkers expression determination by flow cytometry in other studies.