Bevacizumab is a humanized monoclonal antibody which mediates its activity through high-affinity binding to vascular endothelial growth factor (VEGF). VEGF, a signaling protein, is involved in the initiation of vasculogenesis and angiogenesis of blood vessels. Due to the reliance of solid tumors on efficient angiogenesis to grow beyond a few mm^3 in volume, bevacizumab is used as a combination therapy for a number of malignancies, including metastatic colorectal cancer and metastatic kidney cancer.

This *in vitro* cell-based binding assay utilized a flow cytometry format to measure antibody binding. The VEGF-expressing target cells were incubated with a dilution series of bevacizumab. The antibody-bound cells were then detected using a fluorescently-labeled secondary anti-IgG antibody. The degree of antibody binding can be quantified using a fluorescence readout on the iQue® Screener PLUS, in approximately 10 minutes per plate.

The data presented here was obtained from an assay qualification performed at Sartorius Stedim BioOutsource. During the qualification study, the data collected demonstrated that the assay is accurate, reproducible, and precise over a linear range of 50 to 200% relative to the reference standard, and was specific to bevacizumab. The methodology was deemed fit for the purposes of evaluating the functional comparability and potency of bevacizumab biosimilar and innovator material. The benefits of this commercially available assay are that it is easy-to-use, highly reproducible, and saves a large proportion of assay development time, translating into overall cost savings in a biosimilar development program.

Flow Cytometry Instrumentation (B136)

428/B136

Light-Scattering Gating and Characterization of Plasma Submicron Particles

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Background: There are many submicron particles in the human blood plasma: microparticles, chylomicrons, extracellular vesicles. These particles all play an important role in different biological processes. However, these particles are very heterogeneous, highly varying by size, composition, and density. The flow cytometry method (FCM) is commonly used for clinical studies of plasma submicron particles. Unfortunately, efficiency of FCM detection, in this case, depends on several factors: instrument optical configuration, mode of triggering (side scattering (SSC), forward scattering (FSC), or fluorescence-based), and the threshold level, along with appropriate use of reference particles and adequate calibration protocols. The goal of this study is to discuss the following issues: 1) assessment of the instrument sensitivity for submicron particle detection based on standard FSC and/or SSC measurements; 2) which particle characteristics can be estimated from these scatter measurements; and 3) additional capabilities provided by angle-resolved light-scattering (LSPs) measurements.

Methods: Standard FSC and SSC, as well as LSPs of individual particles were measured by a scanning flow cytometer (SFC). These signals are used to calculate the particle size, shape, and RI from the solution of the inverse light-scattering problem based on the Mie theory. We worked with the supernatant of platelet-rich plasma formed in a tube within 2 hours after collecting blood in a vacuum citrate tube. Also used were nonfluorescent polystyrene microspheres 0.4, 0.7, 1, 3, and 1.87 μm, to determine the LSP, FSC, and SSC angular ranges of light acceptance, as well as actual measurements together with plasma particles.

Results: We developed the approach to standardize scatter-based detection of plasma particles, taking into account the FCM optical configuration. This includes accurate evaluation of the instrument sensitivity and instrument-independent gating strategy, in absolute scales of size and RI. Moreover, we propose a potential method to determine size and RI of single-spherical plasma particles from the FSC and SSC signals, and analyze its feasibility in terms of uniqueness and accuracy of the solution. This leads to complete understanding of the instrument capabilities in plasma particles detection and characterization. Finally, we contrast those best-of-the-FCM-world results with the capabilities of the SFC, including shape classification and uncertainties of estimated plasma particles characteristics.

Conclusion: The proposed absolute FSC/SSC gating is naturally standardized for any FCM instrument, given the knowledge of its optical system, and leads to instrument-independent analysis of submicron particles. A broad application of this approach to the commercial cytometers currently used in such studies, will improve the robustness and reproducibility of the results. However, some inherent limitations of FSC/SSC analysis remain (assumption of particle sphericity and potential ambiguity in some ranges of particle characteristics), and can only be removed by measurement of additional signals, such as angle-resolved light scattering.

Flow Cytometry Instrumentation (B138)

429/B138

DNA Extracted from Infiltrated T Cells in Human Lung Cancer Specimen is not Stable Using Red Cell Lying Solution containing Certain Fixative

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Introduction: Obtaining high-quality DNA from fresh tissue is essential for successful downstream gene expression analysis. However, under certain conditions, DNA might not be stable as it is commonly believed. In our study, we analyzed different DNA products from T cells in lung cancer specimens using red blood cell lysing solution, with or without, fixative in sample preparation. We found that the fixative from the red blood cell lysing solution had an adverse effect on DNA stability.

Methods: Six lung cancer specimens were surgically resected and prepared into single-cell suspensions using the gentleMACS Octo Dissociator (Miltenyi Biotec, Gladbach, Germany), and followed the instructions by the manufacturer. Cells were stained with following antibodies: CD45-FITC (BD, HI30) and CD3-BUV395 (BD, UCHT1). After staining, samples were lysed using the BD FACS™ lysing solution, with fixative containing formaldehyde (9.99%), ethylene glycol(30.0%), methanol (3.51%) (Catalog No.349202, BD Biosciences, San Jose, CA), or BD Pharm Lyse™ lysing buffer (without fixative) (Catalog No. 555899, BD Biosciences, San Jose, CA), respectively, using no red cells lysing sample as control. After wash with staining buffer, $CD45^+CD3^+$ cells were isolated by BD FACSAria™ IIu (Special Order System) cytometer. DNA was extracted from the sorted cells and measured by NanoDrop 1000 (Thermo, US). PCR amplified products for TCR CDR3 domain of CD3 were analyzed by agarose gel electrophoresis.

Results: The DNA from $3x10^5$–$1.4x10^6$ CD^+CD3^+ cells with lysing solution containing fixative was not detectable by NanoDrop 1000 and showed no DNA band on agarose gel. However, 0.5ug DNA was obtained from $3x10^5$ $CD45^+CD3^+$ cells using lysing solution without fixative, or not using lysing solution, and agarose gel electrophoresis showed clear DNA bands of TCR CDR3 domain.

Conclusion: The stability of DNA extractive from infiltrating T cells in lung cancer specimens was affected by the fixative in red blood cell lysing solution. Therefore, for the downstream gene study, lysing solution without fixative is needed in our study.