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INSTRUMENT QUALITY CONTROL

Microsphere standards aid in defining the instrument's capabilities and limitations in terms of sensitivity, precision, and accuracy, and provide a means for ensuring that the instrument is stable and suitable for use. They are also helpful in understanding the effects of extraneous factors, such as temperature, humidity, and electronic noise. The comparison of daily and historical QC data aids in the identification of random errors (due to electronic noise, air bubbles, etc.) and systematic errors (bias, shifts and trends caused by temperature variation, laser deterioration, misalignment, etc.) so that suitable corrective action may be taken.

General Stability / Status

Fluorophore-labeled microspheres offer a convenient means to check instrument status and monitor stability over time. When beads are run, the median channel values for pertinent fluorescence detectors are recorded, and data are monitored to identify outliers and trends. This type of check can alert the user to problems with the optical and fluidic systems (e.g. diminishing laser power or obstruction/leakage) and the effect of environmental factors, such as temperature, humidity, and vibration on instrument performance.

Alignment

Microspheres with narrow fluorescence coefficients of variation (CVs) are used for alignment. Fixed alignment instruments are verified periodically, while manual instruments are aligned on a daily basis. Some applications, such as DNA content analysis, will also dictate daily alignment verification.

Although reference fluorescence CVs are provided on alignment bead Certificates of Analysis, it is important that instrument-specific tolerances be established. Tolerances should be established on an optimally aligned instrument, i.e. immediately following a service visit. Keep in mind that fluorescence CV is dependent upon flow rate, concentration, and the optical system of the instrument.

Sensitivity: Detection Threshold

Determining an instrument's threshold sensitivity at test-specific settings is important for both qualitative and quantitative fluorescence analyses. Detection threshold may be determined by using fluorochrome-labeled Quantum™ microspheres and the QuickCal® analysis template. QuickCal® constructs a calibration curve associating fluorescence channel values to standardized fluorescence intensity units. The channel value corresponding to the kit's blank bead is converted to the appropriate standardized fluorescence intensity unit (MESF or ABC); this is the instrument's detection threshold for the specific reporter and detector.

As a QC tool, a drifting detection threshold can alert the user to problems, such as contaminated fluidics (e.g. free label or debris adhering to the unstained bead), or a shortcoming within the optical system.

Sensitivity: Resolution

An instrument's sensitivity is also dependent upon its ability to distinguish unlabeled (autofluorescent) from dimly stained samples. Acceptable resolution may be verified using bead kits that contain multiple populations spanning a range of fluorescence intensities. Poor resolution may indicate a problem within the optical or fluidics system, or suboptimal instrument settings (e.g. use of Linear Mode for fluorescence analyses, or an excessive flow rate).

Linearity

The accurate measurement of fluorescence signal is imperative for applications in quantitative fluorescence cytometry, such as surface marker expression or telomere length determination. To this end, the linear response of the PMTs should be assessed regularly.

Fluorochrome-labeled microspheres of differing intensitites are used to generate a standard curve relating channel values to standardized fluorescence intensity units. Our QuickCal® analysis template calculates a regression and reports the regression coefficient (r2), which should be as near as possible to 1.0. Deviations may indicate the need for maintenance or calibration of components of the instrument's optical system.

STANDARDIZATION

In flow cytometry, standardization is essential for achieving consistent results and generating comparable data. The program should be comprehensive, encompassing reagents, protocols, instrument configuration, and, for qualitative analyses, fluorescence intensity units. Our microsphere-based tools for standardization can serve a significant role in supporting flow cytometry programs, and establishing a framework for the comparison of data from different instruments, laboratories, and over time.

Daily Set-up
Flow cytometers are highly configurable, and results can vary dramatically with different instrument settings. Establishing a common "Window of Analysis" for each detector, with upper and lower fluorescence limits defined, allows reference populations to be positioned in approximately the same place on the scale. This type of standardized instrument set-up ensures consistency of results from specific instruments and enables meaningful data comparison between instruments. Standardized instrument set-up products such as, Quantum™ QC or QC Windows® can ameliorate differences in range, relative scale, and reporting units, as well as daily fluctuation due to electronic noise and ambient temperature and humidity.

Compensation
Standards offer a convenient means for establishing compensation settings in multicolor flow cytometry. Our FITC/PE Compensation Standard is suitable for analyses using these common fluorophores. For analyses relying on other or additional fluorochromes, customized compensation bead sets may be easily developed by labeling aliquots of our Simply Cellular® Compensation Standard or Quantum™ Simply Cellular® microspheres with the same antibody/fluorochrome conjugates that are used to label cells.

Standardized Fluorescence Intensity Units
Quantitative fluorescence analyses demand the highest level of standardization. However, cytometers lack internal calibrators for fluorescence intensity, and are limited to reporting results in relative terms. Our Quantum™ MESF and Quantum™ Simply Cellular® microspheres are external standards that enable the standardization of fluorescence intensity units irrespective of instrument and software. Moreover, they are labeled with the actual fluorochromes used to label cells, for synchronous response to the environment. The beads are run on the same day and at the same settings as samples to establish a calibration curve relating instrument channel value and standardized fluorescence intensity units. Unknowns may then be read against the curve for determination of expression (i.e. quantitation of the signal from each cell population).

APPLICATIONS

Quantitative Fluorescence Cytometry

Fluorescence cytometry is an important tool for investigations in cell and molecular biology. This technology is routinely used for immunophenotyping and an expansive array of research applications, such as the study of protein phosphorylation and the determination of telomere length.

Although fluorescence cytometry has proven to be a very powerful and versatile technology, it is not without its limitations. Notably, without a standardized measure of fluorescence intensity, results of analyses can only be described in relative terms, such as "negative," "dim," "intermediate," and "bright," or in arbitrary fluorescence intensity units. The interpretation of fluorescence intensity units can be further complicated by other factors, such as daily instrument variation, differences in hardware (laser power, filter sets), PMT settings, software, environmental factors such as buffer pH, and the fluorochrome labeling density of antibodies F/P ratio).

Bangs' tools for quantitative flow cytometry provide the means to standardize fluorescence intensity measurements, thereby permitting truly quantitative analyses. Our products include Quantum™ MESF (Molecules of Soluble Fluorochrome) and Quantum™ Simply Cellular® (ABC, Antibody Binding Capacity) kits. Fluorochrome-labeled microspheres are used to generate a standard curve relating fluorescence intensity to standardized MESF or ABC values from Quantum™ MESF or Quantum™ Simply Cellular® beads. The MESF or ABC values of labeled cell samples may be determined by measuring their fluorescence intensities, and "reading" the corresponding MESF or ABC values from the standard curve using the QuickCal® analysis template that is provided with the kit.

Cell Counting
Enumeration of cells in a sample may be achieved through the concurrent use of a microsphere count standard, such as our Flow Cytometry Absolute Count Standard™. Cell count may be calculated by determining the ratio of fluorescent count beads to unlabeled cells.

Suspension Arrays
Applications in flow cytometry now extend far beyond traditional cellular analyses. The flow cytometer's ability to perform multiparametric detection of virtually any cell-sized particle has enabled the proliferation of simplex and multiplexed bead-based assays. Suspension assays are statistically robust and additionally offer the benefits of economy, convenience, flexibility, and exceptional binding kinetics.

Our QuantumPlex™ products offer a fully customizable platform for the development of assays that may be run on any standard cytometer. Beads are encoded with varying intensities of our Starfire Red™ fluorophore for the identification of bead populations corresponding to specific capture molecules or sequences. Starfire Red™ has very little carryover into lower wavelengths, leaving other detectors available for determination of positive binding events via common reporters, such as FITC and PE.