TEMPERATURE EFFECTS ON FLUOROPHORE CONJUGATED MICROSPHERE STANDARDS

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ABSTRACT

Background:
Flow cytometry quality assurance programs must encompass both the complexity of the instruments, themselves, and the analyses that are run. Microparticles that are surface conjugated with fluorophore play an important role in such QC programs, and their use is central to quantitative analyses in particular.

Like stained biologic samples, surface-labeled microspheres are sensitive to their environments (buffer pH, osmolarity, temperature). This responsive nature makes them ideal surrogates for stained cell samples; consequently, they can be similarly susceptible to suboptimal handling. Though formal guidelines are provided for handling and storage of Quantum™ MESF microspheres, we undertook studies to better understand the effects of temperature on FITC and R-PE versions of these surface-labeled standards.

Methods:
Three types of trials were undertaken to investigate the effects of different handling conditions on Quantum FITC and Quantum R-PE MESF microspheres. These included:

1.) A single day study of MESF bead populations to assess fluorescence response upon removal from refrigerated (4-8°C) storage and holding at room temperature (RT) thereafter.

2.) A longitudinal study to assess fluorescence stability when utilized in accordance with specified guidelines.

3.) A longitudinal study to assess fluorescence stability of microspheres when repeatedly acclimated to RT.

Internally-labeled (hard-dyed) Full Spectrum™ beads were run as controls, to discern normal instrument fluctuation.

Results:
When samples were prepped and run immediately upon removal from refrigerated storage, only normal fluctuation, and no temperature-dependent effect on fluorescence was observed for each population. However, when surface-labeled microspheres were repeatedly acclimated to RT prior to runs / return to refrigerated storage, PE-labeled microspheres experienced a gradual, yet progressive loss of fluorescence intensity. In contrast, FITC-labeled microspheres were found to be sensitive to the first prolonged (30 min.) exposure to elevated temperature, though they proved to be relatively stable through subsequent exposures. FITC and PE standards exhibited excellent stability when returned to refrigerated storage immediately (< 5 min.) Full Spectrum microspheres exhibited stable fluorescence across conditions.

Conclusions:
Both internally-dyed and surface-labeled microspheres have their place in a comprehensive flow cytometry QC program. It is important to recognize, however, that standards with surface-immobilized fluorophore are more susceptible to sub-optimal handling, including prolonged and/or repeated exposure to room temperature. It has been demonstrated that proper handling is essential for maintaining the integrity of quantitative fluorescence standards. These findings argue for the careful handling and storage of fluorochromes and fluorophore-labeled reagents, as well, and underscore the value of surface-labeled microsphere standards as cell surrogates in quality control programs.

INTRODUCTION

In flow cytometry, a comprehensive program of quality assurance and standardization is essential for achieving accurate and consistent results within a study, and generating comparable data between instruments and laboratories. 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.

A collection of microparticles is typically enlisted to QC the full breadth of components, systems and configurations of an instrument. Microspheres may be of different sizes to approximate various cell types; they may be labeled with
different fluorophores to match specific lasers/detectors; and they may feature
different labeling strategies (internally or surface labeled with fluorochrome).

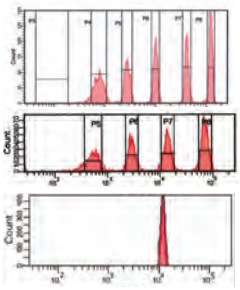
Microsphere standards that are internally-labeled with fluorochrome offer static performance. They are not environmentally-responsive as the fluorophore molecules are entrapped within the bead, and do not come into contact with the suspending solution, where they might be subject to a range of conditions. When these types of standards are run on a routine (daily) basis, fluctuations in fluorescence intensity reflect normal daily variation of the instrument and its macro environment (laboratory temperature / humidity, vibrational 'noise,' etc.).

Surface-labeled microspheres feature the same fluorochromes that are used to label cells. They are dynamic standards, as the surface-immobilized fluorochrome is exposed to the same conditions as are labeled cells, e.g. buffer pH / osmolarity / temperature. Their use is central to quantitative fluorescence analyses as they offer both true spectral matching and true environmental responsiveness. When used as an instrument QC tool, fluctuations in fluorescence intensity reflect instrument-related performance, as well as factors in the microenvironment (buffer / suspending solution).

Fluorochromes each have unique properties, and may be more or less sensitive to different environmental factors. For example, fluorescein is known to be pH-responsive, and both temperature dependency and sensitivity to certain buffer additives has been observed (unpublished data). Though we have found R-Phycoerythrin (R-PE) microspheres to offer extremely stable performance over time, and have observed them to be less sensitive than fluorescein-conjugated microspheres to certain buffer conditions, the effects of temperature (i.e. deviations from refrigerated storage) had not previously been fully characterized.

MATERIALS AND METHODS

Quantum™ MESF FITC (Cat. 555) and PE (Cat. 827) kits were used as the surface labeled populations and feature one blank bead population, and four or five populations surface-labeled with increasing amounts of fluorochrome. Internally-labeled Full Spectrum microspheres (Cat. 885) were run as controls.



Quantum™ FITC MESF microspheres

Quantum™ PE MESF microspheres

Full Spectrum™ microspheres

Three trials were undertaken to investigate the temperature-responsiveness of Quantum™ PE MESF and Quantum™ FITC MESF microspheres. These included:

1.) A single day study of FITC and PE bead populations to assess fluorescence response upon removal from refrigerated (4-8°C) storage and acclimation to / holding at room temperature (RT) thereafter.

2.) A longitudinal study of Quantum™ PE and Quantum™ FITC MESF microspheres to assess fluorescence stability when utilized (removed from and immediately returned to refrigerated storage, < 5min) multiple times in a week.

3.) A longitudinal study assessing fluorescence stability of PE and FITC MESF kits when utilized after removal from refrigerated storage, acclimated to RT (30min), sampled / run, and returned to refrigerated storage. Beads were run ~every day through the work week.

Microspheres were run on a FACSCalibur (488nm laser, FL1 / FITC and FL2 / PE detectors) using CellQuest Pro software and a 1024 scale. Samples include one drop (~50µL) of each bead population and 400µL diluent. Internally-dyed Full Spectrum™ microspheres were run each day and charted alongside the surface-labeled FITC and PE populations to monitor other aspects of instrument fluctuation / stability.

RESULTS

Single Day Study, Response of FITC and PE microspheres to RT (1024 scale used)

Microspheres were run immediately upon removal from the refrigerator (8:00 a.m.), and every 30 minutes thereafter until 4:00 p.m., with the bottles remaining at RT after their initial removal from the refrigerator. FITC - labeled microspheres were found to be sensitive to the first prolonged (30min) exposure to room temperature, but exhibited stable fluorescence thereafter. A temperature effect on the fluorescence intensity of PE was not observed. Both internally-dyed Full Spectrum and surface-labeled PE MESF beads were stable under these conditions and timeframe.

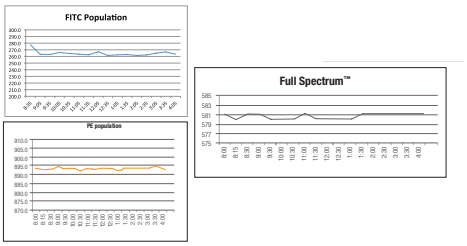
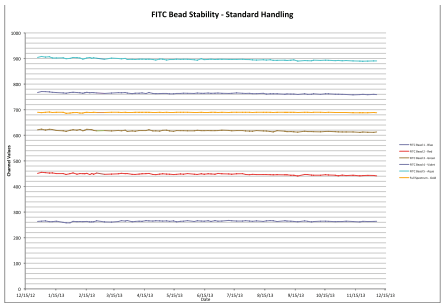


Figure 1: *FITC, PE and Full Spectrum™ microspheres run every 30 minutes following removal from the refrigerator. An initial temperature effect was observed for FITC, but only normal fluctuation, and no temperature-dependent effect on fluorescence, was observed for PE and Full Spectrum populations.*

Longitudinal Study, Response of FITC and PE MESF Kits Using Standard Handling Recommendations (≤ 5min out of refrigerator)

Microspheres were removed from the refrigerator, samples prepped, and stock bottles returned to the refrigerator (≤ 5min). Microspheres were run at a rate of ~2-3 times / week over a 12-month + period. The kits remained stable under these storage and use conditions.



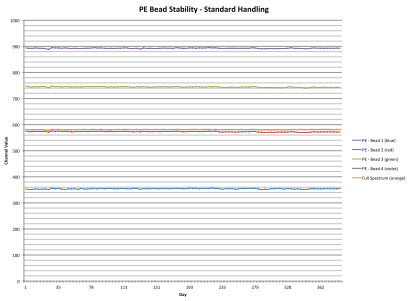


Figure 2: *Quantum™ MESF and Full Spectrum™ microsphere samples were prepped and run immediately upon removal from refrigerated storage. The kits remained stable under these conditions.*

Longitudinal Study, Response of FITC and PE MESF Kits with Acclimation to RT before Each Run (~30min out of refrigerator)

Microspheres were removed from the refrigerator, allowed to acclimate to room temperature, samples prepped, and stock bottles returned to the refrigerator (~30min). The kits were run at a rate of ~4-5 times / week over an 8-month period. Both kits exhibited a progressive loss of fluorescence intensity.

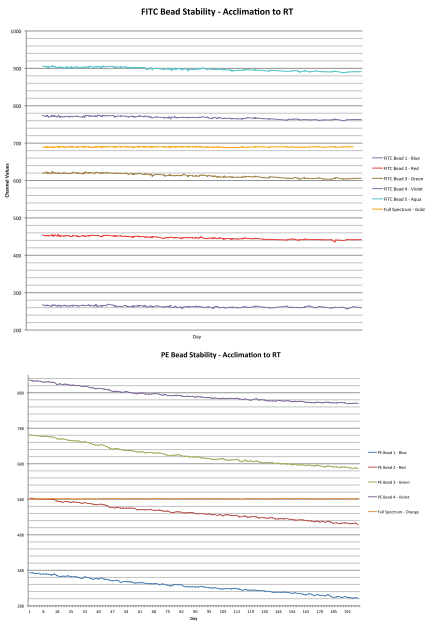


Figure 3: *Quantum™ MESF microsphere samples were prepped and run after removal from refrigerated storage and acclimation to RT. The same bottles were returned to refrigerated storage after the runs. Both FITC and PE-labeled microspheres experienced a gradual, yet progressive, loss of fluorescence intensity, though the loss was much more extreme for PE. This effect is highlighted by the fluorescence profile of the internally-labeled Full Spectrum™ population.*

CONCLUSIONS

Microsphere standards aid in defining a flow cytometer'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 ambient temperature, humidity and electronic noise. Both internally-dyed and surface-labeled microspheres have their place in a comprehensive QC program, and are well-suited to their respective roles, as instrument and (particularly quantitative fluorescence) study standards.

It is important to recognize, however, that standards with surface-immobilized fluorophore are at greater risk than internally-dyed microspheres if subjected to sub-optimal handling. We have demonstrated that PE, typically considered to be one of the most robust and stable fluorochromes, exhibits marked sensitivity to the cumulative effects of temperature cycling. This was demonstrated by an immediate and gradually progressive loss of fluorescence intensity when beads were acclimated to RT and then returned to refrigerated storage each day. In contrast, FITC microspheres were found to be sensitive to the first prolonged (30 min) exposure to room temperature, though they proved to be relatively stable through subsequent exposures. These findings highlight that negligent or sub-optimal handling of microsphere standards, particularly those with surface-immobilized fluorophore, may impact long-term stability and introduce a source of variation. Standards must be handled in accordance with their specific recommendations for highest confidence and stability. Both types of surface labeled standards exhibited excellent stability when handled as recommended.