A PC BASED PROGRAM TO RECORD AND MONITOR FLOW CYTOMETRY OPTICAL AND FLUORESCENT STANDARDIZATION

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Introduction

ASHI Standards require that optical and fluorescent standards be run and recorded in a separate, non-instrument daily quality control (QC) log. This QC data must be reviewed for acceptable performance and trends and must be used as a trigger for corrective action. The QC program supplied by our flow cytometer vendor does not provide easy access to the data in graph form following each data entry. Rather it is more amenable to retrospective data analysis of whole lots of standardization materials that does not allow emerging trends to be easily identified.

We developed a software program that allows daily electronic data transfer or manual data entry onto a spreadsheet and immediate display of data points on Levey Jennings plots. The program allows immediate review and documentation of acceptable instrument performance after each data entry.

Materials and Methods

The program is a custom Microsoft Excel™ application that is run on a Windows 2000 based network system. Automated network backup is performed nightly for data protection. The program calculates and plots cumulative means and standard deviations daily. However, data points from failed QC runs are not figured into the mean or standard deviations for the lot.

Applicable Westgard QC rules were identified in consultation with the flow cytometer vendor for each parameter. These rules are applied to the data to assist in identification of significant single event outliers or emerging trends. Such anomalies may indicate the need for instrument maintenance, fluidics system cleaning or laser replacement. In addition

to daily review, final reports of the composite data and attending plots are printed for each lot of standardization material tested and reflect the instrument's performance over time.

Results

The parameters monitored, applicable Westgard QC rules, critical trends, possible causes, and appropriate responses are summarized in **Table 1**. In the Levey Jennings plot for each parameter, data values that violate Westgard rules are plotted in red, diamond shaped points. Westgard rule violations are explained in a dialog box, and a Comments window is provided to record remedial actions. Certain violations trigger a remedial response even when the standardization run meets the minimum passing criteria for the vendor's calibration software. Such violations include those indicating a need for instrument cleaning or maintenance or impending laser failure.

Figure 1 shows a Westgard violation in forward scatter (FSC) separation data for runs 18 and 19. **Figure 2** shows that side scatter (SSC) separation for run 20 failed to meet the minimum passing criteria established by the manufacturer of the standardization material. A drop in FSC or SSC separation usually indicates an increase in "noise," which can be caused by a dirty flow cell, fluidics system, or sheath fluid. In both cases, the appropriate remedial action is backflushing, draining, and filling the flow cell. In addition, fresh standardization bead suspensions were prepared in response to the SSC failure. For both violations, the separation values returned to normal within two consecutive runs.

Figure 3 illustrates an example of laser current values that suddenly and dramatically increase. While the current normally rises very gradually as the laser ages, any pronounced change indicates a serious problem. Even if the QC run meets the manufacturer's minimum criteria, if laser power or current exceed the historic minimum or maximum the instrument vendor should be contacted immediately to determine the need for immediate laser replacement.

Table 1. This table summarizes the flow cytometry standardization data, Westgard QC rules, critical problematic outliers/trends, possible causes, and appropriate remedial responses monitored by this computer program.

Data	Westgard QC Rules Applied	Critical Outliers/Trends	Possible Causes	Appropriate Response
Separation Data (FSC, SSC, FL1, FL2, FL3)	Single measurement exceeds 3 SD in this and one or more other parameters for a single run, or seven consecutive measurements trend in same direction	Drop in separation with corresponding rise in "Low" (noise) signal for that parameter – esp. FSC/SSC	Possible random outlier Dirty flow cell/fluidics system/sheath fluid (FSC, SSC) Residual fluorescent dyes in system (FL1, FL2, FL3)	**For Westgard violations involving Separation, Detector (PMT), or Compensation data, perform these remedial actions as appropriate for the suspected cause:** 1) If the Calibrite beads are approaching their outdate, open a new box. Replace sheath fluid if cloudy. 2) For possible random outliers: backflush, drain and fill flow cell, repeat FACSComp. 3) For a dirty flow cell or fluidics system: run fresh 10% bleach for 10 minutes. If problem does not resolve, perform monthly instrument maintenance. 4) Repeat FACSComp after remedial action. 5) Inform Supervisor or Director if outlier/trend is observed again. 6) Call manufacturer.
Detector Data (PMT voltages)	Same as above	Increased frequency of outliers or upward / downward trends in PMT voltages	Possible random outlier Calibrite beads approaching outdate Dirty flow cell/fluidics system/sheath fluid Problem with photomultiplier tubes	
Compensation Data	Same as above	Increased frequency of outliers or upward / downward trends in compensation percentages	Possible random outlier Calibrite beads approaching outdate Residual fluorescent dyes in system (commonly sticky dyes like propidium iodide)	

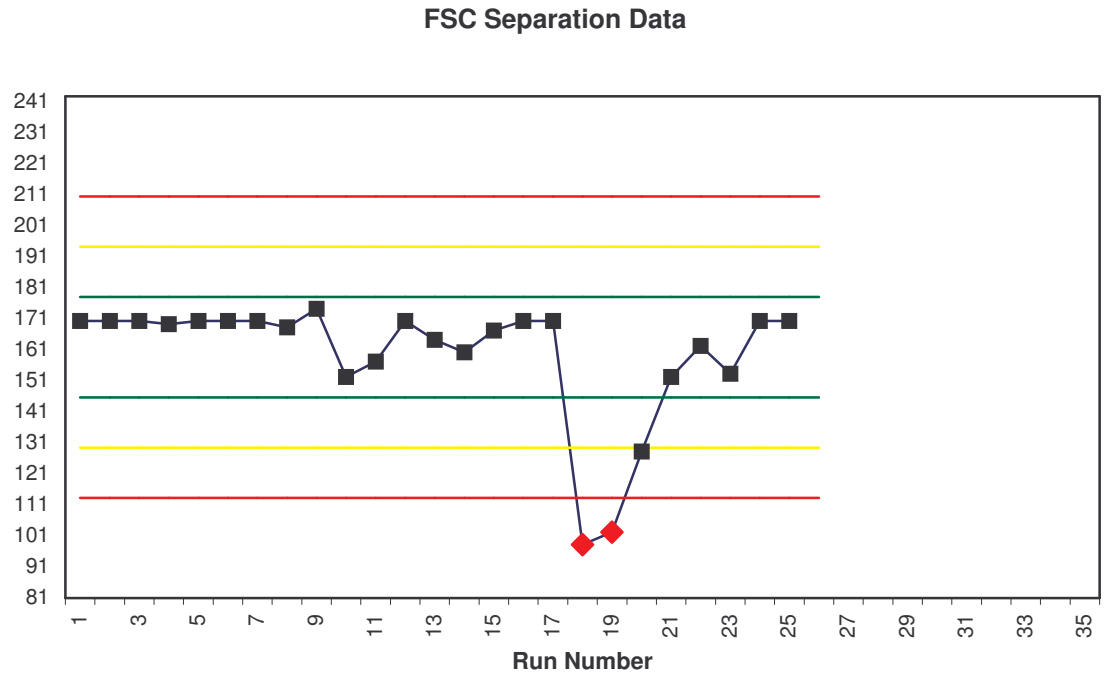

Figure 1. This graph of forward scatter separation data shows Westgard QC rule violations for runs 18 and 19. The appropriate remedial action was backflushing, draining, and filling the flow cell to eliminate debris.

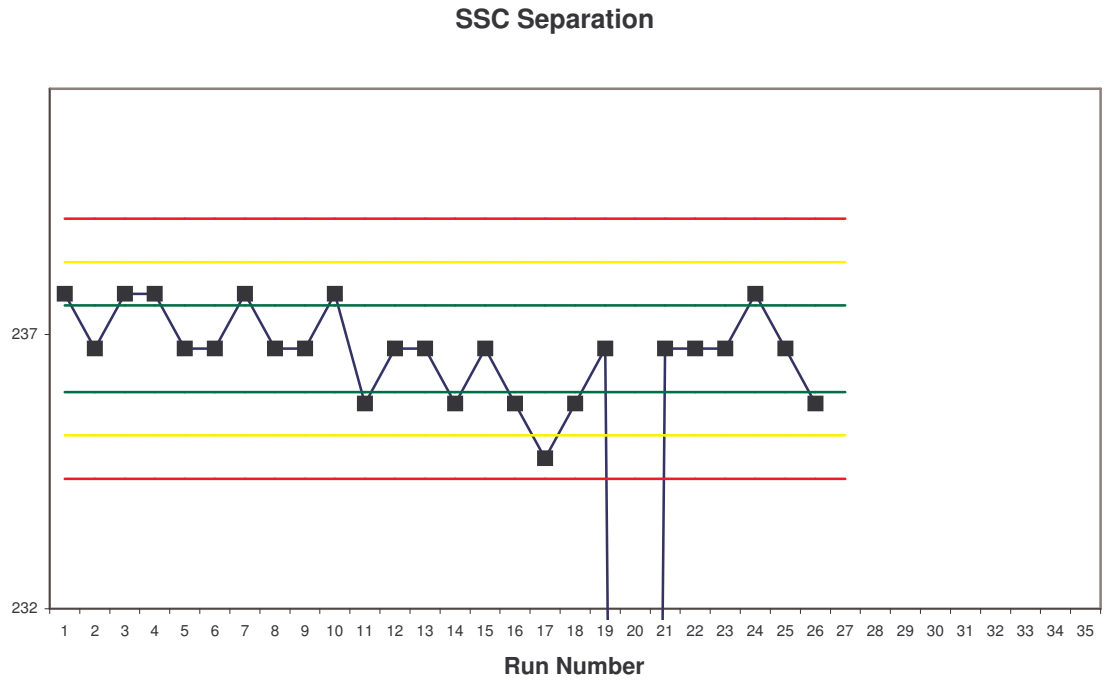

Figure 2. This graph of side scatter separation data shows run 20 that failed to meet the minimum passing criteria established by the manufacturer. The failed data point is not figured into the mean or standard deviations for the lot. The appropriate remedial action was backflushing, draining, then filling the flow cell, followed by preparation of new suspensions of QC beads.

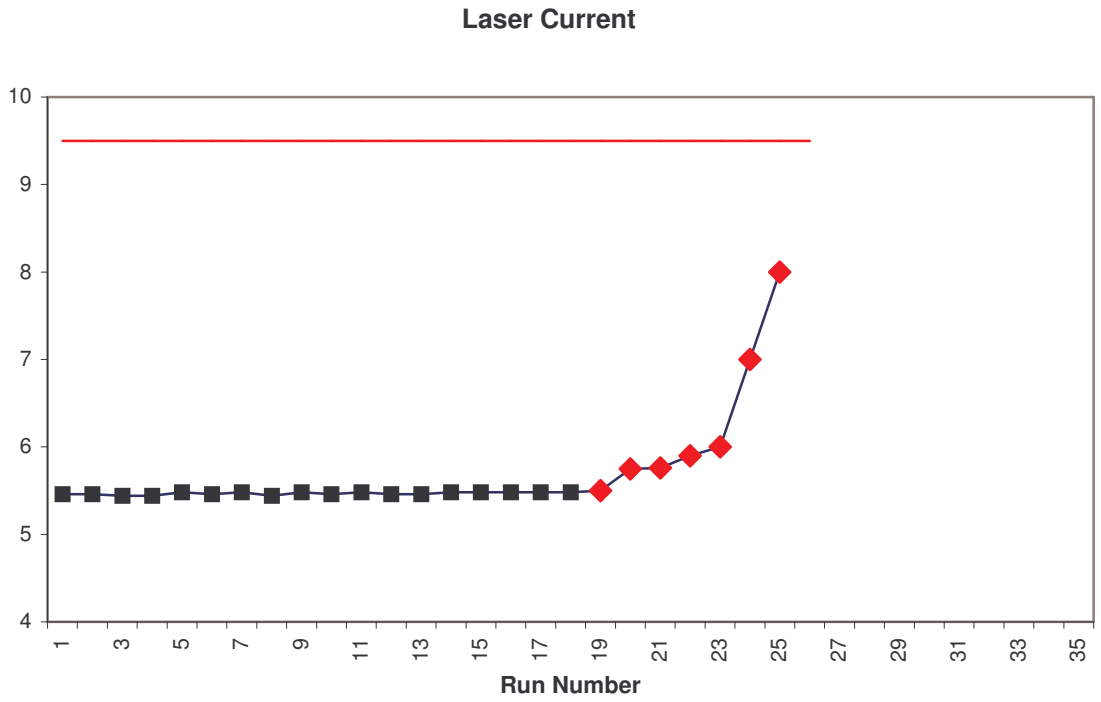

Figure 3. This graph shows a dramatic increase in laser current values flagged by the QC software. If laser power or current exceed the historic minimum or maximum, the instrument vendor should be contacted immediately to determine the need for immediate laser replacement.

Conclusion

This QC program's Levey Jennings plots provide a "snap-shot" of instrument status on a daily basis, as well as a platform for tracking and trending instrument performance over time. In addition to fulfilling the ASHI requirement for a non-

instrument daily QC log, this program functions as a useful indicator tool within the overall maintenance program for a flow cytometer.