Correlation of Bio-Plex Suspension Array Reader Validation With Multiplex Cytokine Assay Performance

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Introduction

The Bio-Plex suspension array system is an integrated system consisting of hardware, software, and assay kits. The system employs bead-based Luminex technology to simultaneously analyze up to 100 targets in a single microplate well. The Bio-Plex validation kit is a tool designed for operational qualification of the system. The kit validates the performance of the fluidics and optics systems, the two primary components of the suspension array reader. A high correlation exists between the validation kit parameters and assay performance; therefore, proper validation of the system is essential for ensuring optimal assay performance. In this technical study, the optics system of a reader was deliberately misaligned to varying degrees to demonstrate the effect of a poorly maintained system on assay performance. At varying degrees of misalignment, reporter channel performance was evaluated using the Bio-Plex validation kit. The effect of misaligned optics was further illustrated by analyzing a 3-plex cytokine assay at each degree of system misalignment.

Validation Kit Parameters

Qualification of analytical instruments is a formal process of documenting that an instrument is fit for its intended use and that it is kept maintained and calibrated (Bedson and Sargent 1996). The Bio-Plex validation kit is used for operational qualification of the Bio-Plex suspension array system. The validation kit consists of four sets of beads that are used to evaluate the performance parameters listed in Table 1.

The reporter validation test is designed to confirm that the reporter channel is correctly measuring assay signals by simulating the results of a typical assay. This test utilizes a set of beads containing various intensities of a dye similar to R-phycoerythrin, the fluorochrome used in Bio-Plex assays.*

* The terms fluorophore, fluorochrome, and fluorescent dye are used interchangeably.

Table 1. Validation kit parameters.

Test	Function Assessed
Optics validation	Optics alignment of the system, by measuring the % coefficient of variation (CV) of the peak signal in each fluorescence channel
Reporter validation	Performance of the assay detection channel using a set of beads designed to simulate results of a typical assay
Classify validation	Ability of the system to accurately distinguish, or classify, beads into their respective regions of the bead map
Fluidics validation	Degree of bead carryover from well to well

The fluorescence of each bead within the set is expressed as molecules of equivalent soluble fluorescence, or MESF, a unit that corresponds to the fluorescence intensity of a given number of pure fluorochrome molecules in solution (Henderson et al. 1998). The beads are analyzed on the Bio-Plex system and a plot of fluorescence intensity versus concentration is created. The plot is used to calculate a series of parameters that are typically used to evaluate assay performance, including dynamic range, accuracy, linearity, sensitivity, and slope. Table 2 lists each of the parameters and their respective definitions.

Table 2. Reporter validation parameters.

Parameter	Definition
Dynamic range	Calculated number of decades covered by a series of samples
Linearity (r^2)	A linear relationship determined by calculating the best straight line through a set of data using the least squares method. Linearity is expressed as r^2, the square of the Pearson product moment correlation coefficient, r
Accuracy	Closeness of a measured value to the actual value
Slope	Rate of change along a regression line, determined by dividing the vertical distance between any two points on a line by the horizontal distance
Sensitivity	Lowest detectable limit of an analyte, expressed as molecules of equivalent soluble fluorescence (MESF) in the reporter validation test

Table 3. Effect of misaligned optics on reporter channel performance. Values shown in red and results in orange shaded cells were outside acceptable specifications.

	Reporter Channel CV				Specification
Reporter Channel Performance	**7.6%**	**10.5%**	**16.0%**	**18.0%**	**5–10%**
Dynamic Range	4.23	4.18	**3.89**	**3.23**	4.15–4.28
Linearity	0.9998	0.9995	0.9998	0.9987	>0.995
Accuracy of Response	93.3	**90.9**	**86.7**	**64.7**	>90%
Slope of Response	0.0732	0.0649	**0.0335**	**0.0073**	0.0593–0.0799
Sensitivity	136	185	**358**	**1,498**	<200 MESF

Methods and Results

Effect of Misaligned Optics on Reporter Channel Performance

If the Bio-Plex system is moved or jarred, its optics may become misaligned, adversely affecting assay results. To demonstrate this phenomenon, the optics path of the reporter channel of a Bio-Plex system was deliberately misaligned to different degrees. The degree of misalignment of the system was verified using the reporter channel %CV of the optics validation test (7.6%, 10.5%, 16.0%, and 18.0%). A reporter channel %CV of <10% is considered acceptable. At each degree of misalignment, a reporter validation test was performed and each of the reporter validation parameters (dynamic range, linearity, accuracy, slope, and sensitivity) was evaluated to assess the effect of optics misalignment on reporter channel performance, as shown in Table 3. In each case where the optics validation test fell outside acceptable specifications, certain parameters of the reporter validation test also showed unacceptable results. The slope decreased when the reporter %CV increased to 16%. When the slope decreased, the dynamic range also decreased. Sensitivity was significantly reduced at a reporter %CV of 16%. The accuracy was affected most significantly, with a value outside of acceptable specifications when the reporter %CV was >10.5%. These data indicate a high correlation between optics alignment and reporter channel performance.

Table 4. Parameters measured at different degrees of array reader misalignment for three cytokines. An instrument with a reporter channel %CV >10% (as indicated in red) does not pass the Bio-Plex validation kit test for reporter channel performance. Results in orange shaded cells were <80% of results obtained for the validated reporter channel (7.6% CV).

	Reporter Channel CV			
Cytokine Assay	**7.6%**	**10.5%**	**16.0%**	**18.0%**
IL-6				
LOD	1.95	1.95	1.95	1.95
Low signal (background)	42	36	**23**	**14**
High signal (10,000 pg/ml standard)	26,060	24,211	**16,733**	**3,698**
Dynamic range	3.57	3.31	**2.29**	**0.51**
Signal/noise (3.9 pg/ml standard)	3.68	3.06	**2.59**	**1.39**
IL-8				
LOD	1.95	1.95	1.95	1.95
Low signal (background)	22	21	**16**	**12**
High signal (10,000 pg/ml standard)	27,905	25,369	23,453	**6,163**
Dynamic range	3.8	3.5	3.2	**0.8**
Signal/noise (3.9 pg/ml standard)	7.94	6.46	**4.41**	**1.89**
GM-CSF				
LOD	1.95	1.95	1.95	**62.50**
Low signal (background)	22	19	**15**	**12**
High signal (10,000 pg/ml standard)	14,157	12,006	**6,821**	**1,443**
Dynamic range	1.94	1.64	**0.93**	**0.19**
Signal/noise (3.9 pg/ml standard)	1.48	1.32	1.18	**1.09**

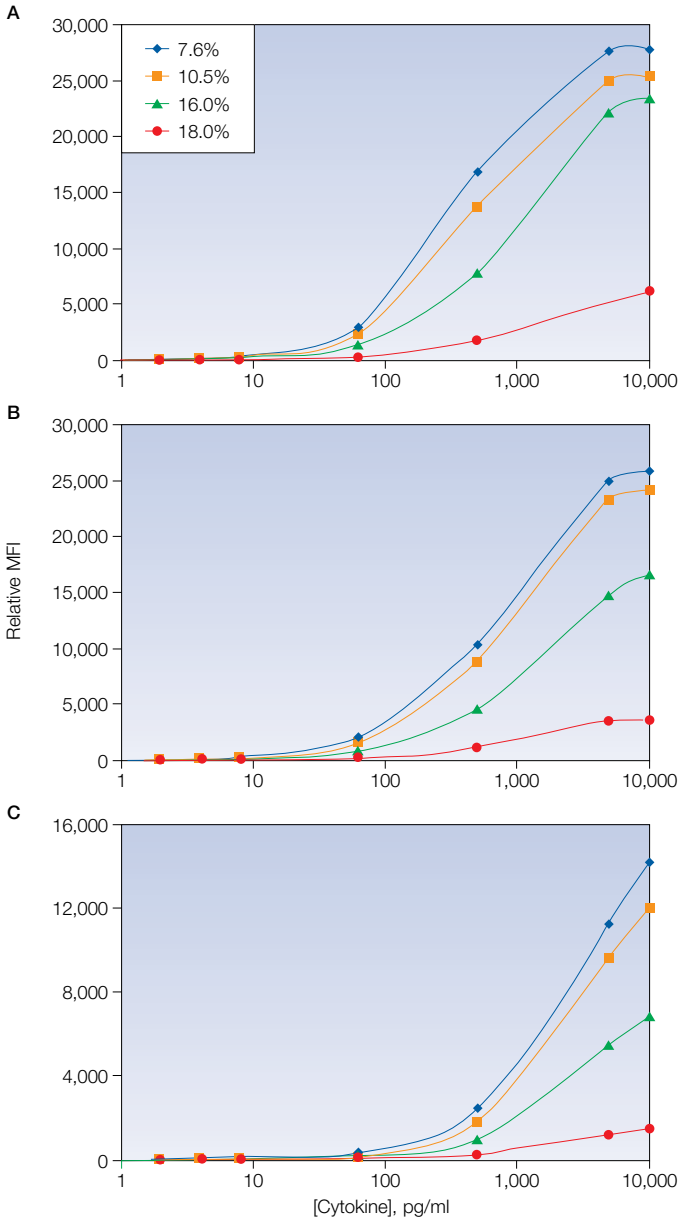
Effect of Misaligned Optics on Cytokine Assay Performance

The effect of misaligned optics was also analyzed using a 3-plex cytokine assay (IL-6, IL-8, and GM-CSF) on a system at varying degrees of misalignment. The same parameters measured in the reporter validation kit were also evaluated for actual assays, including sensitivity, slope, and dynamic range. More specifically, five parameters were evaluated: 1) limit of detection (LOD) as defined by 3 standard deviations (SD) above the mean of the background, 2) low fluorescence intensity signal (equal to background), 3) high fluorescence intensity signal (10,000 pg/ml standard concentration), 4) dynamic range of the standard curve, and 5) signal-to-noise ratio of the 3.9 pg/ml standard. The results of the analyses of the three cytokines are shown in Table 4. When the optics alignment and reporter validation values fell outside acceptable specifications, the assay parameters changed significantly (Table 3). The signal-to-noise ratio and dynamic range were affected, while the LOD remained constant for two of the three cytokines. As the instrument was misaligned to a greater degree (that is, the reporter %CV increased), the overall signal of the assay decreased across the entire standard curve. This is evident when comparing the low and high assay signals for all three cytokines. For example, the IL-6 assay showed a reduction in relative fluorescence intensity of the 10,000 pg/ml standard from 26,060 to 3,698 when the instrument was misaligned to a reporter channel %CV of 18%. As the overall signal of the assay decreased, the slope decreased significantly and the dynamic range of the assay also decreased. These parameters indicate that assay performance is significantly negatively affected by misalignment of the optics.

The effects of misalignment on assay performance are further shown when comparing the standard curves from each of the three cytokines (see figure). The standard curve for all three cytokines was shifted downward even when the reporter %CV was shifted from 7.6% to 10.5%.

Discussion

A number of conclusions may be drawn from this study. When a system is misaligned and the optics validation is affected (as determined by the optics validation reporter %CV), reporter validation parameters are also affected, indicating a high correlation between the optics and reporter validation parameters. The performance of a cytokine assay is affected in the same manner as the reporter validation kit parameters, indicating that the validation kit parameters correlate with cytokine assay performance. Finally, cytokine assay performance parameters are directly affected by the alignment of the optics, indicating that proper validation of the system is critical. Overall, these data suggest that the Bio-Plex validation kit is an essential tool for validating the performance of the Bio-Plex system, thereby ensuring optimal assay performance.



Assays of cytokines on a Bio-Plex system reader with varying degrees of misalignment. A, IL-6 assay; **B**, IL-8 assay; **C**, GM-CSF assay. MFI, mean fluorescence intensity.

References

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