Correlation of Multiplex Cytokine Assay Performance with Bio-Plex™ Protein Array Reader Validation

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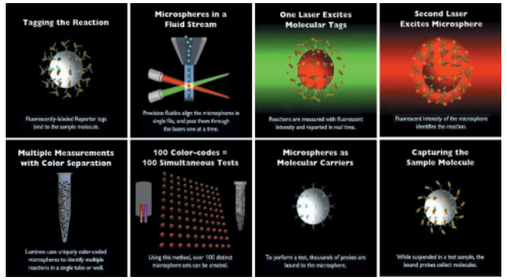
Abstract

The Bio-Plex protein array system is an integrated detection system of hardware, software, and assay kits. It employs Luminex® technology to simultaneously analyze up to 100 targets in a single microtiter well. The Bio-Plex array reader contains an optical bench with lasers and filters that must be correctly aligned for optimal performance. Therefore, we have developed a complete system for the validation of the array reader. The Bio-Plex validation kit has procedures and reagents to assess the optical alignment of the Bio-Plex array reader, and to qualify the typical instrument performance parameters of a fluorescence detection system. Following deliberate misalignment of the optical elements, instrument performance using the validation kit and assay performance using a Bio-Plex 3-plex cytokine assay (IL-6, IL-8, and GM-CSF) were assessed. We present data demonstrating that Bio-Plex cytokine assay performance is adversely affected when validation kit parameters are outside of specifications. The high correlation of instrument validation with assay results for IL-6, IL-8, and GM-CSF demonstrates the importance of system validation for optimal assay performance in the Bio-Plex protein array system.

Introduction

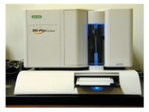
The Bio-Plex protein array system is an integrated detection system of hardware, software, and assay kits. This system employs Luminex technology to simultaneously analyze multiple targets in a single microtiter well. The primary components of the Bio-Plex array reader include an optical bench and a fluidics system. The optics system is comprised of lasers and filters that must be correctly aligned for optimal performance. There are two types of analysis channels in the system: the classify channels are used to discriminate each analyte within a single well and the reporter channel is used to quantitate the amount of analyte present on each bead. We have developed a complete system for the validation of the primary components of the Bio-Plex array reader. Optical alignment is assessed by measuring the distribution CV% of the optical beads. Validation of the reporter channel is assessed using a series of R-PE-like beads with varying intensities to evaluate linearity, slope, dynamic range, sensitivity, and accuracy. These parameters are all related to assay performance. Therefore, validation using these tools ensures that assay performance is optimal. To demonstrate the importance of the use of the Bio-Plex validation kit within the Bio-Plex protein array system, we deliberately misaligned the optics to different degrees of severity and performed validation of the reporter channel optical alignment and reporter channel performance. We analyzed a 3-plex Bio-Plex cytokine assay and correlated the results between the validation kit and the cytokine assay.

Technology



The Bio-Plex protein array system uses Luminex technology, which allows multiple measurements via color separation.

Bio-Plex Protein Array System



Bio-Plex 96-Well Plate Reader

Bio-Plex Protein Array Assay

Worldwide Technical Support

Bio-Plex Manager V1.0

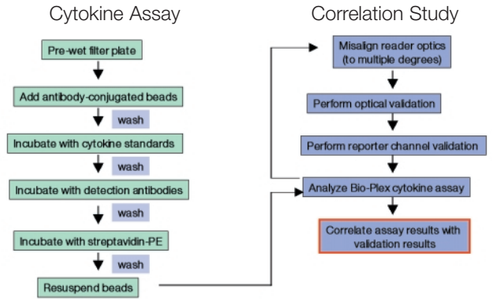
Bio-Plex Validation System

Bio-Plex Validation System

(box indicates parameters measured in this study)

- **Validation of Optical Alignment**
  Method for assessing the alignment of the reporter channel and classify channel alignment. Expressed in terms of a distribution CV% of optical beads 1 and 2
- **Validation of Reporter Channel Performance**
  Method for assessing the performance of the channel where assays are quantitated. Includes measurement of linearity, dynamic range, accuracy, sensitivity, and slope
- **Validation of Classify Efficiency**
  Method for assessing the ability of the Bio-Plex array reader to optically distinguish multiple assays within a single sample
- **Validation of Fluidics Integrity**
  Method for assessing the integrity of the fluidics of the Bio-Plex array reader. Determines if major obstructions are present in the fluidics pathway of the reader
- **Validation of Reading Precision**
  Method for assessing the ability of the Bio-Plex array reader to optically distinguish multiple assays within a single sample

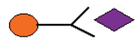
Methods — Overview



Cytokine Assay Procedure

(for Bio-Plex mouse or human cytokine kits)

1. Incubate antigen (standards or unknown samples) with polystyrene beads conjugated with capture antibody



2. Incubate with detection antibody (biotinylated antibody)

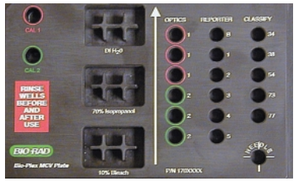
3. Incubate with streptavidin-PE

4. Analyze on Bio-Plex protein array reader

Validation System Procedures

(for optical and reporter channel validation)

1. Place 5 drops each of optical beads and reporter beads in MCV plate
2. Place MCV plate on Bio-Plex array reader
3. Select Optical Validation in Bio-Plex Manager



4. Optical validation results are automatically generated
5. Select Reporter Validation from Bio-Plex Manager, select OK
6. Reporter Validation results are automatically generated

Results

Reporter Channel Performance	Reporter CV% (Optical Validation) 7.6%	10.5%	16.0%	18.0%	Specification 5-12%
Dynamic Range	4.23	4.18	3.89	3.23	4.146 – 4.278
Linearity	0.9998	0.9994	0.9998	0.9987	>0.9994
Accuracy of Response	93.3	90.9	86.7	84.7	> 90%
Slope of Response	0.0732	0.0649	0.0335	0.0073	.0593 - .0799
Sensitivity	136.54	184.83	357.87	1498.36	< 200 MESF

Table 1. Correlation of Optical Validation with Reporter Channel Validation
The optics were deliberately misaligned to different degrees and the optical alignment and reporter validation were measured. When the optical validation fell outside of acceptable specifications, the reporter validation also showed unacceptable results. The data indicate that there is a high correlation between the optical validation specification and the reporter validation specification.

IL-6 ASSAY	Reporter Channel CV% 7.40%	10.50%	16.00%	18.00%
LOD (pg/ml)	1.95	1.95	1.95	1.95
Background	42	36.33	23.33	13.67
RP1 at 10,000 pg/ml	26060.67	24211.83	16733.5	3698.5
Dynamic Range	3.57	3.31	2.29	0.51
S/N (3.9 pg/ml)	3.68	3.06	2.59	1.39
GM-CSF ASSAY	**Reporter Channel CV%**			
	7.40%	10.50%	16.00%	18.00%
LOD (pg/ml)	1.95	1.95	1.95	62.5
Background	22	19	14.67	11.67
RP1 at 10,000 pg/ml	14156.7	12005.8	6820.7	1442.7
Dynamic Range	1.94	1.64	0.93	0.19
S/N (3.9 pg/ml)	1.48	1.32	1.18	1.09
IL-8 ASSAY	**Reporter Channel CV%**			
	7.40%	10.50%	16.00%	18.00%
LOD (pg/ml)	1.95	1.95	1.95	1.95
Background	22.33	21.33	15.67	12
RP1 at 10,000 pg/ml	27905	25369.17	23453.83	6163.17
Dynamic Range	3.8	3.5	3.2	0.8
S/N (3.9 pg/ml)	7.94	6.46	4.41	1.89

Table 2. Effect of Misalignment of Optics on Cytokine Assay Performance
A 3-plex cytokine assay was analyzed on a Bio-Plex array reader in various degrees of alignment. Data indicate that when alignment specifications fall outside of acceptable limits, assay performance is negatively impacted.
Dynamic range = log (high standard - low standard)
LOD = 2 SD above background mean

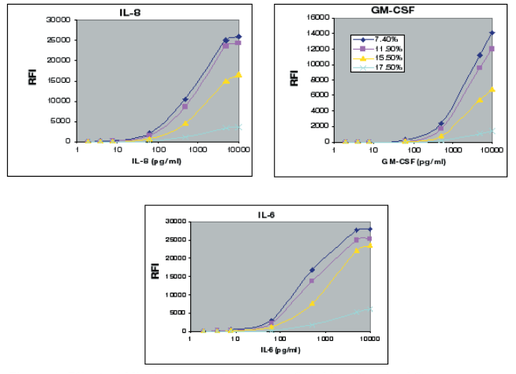


Figure 1. Effect of Misalignment of Optics on Cytokine Standard Curves
Misalignment of optics causes distinct changes in the standard curves. Dynamic range and slope are negatively impacted.

Discussion

The reporter channel optics of the Bio-Plex protein array reader were deliberately misaligned so that the distribution CV% of optical beads 2 increased to 10.5%, 16.0% and 18.0% from a starting point of 7.4%. The optics alignment of the reporter channel was correlated with the reporter channel performance. Table 1 shows the comparison between the reporter channel CV% and reporter channel performance. The data shows a high correlation between the degree of misalignment and the performance of the reporter channel.

At each degree of misalignment, a 3-plex cytokine assay standard curve was analyzed (IL-6, IL-8, GM-CSF). Each point in the standard curve was prepared in triplicate. Five parameters were evaluated: 1) limit of detection (LOD) as defined by 3 SD above the mean of the backgound, 2) background, 3) channel value of high standard curve point, 4) dynamic range of the standard curve, 5) signal-to-noise ratio (S/N) of the 3.9 pg/ml standard. When the optical alignment and reporter validation fell outside of acceptable specifications, certain assay parameters changed significantly (Table 2). S/N and dynamic range changed while LOD remained constant for two of the three cytokines. This is demonstrated dramatically when comparing the standard curves for each cytokine (Figure 1). Both the slope and the dynamic range are negatively impacted.

Conclusions

1. A high correlation exists between the optical alignment of the Bio-Plex array reader and the reporter validation kit parameters
2. Cytokine assay performance is directly affected by the alignment of the optics
3. The reporter validation kit parameters correlate highly with the assay parameters
4. The Bio-Plex validation kit is an essential tool for ensuring optimal assay performance

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