Bio-Plex™ suspension array system tech note 3192

Development of a Multiplex Bead-Based Assay for Antibody Screening of a Nonhuman Primate Colony on the Bio-Plex System

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Introduction

We have been using four separate enzyme-linked immunosorbent assays (ELISAs) to screen our primate colony for the viruses of greatest concern: simian retrovirus (SRV), simian T-cell leukemia virus (STLV), herpes B, and simian immunodeficiency virus (SIV). To minimize the labor involved, testing of antibody responses to these viruses requires that these individual assays be performed on a limited sample volume. As we add more viruses to our screening protocol, serological testing by ELISA will become more time-consuming.

The Bio-Plex suspension array system, which is based on Luminex xMAP technology, is a multiplex flow cytometric-based system that utilizes up to 100 color-coded 5.6 µm polystyrene bead sets. Each bead set is internally dyed with different ratios of two spectrally distinct fluorophores. Each of the bead sets can be conjugated with a unique protein, peptide, antibody, or, in our case, disrupted whole virus. The conjugated beads are pooled together in the wells of a microplate with the sample to be tested (plasma), followed by the addition of a detection antibody, forming a capture immunoassay that is then read by the Bio-Plex suspension array reader. Each separate reaction is identified and quantitated based on the bead color. This allows a single sample to be tested simultaneously for reaction to a number of different viruses.

Our goal was to determine whether the Bio-Plex system yields results comparable to those of our current ELISA assays. The capability to multiplex would allow us to screen animals for multiple viruses simultaneously, greatly reducing the time and labor required to perform our assays while also conserving limited samples.

Methods

Viral Disruption for Conjugation

To develop a method for viral conjugation, we began with our whole-virus ELISA protocol, in which the virus is disrupted using SDS. For the first bead conjugation, SRV was disrupted with SDS. We also tried undisrupted whole virus and whole virus disrupted with Triton X-100 detergent. To compare the results obtained with the various disruption methods, the beads were run on the Bio-Plex system.

Optimizing Conjugation by Virus/Plasma Titration

After determining that we could successfully conjugate whole disrupted virus to the beads, the next step was to optimize the amount of virus for bead conjugation, as well as to determine the optimum plasma concentration. The protocol provided with the Bio-Plex amine coupling kit suggested using 5–12 µg of protein for conjugation. We conjugated beads with 2, 5, 8, 10, and 12 µg of virus (disrupted with Triton X-100), and then reacted the conjugated beads with both positive and negative plasma at dilutions of 1:50, 1:100, and 1:200.

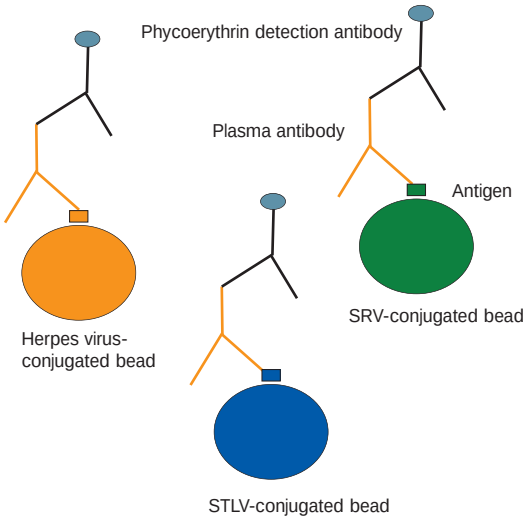

Fig. 1. Conceptual illustration of viral-conjugated bead recognition by phycoerythrin detection antibody.

ELISA Assay

ELISA assays followed conventional ELISA protocols.

Bio-Plex Assay

Multiplexing was performed according to the following protocol:

1. Prepare plasma samples.
2. Load beads into wells.
3. Wash beads.
4. Incubate plasma with antigen-conjugated beads.
5. Wash beads.
6. Incubate beads with phycoerythrin detection antibody (Figure 1).
7. Wash beads.
8. Read on Bio-Plex suspension array reader.

Table 1. Correlation between Bio-Plex assays and ELISA. The correlation coefficients of results obtained with replicate viral-positive and -negative plasma indicated a strong correlation between the methods. The CV obtained with the Bio-Plex system was lower than with ELISA.

Sample	Correlation Coefficient*	*P* Value**	CV***	
			Bio-Plex	ELISA
SRV$^+$				
1	0.99	0.005	1.5	6.0
2	0.99	0.003	2.4	13.0
3	0.98	0.003	2.1	3.9
STLV$^+$				
1	0.99	0.014	3.6	9.5
2	0.98	0.014	3.4	4.3
3	0.99	0.008	3.8	7.8
HSV$^+$				
1	0.97	0.008	1.5	7.1
2	0.96	0.008	3.6	5.3
3	0.99	0.008	2.8	7.8
SIV$^+$				
1	0.99	0.025	3.2	4.2
2	0.99	0.008	4.7	5.1
SRV$^-$	0.98	0.083	2.2	6.9
STLV$^-$	0.99	0.083	5.2	6.7
HSV$^-$	0.96	0.045	0.72	6.6
SIV$^-$	0.99	0.051	5.2	6.9

* Linear regression.

** Spearman correlation ($p < 0.05$ is significant).

*** Coefficient of variation (<20% indicates the assays perform consistently).

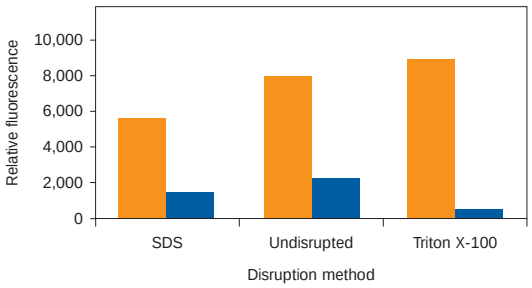

Fig. 2. Comparison of viral disruption method on signal. Results obtained for SRV-positive ( ) and SRV-negative plasma ( ).

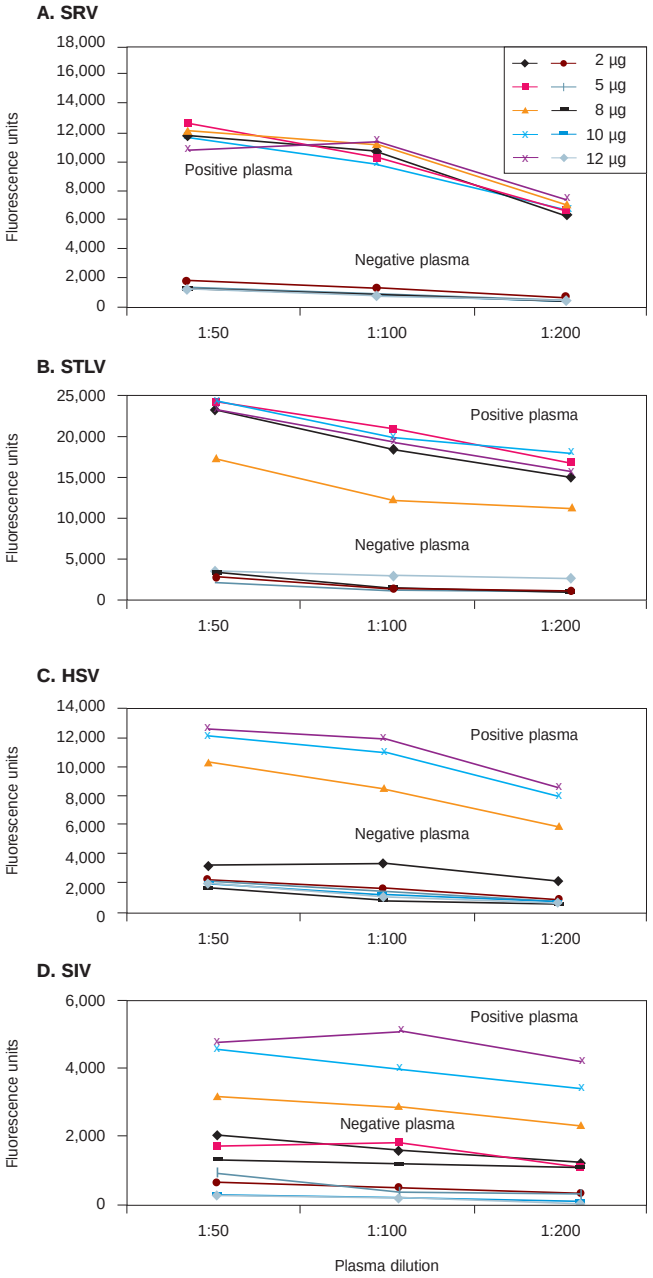

Fig. 3. Titration of plasma with antigens to different viruses.

Table 2. Evaluation of the cross-reactivity of antibodies directed to particular viruses to different beads. Values are in fluorescence units. Shaded values indicate reactive result. Dashes indicate no detectable reaction.

Sample	SRV Beads	STLV Beads	HSV Beads
SRV$^+$	7,107.5	1,248.3	777.1
STLV$^+$	—	17,120.0	—
HSV$^+$	—	1,286.8	16,678.8
SRV$^+$/STLV$^+$	6,580.5	11,264.0	1,009.3
SRV$^+$/HSV$^+$	8,789.3	2,006.3	17,457.0
SRV$^-$	1,253.0	356.8	1,112.0
STLV$^-$	—	286.5	—
HSV$^-$	1,210.8	993.3	1,115.0
SRV$^-$/STLV$^-$/HSV$^-$	748.8	1,407.8	1,147.8

Table 3. Comparison of sensitivity of single-bead and multiplex assays. Results are of several replicates. Values are in fluorescence units.

Sample	Single-Bead Assay	Multiplex Assay
SRV$^+$; average of all replicates	7,384.5	7,916.7
SRV$^+$; control standard	720.8	757.4
STLV$^+$; average of all replicates	19,413.9	19,141.9
STLV$^+$; control standard	3,669.7	3,674.8
HSV$^+$; average of all replicates	12,983.6	17,893.8
HSV$^+$; control standard	1,867.5	2,073.7

Table 4. Comparison of assay sensitivity and specificity.

	SRV		STLV		HSV	
	Bio-Plex	ELISA	Bio-Plex	ELISA	Bio-Plex	ELISA
Samples tested	159	154	159	159	85	89
# positives	11	17	22	21	20	15
# true positives*	8	7	20	20	17	11
# false positives	3	10	2	1	3	4
# true negatives**	148	137	137	138	65	74
# false negatives	0	0	0	0	0	0
Specificity***	100%	100%	100%	100%	100%	100%
Sensitivity†	98%	93%	99%	99%	96%	95%
PPV††	73%	41%	91%	95%	85%	73%
NPV†††	100%	100%	100%	100%	100%	100%

* Positive confirmed by western blot.

** Negative confirmed by ELISA, western blot, or both.

*** Percentage of virus-free animals that test negative, calculated by # true positives/(# true positives + # false negatives).

† Percentage of virus-positive animals that test positive, calculated by # true negatives/(# true negatives + # false positives).

†† Positive predictive value (PPV), or percentage of positive results that are true positives, calculated by # true positives/(# true positives + # false positives).

††† Negative predictive value (NPV), or percentage of negative results that are true negatives, calculated by # true negatives/(# true negatives + # false negatives).

Results and Discussion

Viral Disruption

We were unable to find any reports of whole virus being conjugated to beads for use in this type of multiplex assay. While our approach using SDS-disrupted whole virus did work, it resulted in multiple fragmented beads. Comparing all three approaches, undisrupted, SDS-disrupted, and Triton X-100-disrupted virus, the Triton X-100 treatment gave lower background (as determined by the negative plasma) and a higher signal to the positive plasma than either the SDS-disrupted or undisrupted virus, and with no bead fragmentation (Figure 2).

Virus/Plasma Titration

The results of the virus/plasma titration studies are shown in Figure 3.

Correlation With ELISA

The results we obtained showed a strong correlation between the Bio-Plex and ELISA assays of positive and negative control plasma (Table 1).

Cross-Reactivity

When control plasma was incubated with the bead mixture and then analyzed for each separate bead type, there was no cross-reactivity between the different bead types (Table 2).

Single-Bead vs. Multiplex Assays

Because we were concerned that sensitivity might be lost when multiplex assays were compared to a single-bead assay, both single- and multiple-bead assays were performed; however, as shown in Table 3, we found no loss of sensitivity.

Sensitivity and Specificity

We determined the sensitivity and specificity of the assays by evaluating the number of true and false readings (Table 4).

Conclusions

- There was an excellent correlation between the multiplex suspension assay and the ELISA (r^2 = 0.96–0.99)
- The CV was lower in the multiplex assay than in the ELISA
- There was no loss of sensitivity when multiplexing
- Fewer false positives were detected with the multiplex assay than with the ELISA
- The Bio-Plex assay had equivalent specificity to that of the ELISA, and slightly higher sensitivity

Information in this tech note was current as of the date of writing (2004) and not necessarily the date this version (Rev B, 2005) was published.

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