B-068 MULTI-ARRAY® Assay to Discriminate Recent from Long-Standing HIV Infection

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1 Abstract

In order to accurately assess and compare different prevention strategies, the rate at which new HIV infections are acquired in a population needs to be measured accurately. A simple laboratory test that indicates whether an HIV infection was acquired in the recent past (generally 4-12 months) would be very useful to estimate HIV incidence.

We demonstrated feasibility of several assay formats to separate recent from longstanding HIV infection. Using MULTI-ARRAY technology, we measured antibodies against the HIV proteins gp41, gp120, gp160, p17, p24, p55, p66, tat, viv, and nef in a multiplexed format using a very small sample volume (25 µL of a 1,000-fold diluted serum or plasma sample). We used the well-characterized "HIV Incidence/Prevalence Performance Panel" from SeraCare (part # PRB601), which contains plasma samples from 15 HIV positive donors that have been characterized either as "incident" (recent infection) or "prevalent" (longstanding infection) based on consensus results from nine tests. Our MULTI-ARRAY serology format for antibodies against gp120 and gp160 showed ~10-fold separation between the median signals for incident and prevalent samples (and another ~10-fold separation from apparently healthy controls). All samples in each of the three groups were completely separated from the other two groups. We also developed avidity assay formats for antibodies against gp41, gp120, gp160, and p66 that could accurately separate samples from patients with incident versus prevalent HIV infection. The assays were developed in a 96-well high-throughput assay format for the MESO® SECTOR S 600 Imager and the MESO QuickPlex® SQ 120.

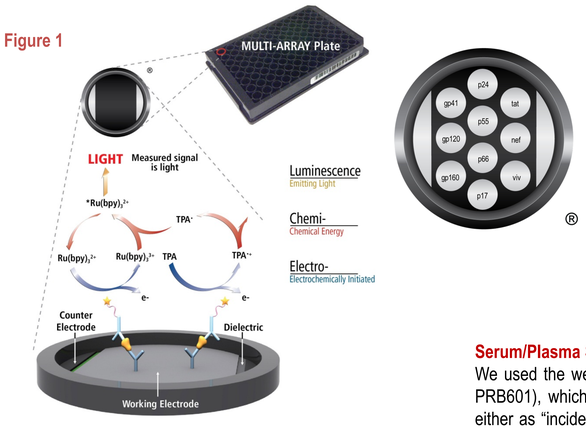
We demonstrated feasibility for transfer of the assay format to a point-of-care (POC) platform. The POC assay is fully automated and simultaneously measures concentrations of antibodies against eight HIV proteins. Time to result is 25 minutes, and CVs are approximately 13%. The magnitude of the antibody response against gp120 and against gp160 accurately separates patients with incident HIV infection from patients with prevalent HIV infection, equivalent to the plate-based results.

In conclusion, we demonstrated feasibility for development of high-throughput and point-of-care assays to discriminate recent from longstanding HIV infection.

2 Methods

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY and MULTI-SPOT® microplates.

Figure 1



Electrochemiluminescence Technology

- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

Serum/Plasma Samples

We used the well-characterized "HIV Incidence/Prevalence Performance Panel" from SeraCare (part # PRB601), which contains plasma samples from 15 HIV positive donors that have been characterized either as "incident" (recent infection) or "prevalent" (longstanding infection) based on consensus results from nine tests. In addition, serum and plasma samples from apparently healthy donors and from HIV positive donors were used.

Plate-Based Standard Serology Assay Format:

For the standard serology format (Figure 2a to the left), recombinant HIV proteins from commercial vendors (including ImmunoDiagnostics, United States Biologicals, Fitzgerald, and Meridian) or from the NIH AIDS Reagent Program were immobilized on 96-well 10-spot MULTI-ARRAY plates. Serum or plasma samples were diluted 1,000x in MSD® Diluent 100. 50 µL of diluted sample and SULFO-TAG labeled anti-human IgG antibody was added to each well and incubated for 30 minutes with shaking. The plate was washed, MSD Read buffer T was added, and the plate was read on a SECTOR® Imager.

Bridging Format

Diluted serum was incubated with biotinylated recombinant HIV antigen and SULFO-TAG labeled antigen off-line for 30 minutes, added into a 96-well MULTI-ARRAY plate coated with streptavidin, and incubated for another 30 minutes with shaking (see Figure 2c to the left). The plate was washed, MSD Read Buffer T was added, and the plate was read on a SECTOR Imager.

Alternative Assay Formats

Antigens were also immobilized through biotin/streptavidin (see Figure 2b on the left) or using MSD's U-PLEX® linkers (not shown). A format comparable to the BED-CEIA assay was developed (see Figure 2d on the left): 500-fold diluted sample was incubated for one hour on a plate with immobilized anti-human IgG antibody, and after a wash, SULFO-TAG labeled recombinant HIV antigen was added.

Avidity Assay

In the standard serology assay format, for half the plate, 0.5 M Guanidine HCl (GuHCl) was included in the MSD read buffer. For each sample, the signal in the presence of GuHCl as a ratio of the signal in the absence of GuHCl was defined as the avidity index.

Figure 2a

a.) Standard Serology Format with directly immobilized antigen

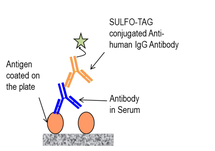


Figure 2b

b.) Serology format with SA-Biotin immobilized antigen

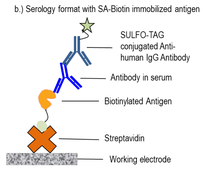
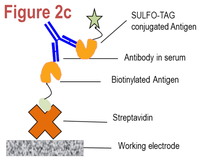
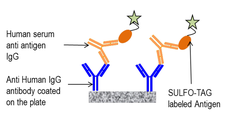


Figure 2c



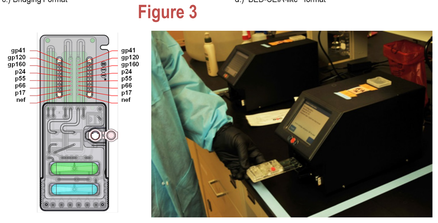
c.) Bridging Format

Figure 2d



d.) "BED-CEIA-like" format

Figure 3



POC Platform

Figure 3 shows the MSD POC single-use cartridge and the cartridge reader. One sample is processed per cartridge; however, the cartridge has two independent detection channels that can be run with the same sample but using different reagent formulations, providing some flexibility for supporting assays with different optimal processing conditions. Two patterned arrays of antigens (or antibodies) are contained in the cartridge, immobilized on screen-printed carbon ink electrodes in the two independent detection channels.

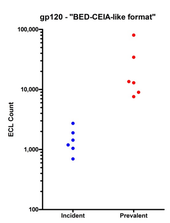
3 MULTI-ARRAY Bridging Assay Format and "BED-CEIA-Like" Format

Feasibility of the bridging format in a single-plex mode was demonstrated using biotinylated antigen and streptavidin coated plates. The data on the right for 125-fold diluted plasma samples from the SeraCare Incident/Prevalent panel show a clear difference between disease states for all four tested HIV antigens.

		Bridging Format; ECL Counts			
		gp41	gp120	gp160	p24
Incident	PRB601-01	16,880	7,089	6,512	7,942
	PRB601-02	13,774	3,270	4,255	9,139
	PRB601-05	5,180	9,962	1,849	7,146
	PRB601-07	41,886	4,606	9,084	2,004
Prevalent	PRB601-03	48,630	76,019	28,284	216,253
	PRB601-04	41,296	144,517	21,137	108,286
	PRB601-06	49,855	93,393	25,083	1,742
	PRB601-08	54,104	125,977	10,533	192,696
Normal Samples	Donor-1	886	537	404	451
	Donor-2	947	543	358	465
	Donor-3	792	480	393	492
	Donor-4	483	551	379	442

Plates were coated with anti-human IgG, and after sample addition SULFO-TAG labeled HIV antigen (e.g. gp120) was added.

This format allows discriminating incident from prevalent HIV infection.

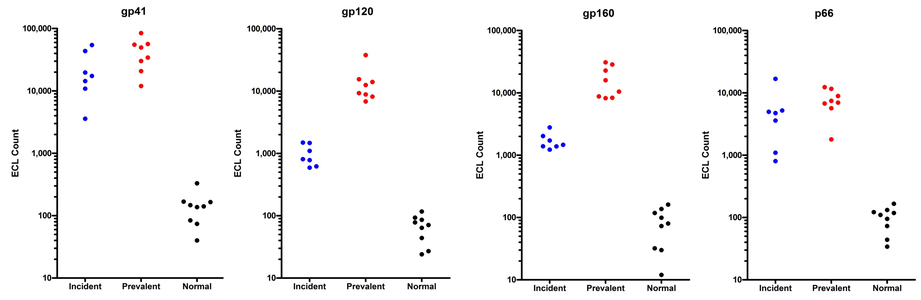


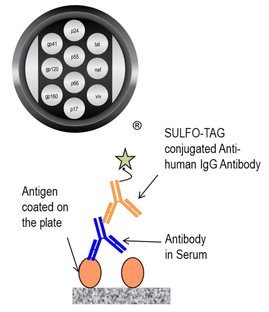
4 Plate-Based MULTI-ARRAY Direct Serology Assay

		MULTI-ARRAY Serology Assay: ECL Counts for 1,000-fold diluted Plasma Sample									
Sample ID	Incident / Prevalent	gp41	gp120	gp 160	p24	p55	p66	p17	tat	nef	viv
PRB601-01	Incident	17,336	1,470	2,782	10,387	144	4,966	246	212	1,109	465
PRB601-02	Incident	19,701	590	1,225	11,153	126	5,207	209	181	900	554
PRB601-05	Incident	14,336	1,489	1,468	8,401	129	1,092	120	122	681	257
PRB601-07	Incident	43,540	620	1,717	2,921	178	801	160	210	841	349
PRB601-09	Incident	54,248	779	2,024	10,619	132	3,594	175	138	1,285	286
PRB601-12	Incident	3,582	1,096	1,387	3,556	144	4,725	180	198	1,133	748
PRB601-14	Incident	10,847	807	1,381	2,661	135	16,753	127	125	458	491
PRB601-03	Prevalent	30,146	9,263	8,204	16,020	268	1,792	177	187	1,450	533
PRB601-04	Prevalent	20,723	13,985	8,335	4,346	159	6,752	189	137	149	1,228
PRB601-06	Prevalent	49,533	8,152	10,460	3,939	166	7,421	123	192	424	312
PRB601-08	Prevalent	54,983	37,798	28,488	72,773	1,282	8,901	3,751	417	524	501
PRB601-10	Prevalent	56,419	12,504	22,759	165	156	6,962	2,160	166	523	416
PRB601-11	Prevalent	34,240	6,815	15,908	348	118	12,329	136	220	4,805	428
PRB601-13	Prevalent	84,232	15,484	30,780	5,291	117	5,659	172	157	490	257
PRB601-15	Prevalent	11,972	8,838	8,752	26,909	154	11,597	165	105	777	279

96-well 10-spot MULTI-ARRAY plates were coated with the ten HIV antigens listed in the table. 50 µL of 1,000-fold diluted serum or plasma and SULFO-TAG labeled anti-human IgG antibody was added to each well and incubated for 30 minutes. The table shows average ECL counts (n=2) for the simultaneously measured antibody response to the ten antigens for the SeraCare HIV Incidence/ Prevalence Performance Panel.

There was a clear difference between ECL counts for patients with incident versus prevalent HIV infection, in particular for the immune response to gp120 and gp160, as shown in the graphs below the table. Also shown in the graphs are ECL counts for samples of apparently healthy individuals, which are clearly lower than for both groups of HIV infected patients.



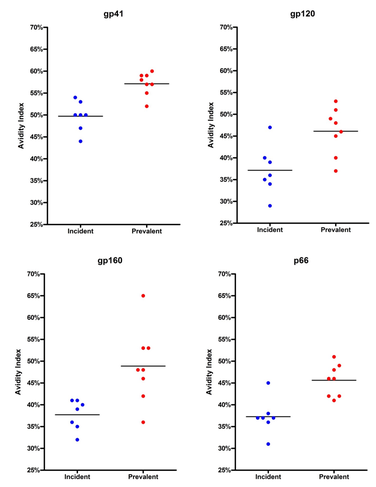


5 Plate-Based MULTI-ARRAY Avidity Assay

		MULTI-ARRAY Serology Assay: Avidity Index									
Sample ID	Incident / Prevalent	gp41	gp120	gp 160	p24	p55	p66	p17	tat	nef	viv
Incident	PRB601-01	50%	39%	41%	43%	45%	36%	51%	39%	40%	49%
Incident	PRB601-02	54%	47%	41%	56%	24%	38%	15%	26%	43%	52%
Incident	PRB601-05	50%	29%	32%	38%	61%	31%	64%	70%	42%	61%
Incident	PRB601-07	47%	34%	36%	31%	42%	37%	36%	22%	42%	34%
Incident	PRB601-09	53%	36%	35%	31%	32%	37%	28%	49%	45%	36%
Incident	PRB601-12	50%	40%	40%	50%	33%	37%	28%	21%	44%	33%
Incident	PRB601-14	44%	35%	39%	27%	45%	45%	2%	34%	40%	47%
Prevalent	PRB601-03	60%	49%	48%	56%	65%	46%	39%	64%	58%	43%
Prevalent	PRB601-04	55%	51%	53%	53%	45%	51%	39%	52%	43%	48%
Prevalent	PRB601-06	59%	40%	42%	30%	31%	42%	53%	36%	35%	50%
Prevalent	PRB601-08	59%	53%	65%	53%	35%	49%	37%	36%	15%	34%
Prevalent	PRB601-10	52%	48%	48%	10%	37%	46%	34%	17%	32%	16%
Prevalent	PRB601-11	57%	45%	46%	41%	33%	48%	31%	15%	50%	20%
Prevalent	PRB601-13	58%	46%	53%	39%	30%	41%	51%	8%	37%	42%
Prevalent	PRB601-15	57%	37%	36%	60%	88%	42%	83%	99%	51%	62%

A MULTI-ARRAY serology assay was performed as in Section 4, but for each sample, an additional condition was tested: 0.5 M of GuHCl was added to the read buffer, followed by a 5-minute incubation. The ratio of the signals in the presence and absence of GuHCl was defined as the avidity index.

The table above shows the avidity index for all ten HIV proteins for the SeraCare HIV Incidence/Prevalence Performance Panel. As the graphs on the right demonstrate, there was a significant difference in the avidity index for incident versus prevalent samples for gp41, gp120, gp160, and p66.

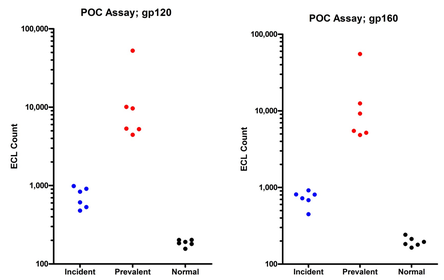


6 Feasibility of Transfer to POC Platform

The MSD POC cartridge includes two independent detection channels, each with eight spots. We immobilized recombinant gp41, gp120, gp160, p24, p55, p66, p17, and nef in both detection areas. SULFO-TAG labeled anti-human IgG detection antibody and buffer were dried in a dedicated area of the cartridge which can be hydrated and mixed by moving the sample back and forth. After a predetermined incubation time, the fluid is then moved to the capture spots for a second incubation followed by a wash and read.

The data on the right are from a 2-step assay format, where diluted sample was allowed to bind to the spots, followed by a wash and incubation with detection antibody. Time to result is 25 minutes, and CVs are approximately 13%.

In this sample set, the POC test could accurately differentiate all incident versus long-standing HIV infections, and could also resolve both groups from non-infected individuals.



7 Conclusion

We demonstrated feasibility for development of high-throughput and point-of-care assays to discriminate recent from longstanding HIV infection.

Based on our results with the SeraCare HIV Incidence/Prevalence Performance Panel, the most promising markers to discriminate incident from prevalent HIV infection are the magnitudes of the antibody responses to gp120 and to gp160. Also promising are avidity to gp41, gp120, gp160, and p66.

As a next step, these results need to be confirmed with a larger set of clinical samples.

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Acknowledgement:

The following reagents were obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: HIV-1 nef Protein (Cat# 11478), HIV-1 viv Protein (Cat# 11050).

This project has been funded in whole or in part with Federal funds from the National Institute of Health, Department of Human Health and Services, under Contract No. HHSN261201000018C.

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