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Development of a High Throughput Multiplex Assay for Researching HIV Incidence

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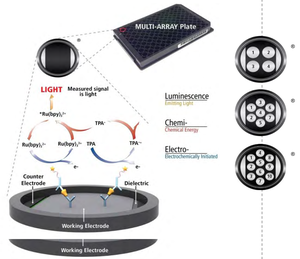
1 Abstract

Background
In order to assess the impact of HIV prevention strategies, it is critical to measure the rate at which new HIV infections are occurring in populations. To achieve needed performance requirements (e.g., long "mean duration of recent infection" [MDRI], low "false recent rate" [FRR]), most programs currently use multi-assay algorithms including avidity-modified antibody and viral load measurements. MULTI-ARRAY is a high throughput technology with a wide dynamic range and low non-specific binding that has been used successfully in a variety of applications. We determined feasibility of an improved multiplex HIV incidence assay to discriminate recent from longstanding HIV infection using the MULTI-ARRAY® platform.
Methods
Using MULTI-ARRAY technology, we measured antibody quantity and avidity to ten HIV proteins. A total of 28 assays were run: standard serology assays with and without disrupting agent, and an assay format with anti-human IgG capture and detection with SULFO-TAG™ labeled HIV antigens. 96 samples were tested: 75 samples from the CEPHIA Developmental Set (http://www.incidence-estimation.com/cephiaqueries/cephiaDB/overview), 15 samples from the SeraCare Incidence/Prevalence Performance Panel, and additional samples from apparently healthy individuals.
Results
Several assays could discriminate recent from longstanding HIV infection even as stand-alone markers if samples from elite controllers were excluded: the standard serology format with and without pretreatment for gp120 and gp160, and the antibody capture format for gp120 showed 100% sensitivity and 100% specificity in this sample set. In a ROC analysis, 13 assays had ROC areas of 0.90 or better (elite controllers excluded). None of the tested antibody assays was able to discriminate elite controllers; thus including a (antigen or nucleic acid) viral load assay would be required. The time dependence of the gp120 and gp160 ECL signal as function of time from seroconversion indicates that the signal continues to increase even after two years, indicating that determining an MDRI of well over a year at a low FRR is feasible.
Conclusion
Assays to discriminate recent from longstanding HIV infection samples have been developed in a 96-well high-throughput assay format for the MESO® SECTOR S 600 Imager and the MESO QuickPlex® SQ 120.

Acknowledgement of Source:
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 Center of Disease Control and Prevention, under Contract No. 200-2013-M-57410.

2 Methods

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



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.

3 Samples

96 Plasma samples were tested in a randomized and blinded format.
a.) CEPHIA Developmental Set:
- 16 Incident
- 35 Prevalent including 5 samples from elite controllers[1]
- 24 HIV negative

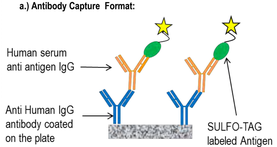
b.) SeraCare Incident/Prevalent Performance Panel:
- 7 Incident[2]
- 8 Prevalent[3]

c.) 9 additional samples from HIV negative individuals

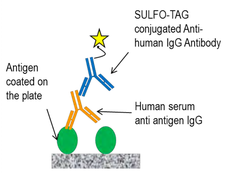
[1]Elite controllers, ~0.5-1% of HIV infected patients, are individuals who are infected for more than a year and maintain low viral load (<75 copies/mL) in the absence of ART treatment.
[2]Incident, defined as <1 year from estimated date of infection.
[3]Prevalent, defined as ≥1 year from infection.

4 Assay Formats

a.) Antibody Capture Format:



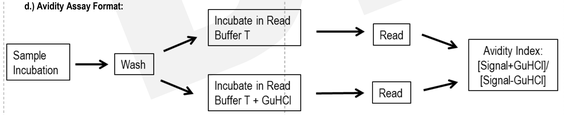
b.) Standard Serology Assay Format:



c.) 10-spot MULTI-ARRAY well



d.) Avidity Assay Format:



Antibody Capture Format:
For the antibody capture format ["BED-CEIA-like" format; Figure a.) on the upper 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. Assays for eight antigens, namely gp41, gp120, gp160, p24, p55, p17, tat & viv were developed.

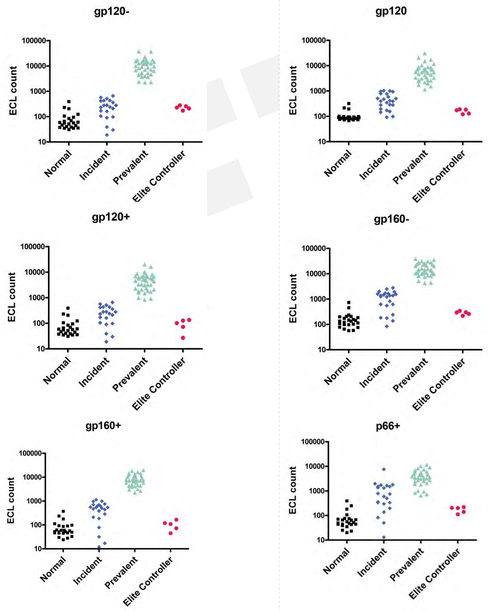
Standard Serology Assay Format:
For the standard serology format [Figure b.) on the left], recombinant HIV proteins from commercial vendors (including ImmunoDiagnostics, United States Biological, Fitzgerald, and Meridian) or from the NIH AIDS Reagent Program were immobilized on 96-well 10-spot MULTI-ARRAY 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.
Ten assays were multiplexed in the standard serology format [see Figure c.)].

Avidity Assay Format:
The standard serology format was modified as shown in Figure d.) below. After the wash, wells were incubated for 5 minutes either in MSD Read Buffer T or in MSD Read Buffer T with 0.5M guanidinium hydrochloride added (GuHCl). For each sample, an avidity index (AI) was defined as the ECL signal in the presence of GuHCl divided by the signal in the absence of GuHCl. The avidity assay format was performed in multiplex with ten HIV proteins.

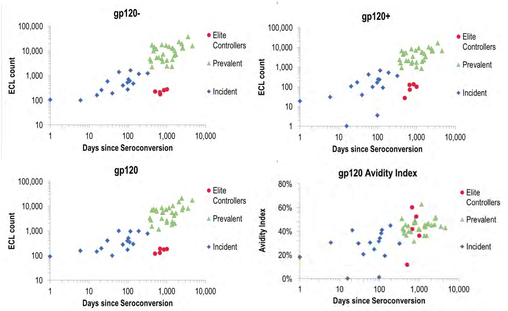
Nomenclature:
In this poster, the standard serology format uses the following nomenclature: gp120 with pretreatment will be referred to as "gp120+", without pretreatment as "gp120-", the avidity index as "gp120AI", and the antibody capture format as "gp120".

5 ECL Counts per sample group for best assays

Assay formats are described in Section 4. The standard serology format nomenclature is used, for example, gp120 with pretreatment is referred to as "gp120+", without pretreatment as "gp120-", the avidity index as "gp120AI", and the antibody capture format as "gp120".
The graphs show that several assays can perfectly separate incident (= recent HIV infection) from prevalent HIV infection if elite controllers[1] are excluded.



6 Signal dependence on time since seroconversion



The graphs show raw ECL counts for several assays as function of time since seroconversion. Assay formats are described in Section 4. The standard serology format nomenclature is used, for example, gp120 with pretreatment is referred to as "gp120+", without pretreatment as "gp120-", the avidity index as "gp120AI", and the antibody capture format as "gp120". Only samples from the CEPHIA Developmental Set were used, since time from seroconversion for these well-characterized samples could be estimated.
The signal for the gp120-, gp120+, and gp120 assay continues to increase even after two years, indicating that an MDRI of well over a year at a low FRR is feasible.

Best Markers – Elite Controllers Excluded

Assay	ROC area	Sensitivity	Specificity	Percent Correct	Incident median	Prevalent median	Cut-off
gp120-	1.00	100%	100%	100%	607	9,868	1,927
gp120+	1.00	100%	100%	100%	233	4,602	748
gp160-	1.00	100%	100%	100%	1,381	13,665	3,556
gp160+	1.00	100%	100%	100%	502	7,355	1,706
gp120	1.00	100%	100%	100%	401	5,554	1,096
gp160	0.97	95%	91%	93%	902	7,956	2,989
p66AI	0.94	82%	91%	85%	34%	46%	42%
gp41+	0.94	95%	83%	90%	7,527	27,997	11,125
gp160AI	0.93	82%	91%	85%	36%	52%	47%
gp41-	0.93	87%	87%	87%	14,607	50,108	27,293
gp41	0.92	84%	91%	87%	3,663	13,082	9,070
p66+	0.91	84%	91%	87%	597	4,112	1,848
gp120AI	0.90	84%	78%	82%	32%	45%	39%
p66-	0.89	79%	91%	84%	1,905	7,781	5,152
gp41AI	0.87	74%	83%	77%	45%	54%	51%

The table shows results from an ROC analysis (elite controllers excluded) with the simplifying (but not necessarily optimal) assumption that false positive and false negative have equal weight. Five assays could perfectly separate incident from prevalent samples.

7 Conclusion

Several high throughput MULTI-ARRAY assays for the MESO SECTOR S 600 Imager and the MESO QuickPlex SQ 120 could discriminate recent from longstanding HIV infection samples even as stand-alone markers if samples from elite controllers were excluded. None of the tested antibody assays was able to discriminate elite controllers; thus including a viral load assay would be required. The time dependence of the gp120 and gp160 ECL signal as function of time from seroconversion indicates that the signal continues to increase even after two years, indicating that determining an MDRI of well over a year at a low FRR is feasible.

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