WEPE105

Characterization of a rapid HIV-1 incidence assay based on a CDC-developed multisubtype antigen in a commercial HIV lateral flow assay format

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Introduction

Estimates of HIV incidence are critical to monitoring the spread of the HIV epidemic, identifying "hot spots" of new infections, and managing and assessing intervention efforts. HIV incidence is optimally determined by large longitudinal cohort studies. However, such studies are time-consuming and expensive, and have their own built in biases. Over the past few years, efforts have been focused on laboratory approaches detecting new HIV infections to estimate HIV incidence. These include commercial assays specifically designed to identify new infections, such as the Sedia™ HIV-1 Limiting Antigen EIA and the Sedia™ BED HIV-1 Incidence EIA. Such assays are more cost effective than longitudinal cohort studies for incidence estimates, but are not easily implemented in resource-limited settings outside of laboratories. This study presents a simple, point-of-care Rapid HIV-1 Incidence Assay (RHIA), similar in principle to rapid blood diagnostic assays, suitable for field use that can be performed with minimal training and can significantly expand access to HIV incidence measurements

Materials and Methods

A simple, lateral flow Rapid HIV-1 Incidence Assay (RHIA) similar to one developed by the CDC[1] was designed based on Sedia's commercial Asanté™ rapid diagnostic format. The Sedia test gives results within 20 to 45 minutes after placing the assay strip into sample comprised of blood, serum or plasma diluted 1:200 in a diluent. Results were read blind by the reader at both 20 and 45 minutes. Our assay incorporates a recombinant multi-subtype antigen developed by the CDC for the HIV-1 LAg-Avidity as an incidence line. The assay has three reaction lines as shown in the photo above: 1) a control line, 2) a test or prevalence line (reactive with all HIV-1 positive specimens) and 3) an incidence line (reactive only with long-term infections). The assay was evaluated against 419 well characterized HIV-1 positive sera obtained from multiple sources including the U.S. CDC (both DGHA and NHAP), CEPHIA, commercial HIV panels, rejected plasma donors, and other commercial sources. Additional specimens were tested beyond those reported in the originally submitted abstract. Samples were characterized by the HIV-1 LAg-Avidity the BED HIV-1 Incidence EIAs and western blot according to the manufacturers' instructions.

Results

The results below are based on the performance of the RHIA relative to the HIV-1 LAg-Avidity EIA and the BED HIV-1 Incidence EIA. Occasionally, there were slight differences in the RHIA between 20 and 45 minute reads, typically due to assay strip clearing and subjective but subtle changes in the incidence line. This is a limitation inherent in rapid test technology well known to manufacturers and frequent users of such rapid assays, where experienced readers may see changes or detect lines that novice readers miss. The RHIA was titered to match the mean duration of recent infection (MDRI) of the LAg-Avidity EIA (130 days). However lower False Recent Rate (FRR) results were observed relative to the BED EIA primarily due to BED EIA's longer MDRI (197 days). The MDRI of the RHIA has not yet been determined using a typical population. Differences in MDRI can affect the reported FRR in direct correlations to other lab assays as used here.

Rapid Incidence Test Status (20' Read)	LAg-Avidity EIA Status: Recent	LAg-Avidity EIA Status: Long Term
Recent	119[a]	17
Long-Term	15[a]	268
Total Lag-Avidity	134[a]	285

False Recent Rate = 17/285 = 5.9%
False Long Term Rate = 15/134 = 11.1%[a]

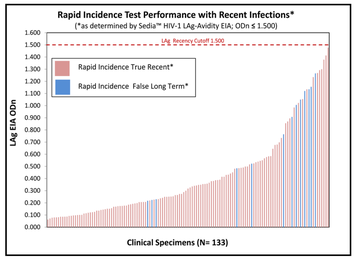
[a] After 45 minutes, 2 LAg-Avidity Recent specimens shifted from "Long-term" to "Recent in the Rapid Incidence Test reducing the False Long Term Rate to 9.7%

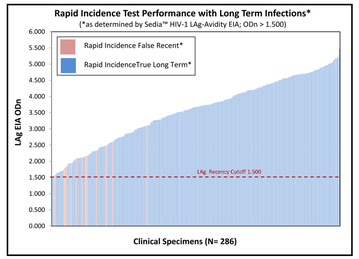
Rapid Incidence Test Status (20' Read)	BED EIA Status: Recent	BED EIA Status: Long Term
Recent	133[b]	3
Long-Term	35[b]	245
Total BED	168[b]	248

False Recent Rate = 3/248 = 1.2%
False Long Term Rate = 35/168 = 21.1%[b]

[b] After 45 minutes, 2 BED EIA Recent specimens shifted from "Long-term" to "Recent" in the Rapid Incidence Test reducing the False Long Term Rate to 19.6%
3 Specimens were not tested on the BED EIA because of insufficient volume.

Sorting the Rapid Incidence Assay results relative to the Sedia™ HIV-1 LAg-Avidity EIA, which has been shown in independent studies to have a lower FRR than other laboratory HIV incidence assays[2,3] reveals that the specimens that were discordant with the HIV-1 LAg-Avidity EIA were generally close to the latter's cutoff (see graphs below). In fact, during repeated testing, many of the specimens close to the cutoff were observed to "give results that would flip" from "recent" to "long-term" and vice-versa in the RHIA.





Examination of specimens giving discordant results between the RHIA and HIV-1 LAg-Avidity EIA (table in next column) reveals that several of the specimens that were scored false recent or false long-term with the RHIA have western blot patterns more similar to true recent or true long-term specimens, respectively, suggesting the comparator assays may be in error.

Conclusions

This study is not intended as a definitive analysis of the performance of the Rapid HIV-1 Incidence Assay. It does, however, demonstrate feasibility of using a commercial rapid assay format for differentiating recent from long term HIV-1 infections. The performance of the RHIA is likely not yet optimal and may be influenced by several factors:

- **Absence of Gold Standard Laboratory Assay.** Neither assay used as the comparator in this study is considered the gold standard by which incidence assays are measured. Although the more recently developed LAg-Avidity EIA improves the accuracy of HIV incidence estimates over the BED EIA, specimens identified as "recent" or "long-term" by either assay may be mischaracterized. There is no "gold standard" laboratory assay for measuring HIV incidence. The best gold standard is a collection of specimens from longitudinal cohort studies of known duration of infection, however such specimens are not widely available.
- **Effect of Window Period on False Recent Rate**. Although the RHIA was designed so that its "window period" (MDRI) was similar to the HIV-1 LAg-Avidity EIA, there is likely to be some difference, which can affect the FRR. This effect is demonstrated by the greater disparity between the RHIA and the BED EIA, since the latter likely has a longer MDRI than the rapid test. The next step in characterizing the rapid HIV Incidence Assay is to assess its MDRI and determine its FRR in a representative population with HIV-infected individuals of known duration of infection.

Discordant Analysis of Rapid HIV-1 Incidence Test vs. HIV-1 LAg-Avidity EIA and BED HIV-1 Incidence EIA

Specimen No.	Sedia BED HIV-1 Incidence EIA: Screen ODn	Sedia BED HIV-1 Incidence EIA: Median Confirmatory ODn	Sedia BED HIV-1 Incidence EIA: Sedia BED Status	Sedia HIV-1 LAg-Avidity EIA: Screen ODn	Sedia HIV-1 LAg-Avidity EIA: Median Confirmatory ODn	Sedia HIV-1 LAg-Avidity EIA: Sedia LAg Status	Sedia Rapid Incidence: Read at 20 min.	Sedia Rapid Incidence: Read at 45 min.	Discordant Class	Western Blot
#042	0.083		R	0.086	0.112	R	LT	LT	False Long Term	
#192	0.076		R	0.245	0.218	R	LT	R	False Long Term	
#138	0.067	0.076	R	0.128	0.224	R	LT	LT	False Long Term	
#132	0.150	0.168	R	0.223	0.230	R	LT	R	False Long Term	
#193	0.168		R	0.464	0.484	R	LT	LT	False Long Term	
#247	0.192	0.222	R	0.509	0.522	R	LT	LT	False Long Term	
#205	0.110		R	0.567	0.539	R	LT	LT	False Long Term	
#199	0.101		R	0.755	0.765	R	LT	LT	False Long Term	
#045	0.382		R	0.550	0.907	R	LT	LT	False Long Term	
#225	0.452		R	1.032	1.006	R	LT	LT	False Long Term	
#181	0.304		R	1.200	1.050	R	LT	LT	False Long Term	
#070	insufficient specimen			1.025	1.120	R	LT	LT	False Long Term	insufficient specimen
#261	0.484	0.431	R	1.226	1.135	R	LT	LT	False Long Term	
#073	0.206	0.210	R	1.172	1.155	R	LT	LT	False Long Term	
#201	0.270		R	1.274	1.266	R	LT	LT	False Long Term	
#110	0.546	0.571	R	1.840	1.558	LT	R	R	False Recent	
#117	0.299	0.376	R	1.562	1.567	LT	R	R	False Recent	
#111	0.533	0.586	R	1.944	1.766	LT	R	R	False Recent	
#380	0.686	0.733	R	1.549	1.806	LT	R	R	False Recent	
#379	0.665	0.732	R	2.114	1.839	LT	R	R	False Recent	
#267	0.355	0.379	R	1.992	1.932	LT	R	R	False Recent	
#265	0.349	0.401	R	2.009	1.939	LT	R	R	False Recent	
#377	0.417	0.496	R	2.252	2.091	LT	R	R	False Recent	
#257	0.588	0.628	R	2.057	2.113	LT	R	R	False Recent	
#273	0.338	0.389	R	2.162	2.162	LT	R	R	False Recent	
#402	0.564	0.554	R	1.979	2.174	LT	R	R	False Recent	
#109	0.556	0.570	R	1.820	2.176	LT	R	R	False Recent	
#252	0.405	0.327	R	2.288	2.328	LT	R	R	False Recent	
#317	1.404	1.267	LT	2.286	2.477	LT	R	R	False Recent	
#084	1.168	1.196	LT	2.745	2.488	LT	R	R	False Recent	
#241	0.645		R	2.619	2.594	LT	R	R	False Recent	
#229	0.853		LT	3.092	3.107	LT	R	R	False Recent	

- **Subjective Nature of Rapid Test Format.** The rapid test format used for this assay is designed and read visually, lending itself to subjectivity that likely affects its variability.
- **Panels Tested Represent an Atypical "Population".** The specimens used for this study are not a random sampling of a "typical" at risk population, but were intentionally selected and skewed toward the HIV-1 LAg-Avidity cut-off to enable us to more finely tune the assay performance. A typical population is likely to have more long- and very long-term specimens which would likely further reduce the FRR.
- **No Testing Algorithm Established.** The use of a multi-test algorithm (such as confirmatory testing of recents and "near" long-terms as done with the BED and LAg-Avidity EIAs, or use of multiple assays to screen and confirm for recent infections) was not done in this analysis. Such an algorithm may further improve the FRR of this assay.

We anticipate our next steps are to collaboratively evaluate the MDRI and FRR for this assay in collaboration with the CDC and CEPHIA, utilizing their specimen banks of samples of known recency of infection.

Literature Cited

[1]Granade TC, Nguyen S, Kuehl DS, Parekh BS. (2013). Development of a novel rapid HIV test for simultaneous detection of recent or long-term HIV type 1 infection using a single testing device. AIDS Res Hum Retroviruses 29(1):61-67.

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Acknowledgments

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