Theranos HIV-1 Antibody Assay

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1 Purpose

This document describes the studies conducted during the development of Theranos HIV-1 Antibody assay.

2 Scope

This report includes relevant studies conducted during the development of the Theranos HIV-1 Antibody assay.

3 Definitions

Term	Definition
HIV	Human Immunodeficiency Virus
RF	Rheumatoid factor
HAMA	Human anti-mouse antibodies
NPA	Negative Percent Agreement
PPA	Positive Percent Agreement

Table 1: Definitions

4 Assay Specifications

Theranos HIV-1 Antibody assay is an in vitro diagnostic immunoassay for the qualitative determination of IgG antibodies to HIV in human serum, plasma and whole blood. In this assay, sample is pre-incubated with HIV-1 antigen peptide conjugated to a reporter enzyme. Antigen-antibody complexes thus will form if the patient sample contains HIV-1 antibodies. This reaction mixture is further co-incubated with solid surface coated with HIV-1 antigen. After a number of washing steps to remove unbound anti-HIV antibody, a chemiluminescent substrate is added and signal is measured using Theranos Analyzer. The sample data is used to calculate each sample's index value by comparison with cut off calibrator.

5 Reference Assay

Advia Centaur HIV 1/2/0 Enhanced (EHIV) is used as a predicate method for Theranos HIV-1 Antibody assay.

6 Calibrator Verification

For calibration of Theranos HIV-1 Antibody assay, negative and cut off calibrators are used. Negative calibrator is a pooled normal human serum (confirmed negative for HIV-1/HIV-2, HCV and HBsAg).Contrived Cut off calibrator is prepared by diluting pooled HIV positive sample with the negative calibrator. Antibody index of the contrived calibrator is verified to be 1 by getting it tested on Advia Centaur EHIV assay.

7 Method Comparison

7.1 Theranos HIV-1 assay performance was evaluated by running 16 commercially available sero-conversion panels, 82 clinical samples (normal and pathological donors), as well as 5 performance panels.

7.2 The overall positive percent agreement was 93% and negative percent agreement was 100%. The data is summarized in the following table. A total of 8 discrepant samples (negative on Theranos and positive on Centaur) were found that contributed to 93% PPA. However, when tested on more sensitive Theranos p24 antigen assay they were all reactive and would bring up the PPA for Theranos HIV-1 combined assays to 100%.

	Clinical Samples (Normal + pathological donors)						
		Theranos					
Centaur EHIV		Positive	Negative	Equivocal	Total		
	Positive	42	0	0	42	**100%**	**PPA**
	Negative	0	40	0	40	**100%**	**NPA**
	Equivocal	0	0	0	0		

	Performance panels						
		Theranos					
Centaur EHIV		Positive	Negative	Equivocal	Total		
	Positive	39	4	0	43	**91%**	**PPA**
	Negative	0	27	0	27	**100%**	**NPA**
	Equivocal	0	0	0	0		

	Sero-Conversion panels						
		Theranos					
Centaur EHIV		Positive	Negative	Equivocal	Total		
	Positive	27	4*	0	31	**87%****	**PPA**
	Negative	0	57	0	57	**100%**	**NPA**
	Equivocal	0	0	0			

	All of the above included						
		Theranos					
Centaur EHIV		Positive	Negative	Equivocal	Total		
	Positive	108	8	0	116	**93%**	**PPA**
	Negative	0	124	0	124	**100%**	**NPA**
	Equivocal	0	0	0	0		

* these samples are tested reactive on more sensitive Theranos HIV-1 p24 antigen assay

** PPA would be 100% if we consider reactive results from HIV-1p24 antigen assay

Table 2: Method Comparison Summary

8 Cross Reactivity

8.1 In order to test the Theranos HIV-1 Antibody assay for cross reactivity to medical conditions unrelated to HIV, about 163 samples from 28 unrelated medical conditions were tested.

8.2 All the samples were tested on Centaur EHIV assay to compare their antibody index.

8.3 No cross reactivity was observed on Theranos HIV-1 Antibody assay demonstrating 100% specificity.

Medical Condition / Potential Cross Reactant	No. of samples tested	Discrepant Samples
HIV-2	14	0
HCV	5	0
HBsAg	4	0
Flu vaccinated	5	0
CMV	3	0
EBV	3	0
Rubella	4	0
SLE	4	0
Grave's disease	4	0
HTLV-1	5	0
HTLV-2	5	0
Candida	4	0
RF	14	0
HAMA	13	0
High IgG	22	0
High IgM	2	0
Mixed connective tissue disease	3	0
Monoclonal gammopathy	3	0
Scleroderma	4	0
Syphilis	3	0
Crohn's disease	6	0
Hemodialysis	5	0
Toxoplasma IgG	3	0
Hashimoto's disease	1	0
Chlamydia	2	0
HSV 1/2	8	0
ANA	10	0
VZV	4	0
Total	163	0

Table 3: cross-reactivity to different medical conditions

9 Interference

9.1 To test the effect of interference from lipemic, hemolyzed, icteric, high-protein and high RF and HAMA samples on Theranos assay, commercially available stocks were spiked into pooled normal human serum. The antibody index were compared to Centaur EHIV assay.

9.2 No interference was observed.

Interferents Tested	Centaur Ab index	Theranos Ab Index
RF (2000 IU/mL)	<0.05	0.4
HAMA (622ng/mL)	<0.05	0.1
Hemoglobin (1000mg/dL)	<0.05	0.1
Triglycerides (1500 mg/dL)	<0.05	0.1
Total protein (1g/dL)	<0.05	0.1
Bilirubin (40mg/dL)	<0.05	0.1

Table 4: Interference to endogenous compounds

10 Matrix comparison

10.1 Theranos assay was evaluated for matrix comparison by comparing plasma collected from Theranos BCD (finger stick) to venous serum and venous EDTA.

10.2 20 unique patients each donated 1 venous SST tube, 1 venous K_2EDTA tube, and 2 finger stick samples in Theranos K_2EDTA BCDs. Plasma from all BCDs from the same patient were pooled. Due to the fact that all in-house collected samples were HIV negative, the matched samples from all patients were spiked using different amounts of an HIV-positive serum sample to get samples across a broad assay range. HIV levels were determined by Theranos method from all 3 matrices and compared to each other.

10.3 The following plots show excellent correlation when 2 matrices are plotted against each other, with slopes in the acceptable range (between 0.9 and 1.1)

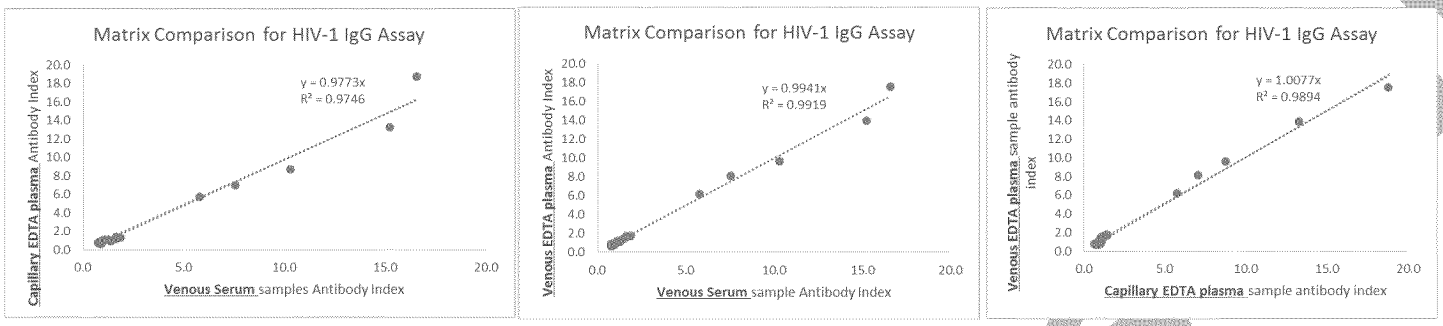

Figure 1: Matrix comparison plots

11 Hook effect

11.1 The goal of this experiment was to test for hook effect by diluting a neat sample and testing it on Theranos assay.

11.2 A severe hook effect was initially observed due to extremely high antibody titers in HIV-1 positive patients.

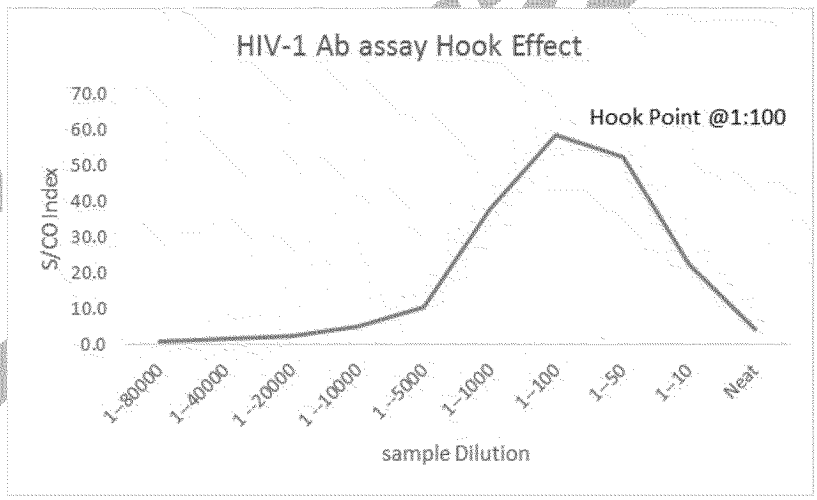

Figure 2: Hook effect observed in HIV-1 Antibody Assay

11.3 Hook effect was later minimized by changing some of the assay parameters. Several HIV positive samples (previously showing low antibody index due to hook effect, yet reactive on Theranos HIV-1 Antibody Assay) were run with new assay parameter changes. They indeed show improved antibody index with new changes and minimizes our risk of missing any HIV-1 antibody reactive sample.

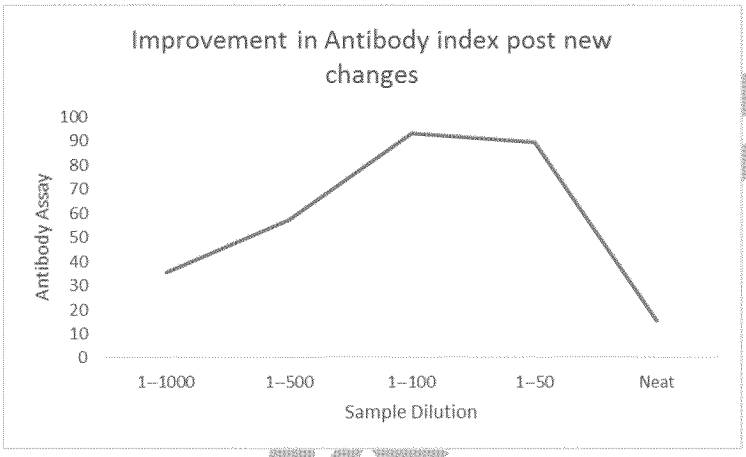

Figure 3: Improvement to minimize Hook effect in HIV-1 Antibody Assay

11.4 A hook effect is still observed with new assay parameter changes. However, the risk of high antibody titers being non-reactive on Theranos assay is minimal with the new changes.

12 Conclusions

Theranos HIV-1 Antibody assay is a robust bridging ELISA assay for detection of antibodies from serum, plasma and / or finger stick specimens of HIV positive patients. The assay shows excellent specificity (100%) and comparable sensitivity (93%) to currently available FDA approved methods for HIV-1 detection.