Theranos HIV -1 p24 antigen Assay

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1 Purpose

This document describes the studies conducted during the development of Theranos HIV-1 p24 antigen assay.

2 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

3 Assay Specifications

3.1 Theranos HIV-1 p24 antigen assay is an in vitro diagnostic immunoassay for the qualitative determination of HIV-1 p24 antigen in human serum, plasma and finger stick whole blood. It can be used a screening test for detecting an early HIV infection. HIV-1 p24 antigen is a structural protein that constitutes the majority of the HIV virus core. This protein is in very high concentration during very early stage of infection where antibodies haven't developed yet. High serum levels of this protein during a short window period of infection and sero-conversion helps p24 antigen detection as a screening test for primary infection.

3.2 In this assay, patient sample is treated with propriety reagents to release bound p24 antigen from antibodies if any. The treated sample is later pre-incubated with a p24 antibody conjugated to a reporter enzyme. Antigen-antibody complexes thus will form if the patient sample contains p24 antigen in detectable amount. This reaction mixture is further co-incubated with solid surface coated with another p24 antibody. After a number of washing steps to remove unbound p24 antigen, 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.

4 Reference Assay

No FDA approved yet sensitive method was available during the development of Theranos HIV-1 p24 antigen assay. Non- FDA approved Alliance p24 antigen ELISA kit was used as a predicate method due to its higher sensitivity than FDA approved Allere Determine HIV 1/2 Ag/Ab Combo.

5 Calibrator Verification

For calibration of Theranos HIV-1 Antibody assay, negative and cut off calibrators are used. WHO p24 reference analyte (90/636) is used for preparing the assay calibrators. Negative calibrator is a pooled normal human serum (confirmed negative for HIV-1/HIV-2, HCV and HBsAg). Cut off calibrator is prepared by diluting WHO p24 reference analyte serially with the negative calibrator to get to a nominal value of 1 U/mL.

6 Method Comparison

6.1 Theranos HIV-1 p24 antigen assay performance was evaluated by running 57 commercially available sero-conversion panels, 77 clinical samples (normal and pathological donors), as well as 7 performance panels.

6.2 The overall positive percent agreement (PPA) was 96% and negative percent agreement (NPA) was 94%. Method comparison summary is shown in the following table (Table 2). Theranos assay was found to be more sensitive than the PE Alliance reference method. As a result, discrepant samples (reactive on Theranos assay and non-reactive on PE Alliance) were followed up with a confirmatory assay testing and were found to be reactive, bringing up Theranos p24 assay NPA to 100%.

	Clinical Samples (Normal + pathological donors)						
		Theranos					
PE Alliance		Positive	Negative	Equivocal	Total		
	Positive	15	3	0	18	**83%**	**PPA**
	Negative	0	58	0	58	**100%**	**NPA**
	Equivocal	0	0	0			

	Performance panels						
		Theranos					
PE Alliance		Positive	Negative	Equivocal	Total		
	Positive	38	1	0	39	**97%**	**PPA**
	Negative	17	43	0	60	**72%**	**NPA***
	Equivocal	0	0	0			

	Sero-Conversion panels						
		Theranos					
PE Alliance		Positive	Negative	Equivocal	Total		
	Positive	130	3	0	133	**98%**	**PPA**
	Negative	11	351	0	362	**97%**	**NPA***
	Equivocal	0	0	0			

	All of the above included						
		Theranos					
PE Alliance		Positive	Negative	Equivocal	Total		
	Positive	183	7	0	190	**96%**	**PPA**
	Negative	28	452	0	480	**94%**	**NPA***
	Equivocal	0	0	0	0		

* NPA is 100% when all discrepant samples were confirmed reactive with a confirmatory assay discrepant samples were non-reactive on PE Alliance and reactive on Theranos p24 antigen assay

Table 2: Method Comparison Summary

7 Cross Reactivity

7.1 In order to evaluate Theranos HIV-1 p24 antigen assay for cross reactivity to medical conditions unrelated to HIV-1, initially about 124 samples from 21 unrelated medical conditions were tested.

7.2 All the samples were tested on PE Alliance assay to compare their p24 antigen index.

Medical Condition / Potential Cross Reactant	No. of samples tested	Discrepant Samples
HIV-2	15	0
HCV	5	0
HbsAg	5	0
Syphilis	5	0
Candida	5	0
Hemodialysis	5	0
Flu vaccinated	5	0
CMV/EBV/Rub	5	0
SLE	5	0
RF	5	0
HAMA	5	0
HSV-1	5	0
Pregnant -HR	5	0
Multiparous	6	0
MCTD	4	0
Grave's Disease	5	0
Scleroderma	5	0
HTLV1/2	10	0
Sjorgren's Syndrome	3	0
ANA Positive	4	0
Varicella Zoster Virus (VZV)	4	0
Toxoplasma Gondii IgG	4	0
Chlamydia	4	1*
Total	124	1

* This sample was very close the cut off and falls in the equivocal zone.

Table 3: cross-reactivity to different medical conditions

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

8 Interference

8.1 To test the effect of interference from different medical conditions, individual unrelated medical condition samples were spiked with different levels of p24 antigen in order to get final antigen concentration close to the cut off (low positive).

8.2 Although some positive interference was observed, the clinical outcome for any of the samples was not affected due to the interference for low positive.

8.3 In order to assess the positive interference further the same samples that had shown positive interference with spiked low positive p24 antigen levels, were spiked with high negative levels of p24 antigen levels. No change in clinical outcome was observed demonstrating 100% specificity of Theranos p24 antigen assay.

Interference Summary for Theranos HIV-1 p24 Assay			
Sample Category	Samples tested	Reactive samples	Specificity
samples with spiked p24 (Low pos)	104	0	100%
samples with spiked p24 (High neg)	41	0	100%

Table 4: Interference with different medical conditions

8.4 In addition, endogenous interferents and high RF and HAMA samples were also tested on Theranos assay. Commercially available stocks were spiked into pooled p24 high negative and low positive contrived serum. p24 antigen index of these samples was compared with cut off calibrator and a positive control run along with the samples.

Sample type	Interfering substance	Theranos Index
p24 negative	Control	0.7
	triglyceride 1500 mg/dL	0.8
	Hemoglobin 1000 mg/dL	0.6
	Bilirubin 40 mg/dL	0.7
	Total protein 5g/dL	0.8
	RF 2000 IU/mL	0.7
	HAMA 1000ng/mL	0.8
Sample type	Interfering substance	Theranos Index
p24 positive	Control	1.3
	triglyceride 1500 mg/dL	1.5
	Hemoglobin 1000 mg/dL	1.0
	Bilirubin 40 mg/dL	1.7
	Total protein 5g/dL	1.3
	RF 2000 IU/mL	1.6
	HAMA 1000ng/mL	1.2

Table 5: Interference to endogenous interferents

8.5 No interference was observed with endogenous interferents spiked with high negative p24 antigen levels. Some interferents (hemoglobin and bilirubin) however showed greater than 20% interference when spiked with low positive levels of p24 antigen and should be re-tested at lower levels of these interferents.

9 Matrix comparison

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

9.2 20 unique patients donated 1 venous SST tube, 1 venous K_2EDTA tube, and 2 finger stick samples each 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 p24 antigen negative, the matched samples from all patients were spiked using different amounts of p24 antigen WHO reference analyte to get samples across a broader assay range. p24 antigen levels were determined by Theranos method from all 3 matrices and compared to each other.

9.3 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)

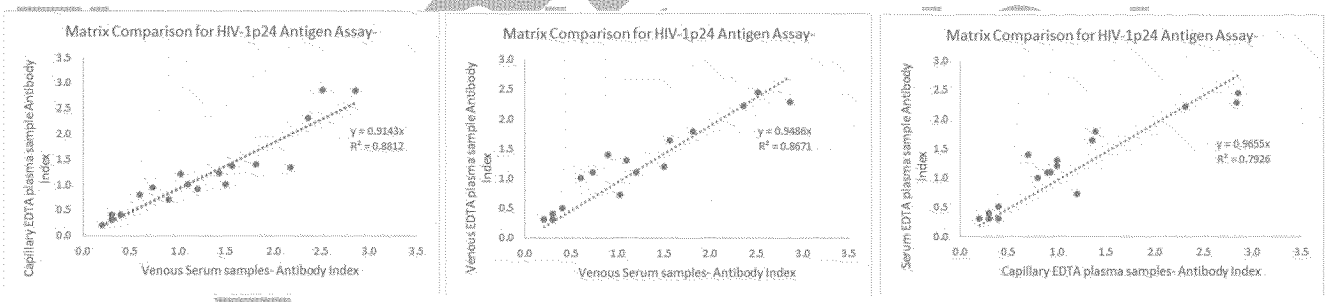

Figure 1: Matrix comparison plots

10 Hook effect

10.1 The goal of this experiment was to test for hook effect by testing very high levels of p24 antigen on Theranos assay. Commercially available pure p24 antigen was spiked in pooled normal human negative serum and the antigen index was determined in comparison to the cut off calibrator.

10.2 No hook effect was observed up to p24 antigen levels as high as 600 ng/mL.

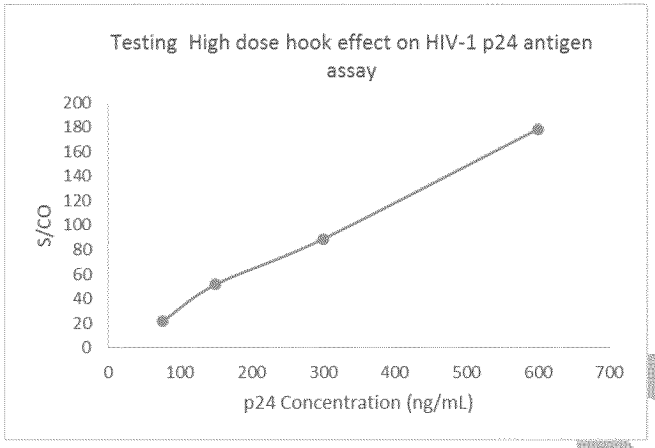

Figure 2: Hook effect Study for HIV-1 p24 antigen Assay

11 Conclusions

Theranos HIV-1 p24 assay is a sensitive assay for early detection of HIV infection from serum, plasma and / or finger stick specimens. It could be used as a screening test for detecting primary infection. The assay shows excellent specificity (100%) and sensitivity (96%).