HIV-2 Assay Development Report

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1 Purpose

This document describes the studies conducted during the development of the HIV-2 IgG assay.

2 Scope

This report includes relevant experiments in the development of the HIV-2 IgG assay.

3 Definitions

Term	Definition
HIV-2	Human Immunodeficiency Virus Type 2
RF	Rheumatoid factor
RLU	Relative light unit
IgG	Immunoglobulin G
HAMA	Human anti-mouse antibodies
NPA	Negative Percent Agreement
PPA	Positive Percent Agreement

*Table [SEQ Table * ARABIC]: Definitions*

4 Assay principle

The Theranos HIV-2 assay is an in vitro diagnostic immunoassay for the qualitative determination of IgG antibodies to HIV-2 in human serum, plasma and whole blood. In this assay, the sample is incubated with a solid surface coated with HIV-2 antigens. Antigen-antibody complexes will form if the patient sample contains anti-HIV-2 antibodies. After a number of washing steps to remove unbound sample, the solid surface is incubated with an HIV-2 peptide conjugated to a reporter enzyme. After a number of washing steps to remove unbound detection conjugate, the solid surface is incubated with a chemiluminescent substrate and RLUs are measured using the Theranos Analyzer. The RLU data is used to calculate each sample's index value by comparison with on-board calibrators.

5 Reference Assay

5.1 The following assay was used as reference method:
HIV-2, ADVIA Centaur XP

6 *Preliminary clinical study*

6.1 The goal of this experiment is to evaluate the Theranos assay for its ability to detect anti-HIV-2 antibody in a group of individuals.

6.2 Procedure: A total of 281 patient samples, including individuals at high risk, pregnant individuals and individuals with signs and symptoms of HIV-2 infection, were analyzed using the Theranos system and compared with results from the reference method, ADVIA Centaur XP HIV-2 IgG assay.

6.3 Acceptance criteria: Positive percent agreement (sensitivity) should be 100% and negative percent agreement (specificity) should be 100%.

6.4 Results: The overall positive percent agreement was 100% and negative percent agreement was 100%.

	High Risk, including pregnancy					
		Theranos				
Centaur HIV-2 IgG		Positive	Negative	Equivocal	Total	
	Positive	0	0	0	0	
	Negative	0	119	0	119	**100.00%** **NPA**
	Equivocal	0	0	0	0	

	Symptomatic subjects + Positive samples					
		Theranos				
Centaur HIV-2 IgG		Positive	Negative	Equivocal	Total	
	Positive	36	0	0	36	**100.00%** **PPA**
	Negative	0	0	0	0	
	Equivocal	0	0	0	0	

	Additional panels					
		Theranos				
Centaur HIV-2 IgG		Positive	Negative	Equivocal	Total	
	Positive	15	0	0	15	**100.00%** **PPA**
	Negative	0	111	0	111	**100.00%** **NPA**

	Equivocal	0	0	0	0

All samples						
		Theranos				
Centaur HIV-2 IgG		Positive	Negative	Equivocal	Total	
	Positive	51	0	0	51	**100.00%** **PPA**
	Negative	0	230	0	230	**100.00%** **NPA**
	Equivocal	0	0	0	0	

*Table [SEQ Table * ARABIC]: Clinical Study Results*

6.5 Conclusions: Our results meet the acceptance criteria and additional samples (10000 samples) will be run during validation, which will further indicate and support that the assay meets the acceptance criteria

7 *Analytical specificity (cross-reactivity)*

7.1 The goal of this experiment is to test the Theranos HIV-2 assay for cross reactivity to medical conditions unrelated to HIV-2.

7.2 Procedure: 201 samples from 17 cross-reactive groups were tested using the HIV-2 Theranos Analyzer and compared to ADVIA Centaur.

7.3 Acceptance criteria: minimal cross reactivity to medical conditions unrelated to HIV-2. Positive % agreement should be 100% and negative % agreement should be 100%

7.4 Results: 100% of negative samples were found to be non-reactive to both the Theranos and Reference method (negative % agreement was 100%), and 100% of positive samples were found to be reactive for both assays (Positive % agreement was 100%). Summary is shown in the following table:

Category #	Cross Reactant	Samples Tested	In Agreement
1	Elevated IgG	14	14
2	ANA	14	14
3	EBV IgG	14	14
4	RF+	14	14
5	CMV	14	14
6	Toxoplasma Gondii	14	14
7	HBV	14	14
8	HBsAg	7	7
9	Hemodialysis	12	12

10	Graves Disease	12	12
11	Flu	10	10
12	Scleroderma	14	14
13	Rubella	10	10
14	Lupus	10	10
15	HSV 1/2	12	12
16	Elevated IgM	10	10
17	HCV	6	6

*Table [SEQ Table * ARABIC]: cross-reactivity to different medical conditions*

7.5 Conclusions: No cross-reactivity observed. Acceptance criteria for cross-reactivity was met

8 Interference

8.1 The goal of this experiment was to test the effect of lipemic, hemolyzed, icteric, high-protein, RF+, and HAMA+ samples on Theranos assay

Procedure: An HIV-2 positive and an HIV-2 negative sample were spiked with 750 mg/dL triglycerides, 1000 mg/dL hemoglobin, 20 mg/dL bilirubin, 2g/dL total protein, 500 ng/mL HAMA+, or 2000 IU/mL RF+. These spiked samples were tested on the Theranos Analyzer and compared with non-spiked positive and negative HIV-2 samples to determine percent interference. Percent interference was calculated with the equation below. x_M is the back calculated measured value of the spiked sample on the Theranos assay and x_T is the value of the unspiked sample on the Theranos assay.

- *If reference COI < 0.5, absolute value of the interference must be less than 0.1 COI, i.e.*

$$Interference = x_M - x_T$$

- *If reference COI > 0.5, absolute value of the interference must be less than 20%, i.e.*

$$Interference = {(x_M - x_T)}/{x_T}, \%$$

8.2 Acceptance criteria: The absolute value of the percent interference must be less than 20%.

8.3 Results: All spiked samples showed no difference in diagnosis between Sample and Control. Summary is shown in the following tables:

Sample Type	Interfering Substance	Interference
HIV-2 Positive (COI ~ 1.5)	Hemoglobin (1000 mg/dL)	2%
	Bilirubin (20 mg/dL)	-14%
	Triglycerides (750 mg/dL)	1%
	Total Protein (2 g/dL)	10%
	RF+ (2000 IU/mL)	14%
	HAMA+ (500 ng/mL)	7%
HIV-2 Negative (COI = 0.02)	Hemoglobin (1000 mg/dL)	0.01
	Bilirubin (20 mg/dL)	0.01
	Triglycerides (750 mg/dL)	0.02
	Total Protein (2 g/dL)	0.01
	RF+ (2000 IU/mL)	0.01
	HAMA+ (500 ng/mL)	0.00

*Table [SEQ Table * ARABIC]: Interference to endogenous compounds*

8.4 Conclusions: No interference was observed. The acceptance criteria was met.

9 Matrix comparison

9.1 The goal of this experiment was to use Theranos assay to compare Venous EDTA Plasma with Venous Lithium Heparin Plasma and Venous Serum.

9.2 Procedure: 14 unique patients each donated 1 Serum tube, 1 K_2EDTA tube, and 1 Li-Hep tube. Plasma from all BCDs from the same patient were pooled. Due to the fact that all in-house collected samples were HIV-2 negative, the matched samples from 14 patients were spiked using different amounts of an HIV-2 positive serum sample. HIV-2 levels were determined by the Theranos method from all 3 matrices and compared to each other.

9.3 Acceptance criteria: Each EDTA or Lithium Heparin sample must be within +/- 30% difference from the serum sample.

9.4 Results: Table 5 summarizes the matrix comparison results for the HIV-2 assay. One Lithium-Heparin sample is discrepant when compared to the serum reference.

Sample	Venous EDTA	Venous Li-Hep
1	12.4	-6.9
2	-14.6	-5.8
3	-26.2	-5.2
4	-21.6	-8.0
5	-3.9	-11.9
6	-23.1	-20.1
7	-5.1	-1.2
8	-7.3	2.6
9	-27.8	-22.9
10	-3.1	5.3
11	-12.4	-8.3
12	-25.5	18.3
13	-28.9	-31.3
14	-21.8	-11.7

*Table [SEQ Table * ARABIC]: Matrix Comparison Results*

9.5 Conclusions: Only one discrepant sample compared with the venous serum reference demonstrating matrix equivalence for the HIV-2 IgG assay.

10 Conclusions

We successfully developed an ELISA sandwich assay to detect HIV-2 IgG in different matrices. The assay meets all requirements for sensitivity, specificity, cross reactivity, interference, and matrix effect.