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Hepatitis C Virus IgG Assay Development Report

Theranos, Inc.

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1. ASSAY INFORMATION[TC "ASSAY INFORMATION" \F C \L "2"]

1.1 Assay Specifications[TC "Assay Specifications" \f C \l "3"]

This assay is designed to qualitatively determine the presence of IgG antibodies to Hepatitis C Virus (HCV) in human serum.

1.1.1 Reference Assays [TC "Reference Assays and Standards" \f C \l "3"]

The following commercial ELISA kits have been used in house as predicate methods:

- US Biological, Cat # H1920-17J
- Abnova, Cat # KA0291

1.1.2 Materials and Methods[TC "Materials and Methods" \f C \l "1"]

A protein serves as the capture surface for Hepatitis C Virus IgG antibodies in the sample. After incubation of the appropriately-diluted sample on the capture surface, the surface is washed. A mouse anti-human IgG detection antibody is incubated on the surface. After this incubation period, the surface is washed again. Alkaline phosphatase substrate is incubated on the surface, and then the resulting chemiluminescence is read in Relative Light Units (RLU).

2 ASSAY DEVELOPMENT[TC "ASSAY OPTIMIZATION" \F C \L "2"]

2.1 Specificity tests on the Theranos System-HAMA and Rf samples

Positive disease samples known to cause false positives in this HCV IgG assay were tested on the Theranos system. Both rheumatoid factor (Rf) and Human Anti-Mouse Antibodies (HAMA) positive samples were tested on the Theranos system. One sample out of the 6 Rf positive clinical samples tested, consistently gave high levels of cross reactivity on the Theranos system. This sample was also positive in both the commercial kits indicating that this sample could indeed be positive for HCV IgG.

HAMA Sample #4 came out borderline positive on the Theranos system. This sample also came out positive in the Abnova Kit. Only one out of the sixteen HAMA samples ran on the Theranos system resulted in a borderline positive result.

Table [SEQ Table * ARABIC]: Specificity Test on the Theranos System

Sample Type	Sample #	Mean RLU
Pos CTL	USBiological Kit	84109
Pos CTL	Biochain Kit	79035
Pos CTL	Axell Kit	84474
Low Pos CTL	Abnova Kit	22886
Pos Mean RLU		**67626**
Neg Mean RLU*		**2277**
Mean Pos RLU/Mean Neg RLU		**29.7**
HAMA Positive Clinicals	2	12286
	3	568
	4	22148
	6	11548
	8	7151
	9	6884
RF Positive Clinicals	1	3374
	2	960
	3	1358
	4	830
	5	5439
	6	867094

*Neg Mean RLU –Refers to the Normal Sera Mean RLU.

Table [SEQ Table * ARABIC]: Kit comparison data

Sample Type	US Biological Kit	Abnova Kit	Theranos System*
US Biol Pos CTL	+	+	+
Biochain Pos CTL	+	+	+
Axell Pos CTL	+	+	+
Abnova Pos CTL	+	+	+
Neg CTL	-	-	-
Sample 1	-	-	-
Sample 2	-	-	-
Sample 3	-	-	-
Sample 4	-	-	-
Sample 5	-	-	-
Sample 6	-	-	-
Sample 7	-	-	-
Sample 8	-	-	-
Sample 9	+	+	-
Sample 10	-	-	-
Sample 11	-	-	-
Sample 12	-	-	-
Sample 13	-	-	-
Sample 14	-	-	-
HAMA #2	-	+	-
HAMA #4	-	+	+
HAMA #6	+	+	-
RF #6	+	+	+

* Based on the cut off equation which was statistically determined.

Table [SEQ Table * ARABIC]: Sixteen HAMA Samples Screen

Sample Type	Sample #	Mean RLU
Pos CTL	USBiological Kit	84109
Pos CTL	Biochain Kit	79035
Pos CTL	Axell Kit	84474
Low Pos CTL	Abnova Kit	22886
Pos Mean RLU		**67626**
Neg Mean RLU		**2277**
Mean Pos RLU/Mean Neg RLU		**29.7**
HAMA Positive Clinical Samples	#2	12286
	#3	568
	#4	22148
	#6	11548
	#8	7151
	#9	6884
	#11	5547
	#13	12825
	#14	9202
	#15	1550
	#17	5008
	#20	9253
	#21	2209
	#22	1838
	#23	1351
	#24	4376

2.2 Specificity tests on the Theranos System-WHO Controls

WHO controls representing conditions unrelated to HCV infection and containing potentially interfering substances were tested on the Theranos system. WHO antibody controls for anti-HBV, anti-HAV, anti-HIV-1/2, anti HSV and anti-CMV were all found to not cross react on the Theranos system. Additionally, positive antibody kit controls for diseases such as *Treponema pallidum and Influenza A* were found to not cross react in this assay. The WHO quality control standard for HCV was obtained and evaluated on the Theranos system. While the control is not used to evaluate sensitivity of the assay, the result obtained was ~10 fold higher than the mean negative control sera.

Table [SEQ Table * ARABIC]: Cross reactivity tests

Sample Type	**Mean RLU**	**CV%**
Biochain Pos CTL	54366	8.2
Abnova Pos CTL	20957	14.6
Mean Pos RLU	**37662**	
Abnova Neg CTL	335	11.6
Neg Sample 1	1076	15.9
Neg Sample 2	1640	8.7
Neg Sample 3	1369	15.5
Neg Sample 4	3357	11.8
Neg Sample 5	3469	13.9
Neg Sample 6	2358	11.8
Neg Sample 7	1158	14.9
Neg Sample 8	1780	13.9
Neg Sample 9	551	13.4
Neg Sample 10	951	6.8
Mean Negative RLU	**1771**	
Mean Pos RLU/Mean Neg RLU	**21**	
Hepatitis B WHO Control	1671	10.2
Hepatitis A WHO Control	3137	16.2
Human immunodeficiency virus 1/2 WHO Control	3145	9.5
Cytomegalovirus WHO Control	2513	4.2
Herpes simplex virus WHO Control	1173	14.6
Treponema pallidum- Kit Pos CTL	1661	19.3
Influenza A- Kit Pos CTL	424	19.9

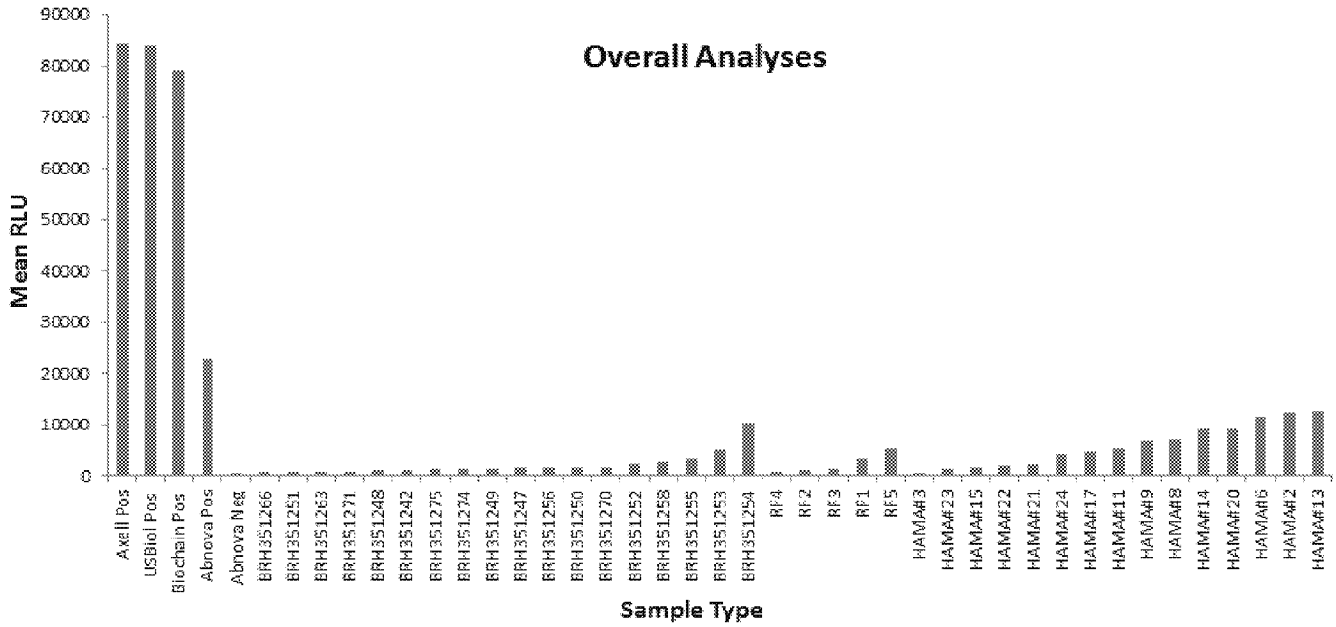
Table [SEQ Table * ARABIC]: Evaluating the HCV WHO Control

Sample Type	**Mean RLU**	**CV%**
Biochain Pos CTL	54366	8.2
Abnova Pos CTL	20957	14.6
Mean Pos RLU	**37662**	
WHO HCV CTL Pos	13727	3.8
Abnova Neg CTL	335	11.6
Neg Sample 1	1076	15.9
Neg Sample 2	1640	8.7
Neg Sample 3	1369	15.5
Neg Sample 4	1189	18.9
Neg Sample 5	770	18.7
Neg Sample 6	3357	11.8
Neg Sample 7	3469	13.9
Neg Sample 8	2358	11.8
Neg Sample 9	1158	14.9
Neg Sample 10	1780	13.9
Neg Sample 11	551	13.4
Neg Sample 12	951	6.8
Neg Sample 13	1425	19.4
Neg Sample 14	848	22.9
Neg Sample 15	1080	18.9
Mean Negative RLU	1535	
WHO HCV CTL Pos CTL/Mean Neg RLU	9	

2.3 Summary of the HCV assay on the Theranos system

A graphical representation demonstrating the HCV assay's performance on the Theranos system. HAMA Sample #4 is not included in the figure.

Figure [SEQ Figure * ARABIC]: Overall analysis



2.4 Stability Studies

Stability of reagents is being monitored.

3 CONCLUSION

We have successfully developed an immunoassay to detect anti-Hepatitis C Virus IgG antibody in human serum and plasma.