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Herpes Simplex Virus 1 IgG Assay Feasibility Report

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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"]

An enzyme linked immunosorbent assay (ELISA) was developed for the qualitative detection of Herpes Simplex Virus type 1 (HSV1) IgG Antibody. HSV1 primarily infects the mouth and nose while Herpes Simplex Virus type 2 (HSV2) primarily infects the genital area. Both are characterized by blistering the epithelia and a dormant phase in the ganglion of sensory neurons. Primary infections of HSV may lead to illness during pregnancy and compromise fetal and newborn health. HSV1 and HSV2 have similar epitope structures, and it is important to differentiate between them. This report describes the assay development and performance of the HSV1 IgG assay as aid to determining immune status.

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

The Liaison HSV-1 IgG CLIA kit was the predicate method. A secondary method was used for confirmation of results.

- Liaison HSV-1 IgG CLIA, Cat. 310830
- EuroImmun HSV-1 (gC1) ELISA (IgG), Cat. EI 2531-9601-24G

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

The HSV-1 IgG ELISA was developed using the HSV-1 gG1M recombinant antigen as the capture surface. Human IgG antibodies in serum or plasma samples bind specifically to the HSV-1 antigen for 5 minutes followed by a wash cycle. Bound human IgG anti-HSV-1 antibodies are detected using an AP labeled mouse monoclonal antibody to human IgG. After incubation with the detector antibody for 5 minutes, another wash cycle was performed and the alkaline phosphatase substrate is incubated on the surface for another 5 minutes. The resulting chemiluminescence is read in relative light units (RLU) on the Theranos system.

Table [SEQ Table * ARABIC]: Materials

Name	Supplier	Catalog #
Herpes Simplex Virus type 1 gG1M antigen	Fitzgerald	30R-AH023
Mouse Anti-hIgG clone 2C11	Novus	NB100-2046
Bio-Rad Kit Calibrator	Bio-Rad	25182
Alkaline Phosphatase Labeling Kit (SH)	Dojindo	LK13-10
Theranos Substrate	Theranos	In House
Assay Diluent (Protein Free)	Surmodics	SM01-1000
Theranos AP Conjugate Stabilizer	Theranos	In House
Tris Buffered Saline with Tween 20	Sigma	T9039-10PAK
Theranos Cartridge	Theranos	In House
Theranos System	Theranos	In House

1.1.3 Labeling of Detector Antibody

The mouse anti-hIgG clone 2C11 was labeled with alkaline phosphatase according to kit instructions (Dojindo, LK13-10).

1.1.4 Preparation of Theranos AP Conjugate Stabilizer

The conjugate diluent was prepared by adding ZnCl and MgCl to the assay buffer TBS 3%BSA 0.05% Sodium Azide at final concentrations of 0.1mM ZnCl and 5mM MgCl.

2 ASSAY DEVELOPMENT

All experiments used three cartridges per sample with two tips per cartridge per condition unless otherwise specified.

2.1 Antigen Capture

Three antigens were tested to determine the best antigen capture. C11 was an HSV-1 gD recombinant antigen, C13 and C17 were both different segments of the HSV-1 gG antigen recombinants. The Theranos tips were coated with each capture in Carbonate-Bicarbonate buffer at 5ug/mL. The assay was performed using the Generic2_10X which is a 10-10-10 incubation protocol at a 10x dilution. The AP-conjugated Novus clone 2C11 antibody was used as the detection antibody at 100 ng/mL in the House Blocking Buffer. C13 and C17 both performed well and were both used in further testing. Samples were run in triplicate with two tips per cartridge.

Table [SEQ Table * ARABIC]: Antigen Capture

		HSV1 C11			HSV1 C13			HSV1 C17		
		gD *(aa266-394 fused to GST-Tag at C-Term) from E. coli*			gG *(amino terminal region Met1-ASP190 fused with SOD) from S. cerevisiae*			gG *(84-175) from E.coli*		
		Inter-Cartridge			Inter-Cartridge			Inter-Cartridge		
Sample ID	Liaison Result	Mean	CV %	RLU/Avg(-)	Mean	CV %	RLU/Avg(-)	Mean	CV %	RLU/Avg(-)
N1	Negative	3636	18.1	0.06	1155	34.7	0.14	3467	27.9	0.22
N3	negative	12281	11.9	0.21	1273	47.0	0.16	8376	24.8	0.53
Z16	negative	10285	1.5	0.18	7876	27.7	0.97	14191	5.4	0.91
Z37	negative	247301	19.5	4.27	6900	26.4	0.85	10972	11.8	0.70
RF6	negative	15941	15.8	0.28	23252	8.2	2.87	41316	19.2	2.64
Z8(HSV1/2+)	positive	15170	10.9	0.26	15429	11.5	1.91	13478	11.9	0.86
SCP	positive	78248	9.0	1.35	70245	13.9	8.68	70822	9.0	4.52
Z6	positive	10285	1.5	0.18	179730	12.7	22.21	274147	14.7	17.50
Z12	positive	217202	6.7	3.75	336536	14.6	41.59	287591	16.4	18.36
RF12	positive	196628	23.6	3.40	89121	22.4	11.01	69964	8.8	4.47
HAMA9	Positive	77392	27.9	1.34	232929	13.6	28.79	364826	18.6	23.29
		Average(-):	57889		Average(-):	8091		Average(-):	15664	

The Average (-) was calculated using the response of the negative samples.

2.2 Capture Surface Titration

Optimum surface coating condition was determined by titration of the capture surface. Tips were coated with C13 and C17 antigen at 10 ug/mL, 5 ug/mL and 2.5 ug/mL. The assay was performed using the Generic2_10x protocol and 2.5ug/mL tip coating of C13. The detector antibody was used at 100 ng/mL. Both C13 and C17 gave best sensitivity and precision at 2.5 ug/mL. For future experiments, tips were coated at 2.5ug/mL. Samples were run in triplicate with two tips per cartridge.

To determine the Antibody Index the following equation was used:

Avg RLU/(stdev(-)*2+avg(-))= Antibody Index

where stdev(-) and avg(-) are calculated using the negative samples within that condition
(This is excluding the HSV2 positive but HSV1 negative samples excluding the HSV2 samples) Green (below 0.9 Index) is considered a negative sample. Red (above 1.1 Index) is considered a positive sample. Yellow (between 0.9 and 1.1) is considered an equivocal sample.

Table [SEQ Table * ARABIC]: Capture Titration of C13

		C13: Fitzgerald gGM1 (amino terminal region Met1-ASP190 fused with SOD) from S. cerevisiae								
		10ug/mL			*5ug/mL*			*2.5ug/mL*		
Sample Id	Liaison HSV1 IgG Result	Avg RLU	%CV	Antibody Index	Avg RLU	%CV	Antibody Index	Avg RLU	%CV	Antibody Index
MT10	Negative	23338	16	0.66	23691	19	0.73	21041	19	0.59
MT12	Negative	1910	28	0.05	1799	11	0.06	3490	23	0.10
MT14	Negative	1139	8	0.03	1210	24	0.04	1317	21	0.04
MT19	Negative	25378	30	0.72	20969	10	0.65	27825	13	0.79
MT20	Negative	6298	18	0.18	6798	12	0.21	7431	24	0.21
Z21 (HSV2)	Negative	12109	21	0.34	9014	26	0.28	10080	21	0.28
Z23 (HSV2)	Negative	28505	30	0.81	21882	9	0.68	21316	22	0.60
Z25	Positive	10433	14	0.30	16856	12	0.52	19345	25	0.55
Z02	Positive	36763	19	1.04	49307	25	1.53	45685	19	1.29
Z07	Positive	34958	11	0.99	74403	23	2.30	91251	15	2.57
Z19	Positive	44685	15	1.27	119007	13	3.68	170657	24	4.81
Z29	Positive	39795	11	1.13	51350	13	1.59	52625	8	1.48
RF01	Positive	14365	21	0.41	31308	33	0.97	29605	15	0.84
RF05	Positive	27554	27	0.78	76033	8	2.35	94156	16	2.66
HA05	Positive	13771	17	0.39	25412	38	0.79	30243	10	0.85
HA06	Positive	70085	7	1.99	136062	20	4.21	197750	23	5.58
Experimental Cutoff		Avg (-)	Stdev (-)	Stdev*2 +avg	Avg (-)	Stdev (-)	Stdev*2 +avg	Avg (-)	Stdev (-)	Stdev*2 +avg
		11613	11822	35257	10893	10707	32307	12221	11612	35445

Table [SEQ Table * ARABIC]: Capture Titration of C17

		C17: Abs online gG(aa74-185) from E.coli								
		10ug/mL			*5ug/mL*			*2.5ug/mL*		
Sample Id	Liaison HSV1 IgG Result	Avg RLU	%CV	Antibody Index	Avg RLU	%CV	Antibody Index	Avg RLU	%CV	Antibody Index
MT10	Negative	29826	23	0.64	31359	25	0.70	27159	24	0.62
MT12	Negative	8908	12	0.19	6225	12	0.14	5173	19	0.12
MT14	Negative	1507	20	0.03	1029	20	0.02	2560	23	0.06
MT19	Negative	35528	14	0.76	30999	15	0.69	33854	8	0.77
MT22	Negative	7255	26	0.16	7163	29	0.16	6452	38	0.15
Z21 (HSV2)	Negative	34439	23	0.74	29927	19	0.67	22014	15	0.50
Z23 (HSV2)	Negative	32004	10	0.68	32147	36	0.72	32899	4	0.75
Z25	Positive	14628	10	0.31	13239	13	0.30	18959	7	0.43
Z02	Positive	61633	15	1.32	72691	24	1.63	101782	19	2.32
Z7	Positive	23832	33	0.51	22624	19	0.51	42620	26	0.97
Z19	Positive	77594	9	1.66	92505	19	2.07	105598	24	2.41
Z29	Positive	356367	23	7.63	346976	29	7.78	246239	25	5.62
RF01	Positive	20926	8	0.45	34405	18	0.77	55512	10	1.27
RF05	Positive	33996	14	0.73	30926	26	0.69	65011	26	1.48
HA05	Positive	43992	16	0.94	36507	12	0.82	28259	29	0.65
HA06	Positive	45183	12	0.97	56758	14	1.27	116685	26	2.66
Experimental Cutoff		Avg (-)	Stdev (-)	Stdev*2 +avg	Avg (-)	Stdev (-)	Stdev*2 +avg	Avg (-)	Stdev (-)	Stdev*2 +avg
		16604	15062	46729	15355	14633	44621	15040	14385	43809

2.3 Detection Antibodies

Three AP-conjugated anti-hIgG antibodies were tested to optimize the detection antibody. Novus clone 2C11(D1), Southern Biotech clones JDC10 (D2) and H2 (D3). The assay was performed using the Generic2_10x protocol and 2.5ug/mL tip coating of C13 or C17. The detection antibodies were tested at 100ng/mL. Both C13 and C17 performed best with D1 where there was the lowest background and the best separation of negatives and positives when compared to the predicate method. Samples were run in triplicate with two tips per cartridge.

To determine the antibody Index the following equation was used:

Avg RLU/(stdev(-)*2+avg(-))

where stdev(-) and avg(-) are calculated using the negative samples within that condition
(This is excluding the IISV2 positive but IISV1 negative samples) Green (below 0.9 Index) is a considered a negative sample. Red (above 1.1 Index) is considered a positive sample. Yellow (between 0.9 and 1.1) is considered an equivocal sample.

Table [SEQ Table * ARABIC]: Detection Antibody Screen on C13

		C13: Fitzgerald gGM1 (amino terminal region Met1-ASP190 fused with SOD) from S. cerevisiae									
		D1				*D2*			*D3*		
Sample Id	Liaison HSV1 IgG Result	Avg RLU	%CV	Antibody Index	Theranos Result	Avg RLU	%CV	Antibody Index	Avg RLU	%CV	Antibody Index
N1	Negative	827	39	0.04	Negative	1694	23	0.07	10552	24	0.02
N3	Negative	751	21	0.04	Negative	1234	13	0.05	37894	15	0.07
N4	Negative	633	26	0.03	Negative	905	13	0.04	7079	31	0.01
N6	Negative	3127	24	0.15	Negative	7765	9	0.33	157675	19	0.31
N7	Negative	1271	20	0.06	Negative	20933	15	0.89	455210	27	0.89
MT10	Negative	20420	33	1.01	Equivocal			0.00			0.00
Z21 (HSV2)	Negative	9093	23	0.45	Negative	27931	6	1.18	154692	17	0.30
Z23 (HSV2)	Negative	17543	7	0.87	Negative	82703	14	3.51	523450	16	1.02
Z15	Positive	13763	13	0.68	Negative	36297	35	1.54	304914	12	0.59
Z19	Positive	146711	16	7.26	Positive	218916	12	9.28	1636083	8	3.19
Z25	Positive	16102	22	0.80	Negative	59391	9	2.52	350329	13	0.68
Z33	Positive	19148	17	0.95	Equivocal	43162	20	1.83	381136	14	0.74
Z39	Positive	150207	30	7.43	Positive	224517	26	9.52	1621248	14	3.16
RF01	Positive	449	33	0.02	Negative	489	35	0.02	1643	28	0.00
RF15	Positive	70544	27	3.49	Positive	88596	28	3.75	1069783	15	2.08
HA05	Positive	46727	40	2.31	Positive	56601	30	2.40	534341	18	1.04
HA06	Positive	140794	23	6.97	Positive	229472	18	9.73	1510418	24	2.94
Experimental Cutoff		Avg (-)	Stdev (-)	Stdev*2 +avg		Avg (-)	Stdev (-)	Stdev*2 +avg	Avg (-)	Stdev (-)	Stdev*2 +avg
		4505	7852	20209		6506	8545	23595	133682	189948	513578

Table [SEQ Table * ARABIC]: Detection Antibody Screen on C17

		C17: Antibodies Online gG (amino region) from E. coli					
		D1			*D2*		
Sample Id	Liaison HSV1 IgG Result	Avg RLU	%CV	Antibody Index	Avg RLU	%CV	Antibody Index
N1	Negative	1752	27	0.06	7498	25	0.09
N3	Negative	1525	23	0.05	9103	26	0.11
N4	Negative	18862	25	0.62	31109	15	0.37
N6	Negative	2025	32	0.07	51463	30	0.61
N7	Negative	22956	20	0.75	65370	20	0.78
Z21 (HSV2)	Negative	13430	29	0.44	54431	22	0.65
Z23 (HSV2)	Negative	19100	18	0.62	67875	11	0.81
Z15	Positive	22149	18	0.72	65693	29	0.78
Z19	Positive	117425	18	3.84	153728	14	1.83
Z25	Positive	16343	4	0.53	44934	24	0.53
Z33	Positive	25766	21	0.84	44248	12	0.53
Z39	Positive	151608	30	4.96	187281	25	2.23
RF01	Positive	49923	19	1.63	49170	28	0.59
RF15	Positive	37140	42	1.21	55976	11	0.67
HA05	Positive	27277	15	0.89	48846	13	0.58
HA06	Positive	119768	15	3.91	148409	13	1.77
Experimental Cutoff		Avg (-)	Stdev (-)	Stdev*2 +avg	Avg (-)	Stdev (-)	Stdev*2 +avg
		9424	10585	30594	32909	25562	84033

2.4 Detection Stabilizer

To test the effect of detection stabilizers, Novus clone 2C11 was diluted in House Stabilizer, Stabilzyme, and Biostab at a concentration of 100 ng/mL. The assay was performed using the Generic2_10x protocol and 2.5ug/mL tip coating of C13 or C17. The best performing diluent was Theranos AP Conjugate Stabilizer. Samples were run in triplicate with two tips per cartridge.

To determine the *2 Index the following equation was used:

Avg RLU/(stdev(-)*2+avg(-))

where stdev(-) and avg(-) are calculated using the negative samples within that condition
(This is excluding the HSV2 positive but HSV1 negative samples) Green (below 0.9 Index) is a considered a negative sample. Red (above 1.1 Index) is considered a positive sample. Yellow (between 0.9 and 1.1) is considered an equivocal sample.

Table [SEQ Table * ARABIC]: Detection Stabilizer on C13

C13		*House Stabilizer*				*Stabilzyme*			*Biostab*		
Sample Id	Liaison HSV1 IgG Result	Avg RLU	%CV	Antibody Index		Avg RLU	%CV	Antibody Index	Avg RLU	%CV	Antibody Index
N1	Negative	982	27	0.03	Negative	788	18	0.06	4320	19	0.06
N3	Negative	840	20	0.03	Negative	849	27	0.06	4017	20	0.06
N6	Negative	4364	13	0.14	Negative	2044	12	0.16	11153	7	0.16
N7	Negative	1505	16	0.05	Negative	1325	18	0.10	3737	11	0.05
MT10	Negative	27862	19	0.91	Equivocal	12107	21	0.92	62722	10	0.92
Z21 (HSV2)	Negative	15774	21	0.52	Negative	8628	32	0.65	39791	20	0.58
Z23 (HSV2)	Negative	25558	13	0.84	Negative	11760	11	0.89	63181	14	0.92
Z03	Positive	45717	13	1.50	Positive	24268	23	1.84	46311	19	0.68
Z05	Positive	29792	15	0.98	Equivocal	14772	7	1.12	50803	12	0.74
Z06	Positive	99845	8	3.27	Positive	45528	13	3.45	137788	18	2.01
Z11	Positive	48668	19	1.60	Positive	23565	19	1.79	65226	15	0.95
Z12	Positive	241358	11	7.92	Positive	110926	7	8.41	280193	19	4.09
Z13	Positive	160562	14	5.27	Positive	77812	20	5.90	151657	20	2.21
Z15	Positive	16344	29	0.54	Negative	9883	24	0.75	45056	15	0.66
Z25	Positive	25120	16	0.82	Negative	16016	26	1.21	39271	32	0.57
Z33	Positive	18689	13	0.61	Negative	11534	13	0.87	37385	27	0.55
Z39	Positive	200825	24	6.59	Positive	57915	16	4.39	260302	15	3.80
RF01	Positive	41385	11	1.36	Positive	28303	30	2.15	72885	5	1.06
RF15	Positive	157110	22	5.15	Positive	49261	29	3.74	124936	9	1.82
HA05	Positive	43128	26	1.41	Positive	32582	12	2.47	64151	21	0.94
HA06	Positive	203528	9	6.68	Positive	121779	18	9.24	181316	11	2.65
Experimental Cutoff		Avg (-)	Stdev (-)	Stdev*2 +avg		Avg (-)	Stdev (-)	Stdev*2 +avg	Avg (-)	Stdev (-)	Stdev*2 +avg
		7110	11688	30487		3423	4881	13184	17190	25641	68471

Table [SEQ Table * ARABIC]: Detection Stabilizer on C17

C17		*House Stabilizer*			*Stabilzyme*			*Biostab*		
Sample Id	Liaison HSV1 IgG Result	Avg RLU	%CV	Antibody Index	Avg RLU	%CV	Antibody Index	Avg RLU	%CV	Antibody Index
N1	Negative	2311	9	0.05	1849	18	0.07	10579	12	0.10
N3	Negative	3257	10	0.07	1827	14	0.07	11684	24	0.11
N6	Negative	28861	23	0.61	21333	25	0.77	34168	24	0.34
N7	Negative	1931	26	0.04	1341	12	0.05	7751	11	0.08
MT19	Negative	35274	28	0.75	16365	13	0.59	91457	10	0.90
Z21 (HSV2)	Negative	18707	10	0.40	9419	23	0.34	66030	11	0.65
Z23 (HSV2)	Negative	27832	2	0.59	14244	#DIV/0!	0.51	82369	15	0.81
Z03	Positive	73768	7	1.57	38857	9	1.40	100423	9	0.99
Z05	Positive	21643	16	0.46	11965	4	0.43	50732	14	0.50
Z06	Positive	179271	14	3.81	120812	18	4.36	226965	13	2.23
Z11	Positive	50004	12	1.06	27406	16	0.99	72663	8	0.71
Z12	Positive	141896	21	3.02	90159	20	3.26	228634	13	2.25
Z13	Positive	96966	23	2.06	47050	15	1.70	121733	19	1.20
Z15	Positive	30884	10	0.66	16402	10	0.59	58292	20	0.57
Z25	Positive	21546	14	0.46	14170	15	0.51	45089	13	0.44
Z33	Positive	31674	13	0.67	17417	18	0.63	52763	8	0.52
Z39	Positive	139256	26	2.96	82228	3	2.97	159127	5	1.56
RF01	Positive	124586	58	2.65	52164	22	1.88	129054	4	1.27
RF15	Positive	84103	8	1.79	49120	21	1.77	199823	6	1.96
HA05	Positive	38642	15	0.82	29732	11	1.07	73195	14	0.72
HA06	Positive	108679	4	2.31	72577	14	2.62	156765	21	1.54
Experimental Cutoff		Avg (-)	Stdev (-)	Stdev*2 +avg	Avg (-)	Stdev (-)	Stdev*2 +avg	Avg (-)	Stdev (-)	Stdev*2 +avg
		14327	16360	47047	8543	9573	27689	31128	35340	101808

At this point it was determined that C17 is a fragment of C13 and C17 was not used for any further optimization.

2.5 Assay Diluent

The effect of the Assay Diluent was tested using six diluents: House Blocking Buffer, House Blocking Buffer with 400ug/mL HBR, Low Cross Buffer, Starting Block, Super Block, and Protein free Assay Diluent. The assay was performed using the Generic2_10x protocol and 2.5ug/mL tip coating of C13. The detection was diluted to 100ng/mL in the Theranos House stabilizer. The Protein free assay Diluent performed best by far with the lowest background, best separation of negatives and positives, and the best clinical correlation to the predicate method. Samples were run in triplicate with two tips per cartridge.

To determine the Theranos Index the following equation was used:

Avg RLU/(stdev(-)*5+avg(-))

where stdev(-) and avg(-) are calculated using the negative samples within that condition
*For *5 Index, the stdev was multiplied by 5 instead of 2. (This is excluding the HSV2 positive but HSV1 negative samples) Green (below 0.9 Index) is a considered a negative sample. Red (above 1.1 Index) is considered a positive sample. Yellow (between 0.9 and 1.1) is considered an equivocal sample.*

Table [SEQ Table * ARABIC]: House Blocking Buffer and House Blocking Buffer +400ng/mL HBR

			House Blocking Buffer				House Blocking Buffer + 400ug/mL HBR			
Sample ID	Liaison Result	EuroImmun Result	Avg RLU	%CV	Antibody Index	Theranos Result	Avg RLU	%CV	Antibody Index	Theranos Result
LN01	Negative	Negative	615	10	0.06	Negative	718	24	0.09	Negative
LN02	Negative	Negative	826	23	0.09	Negative	660	29	0.08	Negative
LN03	Negative	Negative	1011	27	0.11	Negative	1076	18	0.13	Negative
LN4	Negative	Negative	3468	24	0.36	Negative	3471	16	0.42	Negative
EN4	Negative	Negative	4032	16	0.42	Negative	3250	24	0.39	Negative
EN5	Negative	Negative	650	27	0.07	Negative	724	25	0.09	Negative
Z21 (HSV2)	Negative	Negative	13174	22	1.38	Positive	12206	22	1.47	Positive
Z23 (HSV2)	Negative	Negative	23416	13	2.46	Positive	22243	22	2.67	Positive
Z03	Positive	Positive	47170	27	4.95	Positive	37680	22	4.53	Positive
Z05	Positive	Positive	23847	11	2.50	Positive	24679	14	2.96	Positive
Z11	Positive	Positive	40704	11	4.27	Positive	35133	8	4.22	Positive
Z13	Positive	Positive	138101	10	14.49	Positive	152083	18	18.27	Positive
Z15	Positive	Positive	15480	19	1.62	Positive	16028	21	1.93	Positive
Z25	Positive	Positive	19410	13	2.04	Positive	19079	14	2.29	Positive
Z33	Positive	Positive	18223	18	1.91	Positive	20779	14	2.50	Positive
Z39	Positive	Positive	210781	25	22.12	Positive	199009	15	23.90	Positive
RF01	Positive	Positive	42186	21	4.43	Positive	42228	26	5.07	Positive
RF15	Positive	Positive	91895	21	9.64	Positive	80535	20	9.67	Positive
HA05	Positive	Positive	32290	11	3.39	Positive	33318	17	4.00	Positive
HA06	Positive	Positive	164477	15	17.26	Positive	165197	23	19.84	Positive
Experimental Cutoff			Avg (-)	Stdev (-)	Stdev*5 +avg		Avg (-)	Stdev (-)	Stdev*5 +avg	
			1767	1553	9530		1650	1335	8326	

Table [SEQ Table * ARABIC]: Low Cross Buffer and StartingBlock

			Low Cross Buffer				StartingBlock			
Sample ID	Liaison Result	EuroImmun Result	Avg RLU	%CV	Antibody Index	Theranos Result	Avg RLU	%CV	Antibody Index	Theranos Result
LN01	Negative	Negative	708	20	0.47	Negative	1015	22	0.29	Negative
LN02	Negative	Negative	715	26	0.48	Negative	603	34	0.17	Negative
LN03	Negative	Negative	1041	19	0.70	Negative	828	20	0.24	Negative
LN4	Negative	Negative	819	27	0.55	Negative	1539	23	0.44	Negative
EN4	Negative	Negative	911	20	0.61	Negative	1744	28	0.50	Negative
EN5	Negative	Negative	902	21	0.60	Negative	549	23	0.16	Negative
Z21 (HSV2)	Negative	Negative	3873	26	2.60	Positive	2235	24	0.64	Negative
Z23 (HSV2)	Negative	Negative	2709	17	1.82	Positive	3472	8	0.99	Equivocal
Z03	Positive	Positive	21984	27	14.75	Positive	29585	19	8.41	Positive
Z05	Positive	Positive	7892	21	5.29	Positive	13248	20	3.77	Positive
Z11	Positive	Positive	18775	19	12.60	Positive	21682	15	6.16	Positive
Z13	Positive	Positive	60258	13	40.43	Positive	91044	16	25.89	Positive
Z15	Positive	Positive	5218	26	3.50	Positive	4061	23	1.15	Positive
Z25	Positive	Positive	10783	18	7.23	Positive	11124	26	3.16	Positive
Z33	Positive	Positive	7826	22	5.25	Positive	10798	15	3.07	Positive
Z39	Positive	Positive	48680	7	32.66	Positive	167892	27	47.74	Positive
RF01	Positive	Positive	16694	14	11.20	Positive	35882	13	10.20	Positive
RF15	Positive	Positive	39619	6	26.58	Positive	88452	13	25.15	Positive
HA05	Positive	Positive	16334	24	10.96	Positive	18118	22	5.15	Positive
HA06	Positive	Positive	81270	27	54.52	Positive	99365	1	28.25	Positive
Experimental Cutoff			Avg (-)	Stdev (-)	Stdev*5 +avg		Avg (-)	Stdev (-)	Stdev*5 +avg	
			849	128	1491		1046	494	3517	

Table [SEQ Table * ARABIC]: SuperBlock and Protein Free Assay Diluent

			SuperBlock				Protein Free Assay Diluent			
Sample ID	Liaison Result	EuroImmun Result	Avg RLU	%CV	Antibody Index	Theranos Result	Avg RLU	%CV	Antibody Index	Theranos Result
LN01	Negative	Negative	944	18	0.09	Negative	771	44	0.48	Negative
LN02	Negative	Negative	744	27	0.07	Negative	630	24	0.39	Negative
LN03	Negative	Negative	1465	18	0.14	Negative	1075	23	0.66	Negative
LN4	Negative	Negative	3689	25	0.36	Negative	736	33	0.45	Negative
EN4	Negative	Negative	4492	8	0.44	Negative	816	22	0.50	Negative
EN5	Negative	Negative	883	25	0.09	Negative	603	29	0.37	Negative
Z21 (HSV2)	Negative	Negative	15110	3	1.48	Positive	866	8	0.53	Negative
Z23 (HSV2)	Negative	Negative	32209	23	3.16	Positive	1142	16	0.71	Negative
Z03	Positive	Positive	34671	13	3.40	Positive	9043	25	5.59	Positive
Z05	Positive	Positive	28511	11	2.80	Positive	5459	31	3.37	Positive
Z11	Positive	Positive	40231	12	3.95	Positive	6677	25	4.12	Positive
Z13	Positive	Positive	91118	21	8.95	Positive	29653	10	18.32	Positive
Z15	Positive	Positive	27578	10	2.71	Positive	2030	25	1.25	Positive
Z25	Positive	Positive	19008	18	1.87	Positive	4762	30	2.94	Positive
Z33	Positive	Positive	19497	18	1.91	Positive	5210	17	3.22	Positive
Z39	Positive	Positive	244454	19	24.00	Positive	106860	13	66.02	Positive
RF01	Positive	Positive	34916	26	3.43	Positive	11168	10	6.90	Positive
RF15	Positive	Positive	88622	20	8.70	Positive	33574	18	20.74	Positive
HA05	Positive	Positive	27153	21	2.67	Positive	11310	34	6.99	Positive
HA06	Positive	Positive	183953	4	18.06	Positive	82334	20	50.86	Positive
Experimental Cutoff			Avg (-)	Stdev (-)	Stdev*5 +avg		Avg (-)	Stdev (-)	Stdev*5 +avg	
			2036	1630	10185		772	169	1619	

2.6 Detector Antibody Titration

To determine the optimum concentration of Novus 2C11 detector antibody (D1), the assay was run using the best current conditions (10x_10-10-10). The D1 was titrated to 100ng/mL, 50ng/mL, and 25ng/mL. The best detector antibody concentration was determined to be 100 ng/mL. Samples were run in triplicate with two tips per cartridge.

To determine the Theranos *5 Index the following equation was used:

Avg RLU/(stdev(-)*5+avg(-))

where stdev(-) and avg(-) are calculated using the negative samples within that condition
(This is excluding the HSV2 positive but HSV1 negative samples) Green (below 0.9 Index) is a considered a negative sample. Red (above 1.1 Index) is considered a positive sample. Yellow (between 0.9 and 1.1) is considered an equivocal sample.

Table [SEQ Table * ARABIC]: Detection Antibody Titration

			100ng/mL			*50ng/mL*			*25ng/mL*			
Sample	Liaison Result	EuroImmun Result	Avg RLU	CV %	Theranos Index	Avg RLU	CV %	Theranos Index	Avg RLU	CV %	Theranos Index	Theranos Result
LN01	Negative	Negative	463	46	0.23	214	28	0.17	289	37	0.44	Negative
LN02	Negative	Negative	633	34	0.31	492	25	0.38	333	30	0.51	Negative
EN4	Negative	Negative	757	20	0.37	415	31	0.32	335	13	0.51	Negative
EN5	Negative	Negative	768	21	0.38	524	26	0.41	246	28	0.37	Negative
Z16	Negative	Not Tested	1000	29	0.49	526	27	0.41	326	40	0.50	Negative
Z21	Negative	Not Tested	1159	18	0.57	709	20	0.55	444	28	0.67	Negative
Z03	Positive	Not Tested	11057	19	5.40	4926	21	3.81	3268	28	4.96	Positive
Z15	Positive	Positive	1286	24	0.63	706	24	0.55	453	22	0.69	Negative
Z39	Positive	Positive	104431	27	50.98	56657	19	43.88	29192	24	44.32	Positive
RF15	Positive	Positive	29507	22	14.40	17428	28	13.50	7098	23	10.78	Positive
HA06	Positive	Positive	63513	61	31.00	25177	26	19.50	15721	14	23.87	Positive
			avg(-)	stdev(-)	stdev*5 +avg	avg(-)	stdev(-)	stdev*5 +avg	avg(-)	stdev(-)	stdev*5 +avg	
			797	250	2049	480	162	1291	329	66	659	

2.7 Effect of Sample Dilutions

A 10x and 25x sample dilution was tested to determine the effect. Both levels gave good correlation with the predicate method, but the 10x dilution gives considerably higher positives while giving similar negative results. If it were necessary for multiplexing, it would be okay to dilute to 25X. Samples were run in triplicate with two tips per cartridge.

To determine the *5 Index the following equation was used:

Avg RLU/(stdev(-)*5+avg(-))

where stdev(-) and avg(-) are calculated using the negative samples within that condition
(This is excluding the HSV2 positive but HSV1 negative samples) Green (below 0.9 Index) is a considered a negative sample. Red (above 1.1 Index) is considered a positive sample. Yellow (between 0.9 and 1.1) is considered an equivocal sample.

Table [SEQ Table * ARABIC]: Sample Dilutions

			10x			*25x*			
Sample ID	Liaison Result	EuroImmun Result	Avg RLU	%CV	Antibody Index	Avg RLU	%CV	Antibody Index	Theranos Result
LN01	Negative	Negative	1103	31	0.61	1062	5	0.56	Negative
LN02	Negative	Negative	803	23	0.44	726	28	0.39	Negative
Z22	Negative	Not Tested	1027	30	0.56	1103	21	0.59	Negative
Z37	Negative	Not Tested	719	27	0.40	778	24	0.41	Negative
Z03	Positive	Not Tested	27903	42	15.34	7617	27	4.05	Positive
Z05	Positive	Not Tested	9775	36	5.37	2916	15	1.55	Positive
RF15	Positive	Positive	142933	23	78.57	59177	27	31.46	Positive
HA06	Positive	Positive	130149	13	71.54	61352	38	32.62	Positive
Experimental Cutoff			Avg (-)	Stdev (-)	Stdev*5 +avg	Avg (-)	Stdev (-)	Stdev*5 +avg	
			913	181	1819	917	193	1881	

2.8 Assay Protocols

The optimum assay protocol was determined by testing five protocols (10-10-10, 10-10-10 with PSW, 5-5-5, 5-5-5 with PSW, and 2-2-1) on the Theranos system. The best current assay conditions were used to test this. The 2-2-1 protocol did not give sufficient modulation. The 10x_PSW_5-5-5 gave the good modulation and low background, and because it also has a shorter assay run time, this protocol was used moving forward. Samples were run in triplicate with two tips per cartridge. Samples were run in triplicate with two tips per cartridge.

To determine the Antibody Index the following equation was used:

Avg RLU/(stdev(-)*5+avg(-))=Antibody Index

where stdev(-) and avg(-) are calculated using the negative samples within that condition
(This is excluding the HSV2 positive but HSV1 negative samples) Green (below 0.9 Index) is a considered a negative sample. Red (above 1.1 Index) is considered a positive sample. Yellow (between 0.9 and 1.1) is considered an equivocal sample.

Table [SEQ Table * ARABIC]: Assay Incubation and Post Sample Washing 10-10-10

			10-10-10				10-10-10-PSW			
Sample ID	Liaison Result	EuroImmun Result	Avg RLU	%CV	Antibody Index	Theranos Result	Avg RLU	%CV	Antibody Index	Theranos Result
LN01	Negative	Negative	758	30	0.30	Negative	1119	14	0.49	Negative
LN02	Negative	Negative	1324	17	0.52	Negative	1158	28	0.50	Negative
EN4	Negative	Negative	1083	17	0.43	Negative	892	28	0.39	Negative
EN5	Negative	Negative	803	26	0.32	Negative	680	35	0.30	Negative
BRN	Negative	Negative	459	16	0.18	Negative	488	34	0.21	Negative
Z21 (HSV2)	Negative	Negative	1201	18	0.47	Negative	1438	32	0.63	Negative
Z23 (HSV2)	Negative	Negative	1047	20	0.41	Negative	1047	15	0.46	Negative
BRP	Positive	Positive	9621	13	3.79	Positive	30446	24	13.24	Positive
SCP	Positive	Positive	6377	12	2.51	Positive	17921	32	7.80	Positive
Z15	Positive	Positive	1880	28	0.74	Negative	3273	25	1.42	Positive
Z25	Positive	Positive	4089	20	1.61	Positive	12844	15	5.59	Positive
Z33	Positive	Positive	3730	25	1.47	Positive	13995	15	6.09	Positive
Z39	Positive	Positive	87977	16	34.69	Positive	288692	11	125.58	Positive
RF15	Positive	Positive	17169	25	6.77	Positive	144162	13	62.71	Positive
HA06	Positive	Positive	39113	12	15.42	Positive	209277	19	91.03	Positive
Experimental Cutoff			Avg (-)	Stdev (-)	Stdev*5 +avg		Avg (-)	Stdev (-)	Stdev*5 +avg	
			953	297	2439		974	318	2562	

Table [SEQ Table * ARABIC]: Assay Incubation and Post Sample Washing 5-5-5

			5-5-5				5-5-5-PSW			
Sample ID	Liaison Result	EuroImmun Result	Avg RLU	%CV	Antibody Index	Theranos Result	Avg RLU	%CV	Antibody Index	Theranos Result
LN01	Negative	Negative	562	44	0.68	Negative	339	29	0.33	Negative
LN02	Negative	Negative	522	45	0.63	Negative	582	47	0.56	Negative
EN4	Negative	Negative	559	33	0.68	Negative	267	27	0.26	Negative
EN5	Negative	Negative	439	32	0.53	Negative	458	26	0.44	Negative
BRN	Negative	Negative	427	22	0.52	Negative	309	43	0.30	Negative
Z21 (HSV2)	Negative	Negative	453	19	0.55	Negative	304	30	0.29	Negative
Z23 (HSV2)	Negative	Negative	549	31	0.66	Negative	487	22	0.47	Negative
BRP	Positive	Positive	2411	30	2.92	Positive	6986	8	6.78	Positive
SCP	Positive	Positive	1741	25	2.11	Positive	5919	25	5.74	Positive
Z15	Positive	Positive	728	21	0.88	Negative	1236	17	1.20	Positive
Z25	Positive	Positive	1472	24	1.78	Positive	3027	28	2.94	Positive
Z33	Positive	Positive	1286	16	1.56	Positive	3868	25	3.75	Positive
Z39	Positive	Positive	18755	27	22.68	Positive	65455	11	63.50	Positive
RF15	Positive	Positive	7375	8	8.92	Positive	42193	14	40.93	Positive
HA06	Positive	Positive	16517	29	19.98	Positive	52113	27	50.56	Positive
Experimental Cutoff			Avg (-)	Stdev (-)	Stdev*5 +avg		Avg (-)	Stdev (-)	Stdev*5 +avg	
			502	60	801		392	117	978	

Table [SEQ Table * ARABIC]: Assay Incubation 2-2-1

Sample ID	Liaison Result	EuroImmun Result	Avg RLU	%CV	Antibody Index	Theranos Result
LN01	Negative	Negative	161	15.79	0.48	Negative
LN02	Negative	Negative	139	25.17	0.41	Negative
EN4	Negative	Negative	194	14.45	0.58	Negative
EN5	Negative	Negative	205	24.62	0.61	Negative
BRN	Negative	Negative	127	24.16	0.38	Negative
Z21	Negative	Negative	226	29.51	0.67	Negative
Z23	Negative	Negative	172	24.08	0.51	Negative
BRP	Positive	Positive	391	5.29	1.17	Positive
SCP	Positive	Positive	346	9.73	1.03	Equivocal
Z15	Positive	Positive	335	51.60	1.00	Equivocal
Z25	Positive	Positive	355	25.22	1.06	Equivocal
Z33	Positive	Positive	340	36.40	1.02	Equivocal
Z39	Positive	Positive	3285	16.34	9.82	Positive
RF15	Positive	Positive	1647	32.05	4.92	Positive
HA06	Positive	Positive	2052	14.23	6.13	Positive
Experimental Cutoff			Avg (-)	Stdev (-)	Stdev*5 +avg	
			175	36	354	

2.9 Cutoff Determination

To determine the Cutoff (CO) value, 35 samples were screened. Of these, 27 samples were tested negative via predicate method. The average RLU value and standard deviation of these 27 negative samples were used to calculate the cutoff and the Theranos Index. The cutoff was determined to be an RLU 2171 and was used to determine test results in in all following experiments. Samples were run in triplicate with two tips per cartridge.

The calculation of the CO was determined using the following:

Cutoff = Mean RLU + (Standard Deviation)*10

The Theranos index was calculated by using the established cutoff and the following equation:

RLU/Cutoff=Theranos Index

Green (below 0.9 Index) is a considered a negative sample. Red (above 1.1 Index) is considered a positive sample. Yellow (between 0.9 and 1.1) is considered an equivocal sample.

Table [SEQ Table * ARABIC]: Assay Cutoff Determination

Sample ID	Liaison Result	EuroImmun Result	Avg RLU	%CV	Theranos Index	Theranos Result
LN1	Negative	Negative	620	31.0	0.29	Negative
LN2	Negative	Negative	504	18.6	0.23	Negative
LN3	Negative	Negative	812	14.9	0.37	Negative
LN4	Negative	Negative	506	25.7	0.23	Negative
LN5	Negative	Negative	483	6.1	0.22	Negative
EN9	Negative	Negative	586	37.2	0.27	Negative
EN10	Negative	Negative	607	12.6	0.28	Negative
EN11	Negative	Negative	675	19.3	0.31	Negative
EN15	Negative	Negative	400	14.4	0.18	Negative
EN16	Negative	Negative	555	18.4	0.26	Negative
EN17	Negative	Negative	682	12.7	0.31	Negative
EN18 (HSV2)	Negative	Positive	480	18.5	0.22	Negative
EN21	Negative	Negative	600	17.7	0.28	Negative
EN23	Negative	Negative	726	15.7	0.33	Negative
EN25	Negative	Negative	698	11.2	0.32	Negative
EN26	Negative	Negative	374	23.4	0.17	Negative
EN27	Negative	Negative	818	33.3	0.38	Negative
EN28	Negative	Negative	664	27.5	0.31	Negative
MEB1	Negative	Negative	834	25.8	0.38	Negative
MEB2	Negative	Negative	987	16.0	0.45	Negative
MEB3	Negative	Negative	659	28.7	0.30	Negative
MEB4	Negative	Negative	916	16.4	0.42	Negative
FEB1	Negative	Negative	663	19.1	0.31	Negative
FEB2	Negative	Negative	614	26.5	0.28	Negative
FEB3 (HSV2)	Negative	Positive	540	21.9	0.25	Negative
FEB4	Negative	Negative	456	18.5	0.21	Negative
FEB5	Negative	Negative	432	28.6	0.20	Negative
			Avg (RLU)	Stdev (RLU)	Stdev*10 +avg (Cutoff)	
			626	155	2023	

2.10 Hematocrit and anti-coagulant Effects

The hematocrit effect was tested by comparing results from whole blood and plasma in EDTA anti-coagulant. Five male and 5 female samples were used. The hematocrit effect was within a normal range. To test the effect of anti-coagulant, matched Li heparin, EDTA and serum samples were tested. The Li Heparin samples gave high RLUs in the Female samples, but when retested they gave similar results to the EDTA matched samples. The effects of anti-coagulants were negligible. Samples were run in triplicate with two tips per cartridge.

The Theranos index was calculated by using the established cutoff and the following equation:

RLU/2023=Theranos Index

Green (below 0.9 Index) is a considered a negative sample. Red (above 1.1 Index) is considered a positive sample. Yellow (between 0.9 and 1.1) is considered an equivocal sample.

Table [SEQ Table * ARABIC]: Hematocrit Effects

	EDTA Plasma Result		EDTA Whole Blood				EDTA Plasma			
Sample ID	Liaison Result	EuroImmun Result	Avg RLU	%CV	Theranos Index	Theranos Result	Avg RLU	%CV	Theranos Index	Theranos Result
MEB1	Negative	Negative	669	34	0.31	Negative	707	37	0.33	Negative
MEB2	Negative	Negative	373	31	0.17	Negative	987	16	0.45	Negative
MEB3	Negative	Negative	487	18	0.22	Negative	659	29	0.30	Negative
MEB4	Negative	Negative	646	28	0.30	Negative	1041	30	0.48	Negative
MEB5	Positive	Positive	97170	15	44.76	Positive	194595	14	89.63	Positive
FEB1	Negative	Negative	563	25	0.26	Negative	663	19	0.31	Negative
FEB2	Negative	Negative	478	25	0.22	Negative	568	31	0.26	Negative
FEB3	Negative	Negative	664	15	0.31	Negative	540	22	0.25	Negative
FEB4	Negative	Negative	482	14	0.22	Negative	413	31	0.19	Negative
FEB5	Negative	Negative	681	20	0.31	Negative	432	29	0.20	Negative

Table [SEQ Table * ARABIC]: Anti-coagulant Effects

	EDTA Plasma Result		EDTA Heparin Plasma				Li Heparin Plasma				Serum			
Sample ID	Liaison Result	EuroImmun Result	Avg RLU	%CV	Theranos Index	Theranos Result	Avg RLU	%CV	Theranos Index	Theranos Result	Avg RLU	%CV	Theranos Index	Theranos Result
MEB1	Negative	Negative	707	37	0.33	Negative	621	23	0.29	Negative	641	20	0.30	Negative
MEB2	Negative	Negative	987	16	0.45	Negative	548	24	0.25	Negative	625	16	0.29	Negative
MEB3	Negative	Negative	659	29	0.30	Negative	503	20	0.23	Negative	595	5	0.27	Negative
MEB4	Negative	Negative	1041	30	0.48	Negative	480	29	0.22	Negative	634	19	0.29	Negative
MEB5	Positive	Positive	194595	14	89.63	Positive	131155	28	60.41	Positive	136019	24	62.65	Positive
FEB1	Negative	Negative	663	19	0.31	Negative	497	18	0.23	Negative	497	18	0.23	Negative
FEB2	Negative	Negative	568	31	0.26	Negative	583	26	0.27	Negative	583	26	0.27	Negative
FEB3	Negative	Negative	540	22	0.25	Negative	488	21	0.22	Negative	488	21	0.22	Negative
FEB4	Negative	Negative	413	31	0.19	Negative	565	31	0.26	Negative	565	31	0.26	Negative
FEB5	Negative	Negative	432	29	0.20	Negative	377	36	0.17	Negative	377	36	0.17	Negative

2.11 Effect of Interfering Matrixes

Icteric, Lipemic, and Hemolysed samples were tested and compared to the predicate method. All but 2 samples matched; one icteric, and one hemolysed were both found to be positive on the predicate method but negative on the Theranos platform. Samples were run in triplicate with two tips per cartridge.

The Theranos index was calculated by using the established cutoff and the following equation:

RLU/2023=Theranos Index

Green (below 0.9 Index) is a considered a negative sample. Red (above 1.1 Index) is considered a positive sample. Yellow (between 0.9 and 1.1) is considered an equivocal sample.

Table [SEQ Table * ARABIC]: Interfering Matrixes

Sample Type	Sample ID	Liaison Result	Avg RLU	%CV	Theranos Index	Theranos Result
Icteric	I24	Negative	496	33	0.23	Negative
Icteric	I36	Negative	1631	15	0.75	Negative
Icteric	I302	Negative	1196	18	0.55	Negative
Icteric	I447	Negative	867	11	0.40	Negative
Icteric	I525	Positive	483	16	0.22	Negative
Lipemic	L683	Positive	15452	24	7.12	Positive
Lipemic	L684	Positive	115451	9	53.18	Positive
Lipemic	L685	Negative	647	13	0.30	Negative
Lipemic	L687	Positive	11039	26	5.08	Positive
Lipemic	L689	Positive	3509	21	1.62	Positive
Hemolysed	H3	Positive	632	14	0.29	Negative
Hemolysed	H4	Positive	28050	20	12.92	Positive
Hemolysed	H7	Positive	82947	13	38.21	Positive
Hemolysed	H8	Positive	50494	19	23.26	Positive
Hemolysed	H9	Positive	22186	20	10.22	Positive

2.12 Cross Reactivity

The assay was tested for potential cross reactivity from HAMA and RF samples. HAMA and RF samples that were run on the Theranos system correlated well with the Liaison results. Samples were run in triplicate with two tips per cartridge.

The Theranos index was calculated by using the established cutoff and the following equation:

RLU/2023=Theranos Index

Green (below 0.9 Index) is a considered a negative sample. Red (above 1.1 Index) is considered a positive sample. Yellow (between 0.9 and 1.1) is considered an equivocal sample.

Table [SEQ Table * ARABIC]: RF and HAMA

HAMA Samples						
Sample ID	Liaison Result	EuroImmun Result	Avg RLU	%CV	Theranos Index	Theranos Result
HA01	Positive	Positive	28872	20	13.30	Positive
HA02	Equivocal	Negative	803	15	0.37	Negative
HA03	Positive	Positive	74319	20	34.23	Positive
HA04	Positive	Positive	35784	17	16.48	Positive
HA05	Positive	Positive	16537	30	7.62	Positive
HA06	Positive	Positive	105809	12	48.74	Positive
HA07	Positive	Positive	39186	28	18.05	Positive
HA08	Negative	Negative	639	7	0.29	Negative
HA09	Positive	Positive	111421	27	51.32	Positive
HA10	Positive	Positive	43718	24	20.14	Positive
HA11	Positive	Positive	10922	29	5.03	Positive
RF Samples						
Sample ID	Liaison Result	EuroImmun Result	Avg RLU	%CV	Theranos Index	Theranos Result
RF01	Positive	Positive	22574	35	10.40	Positive
RF02	Positive	Positive	113377	25	52.22	Positive
RF03	Positive	Positive	23964	27	11.04	Positive
RF04	Positive	Positive	19641	24	9.05	Positive
RF05	Positive	Positive	76452	22	35.21	Positive
RF06	Negative	Positive	870	30	0.40	Negative
RF07	Positive	Positive	6055	28	2.79	Positive
RF08	Positive	Positive	38127	27	17.56	Positive
RF09	Positive	Positive	82715	24	38.10	Positive
RF10	Positive	Positive	72766	21	33.52	Positive
RF11	Positive	Positive	18828	26	8.67	Positive
RF12	Positive	Positive	25503	28	11.75	Positive
RF13	Positive	Positive	26212	17	12.07	Positive
RF14	Positive	Positive	51008	20	23.50	Positive
RF15	Positive	Positive	89713	16	41.32	Positive

Table [SEQ Table * ARABIC]: Cross Reactive Samples

Sample ID	CLIA	Mean	CV %	Theranos Index	Theranos Result
BRN	Negative	509	17	0.25	Negative
BRP	Positive	23349	21	11.54	Positive
Trep IgG	Positive	15528	27	7.68	Positive
CMV IgG	Positive	9808	21	4.85	Positive
Rubella IgG	Negative	896	12	0.44	Negative
Toxo IgG	Positive	34307	19	16.96	Positive
VZV IgG	Positive	20355	31	10.06	Positive
EBV IgG	Positive	9820	16	4.85	Positive
Ct/Ng	Negative	488	21	0.24	Negative
Toxo IgM	Positive	33981	26	16.80	Positive
EBV IgM	Positive	2039	13	1.01	Equivocal
CMV IgM	Positive	2171	23	1.07	Equivocal
HPV IgG	Not tested	1902	5	0.94	Equivocal
C. albicans	Negative	193	13	0.10	Negative

2.13 Commercial Kit Clinical Correlation

The Theranos assay was tested using samples provided by BioReclamation, Zeptometrix, and SeraCare. The results were compared to the predicate method and a secondary kit. Samples were run in duplicate with two tips per cartridge.

The Theranos index was calculated by using the established cutoff and the following equation:

RLU/2023=Theranos Index

Green (below 0.9 Index) is a considered a negative sample. Red (above 1.1 Index) is considered a positive sample. Yellow (between 0.9 and 1.1) is considered an equivocal sample.

Table [SEQ Table * ARABIC]: Commercial Kit Correlation

Liaison HSV2 Result	Sample ID	Liaison Result	EuroImmun Result	Theranos Index	Theranos Result	Avg RLU	%CV
Negative	BR101	Positive	Positive	29.89	Positive	64884	12
Negative	BR103	Positive	Positive	54.97	Positive	119350	14
Negative	BR104	Positive	Positive	6.03	Positive	13083	10
Negative	BR105	Positive	Positive	3.80	Positive	8239	24
Negative	BR106	Positive	Positive	4.37	Positive	9484	22
Negative	BR107	Positive	Positive	8.77	Positive	19033	14
Negative	BR109	Positive	Positive	87.14	Positive	189177	8
Negative	BR111	Positive	Positive	8.38	Positive	18202	15
Negative	BR112	Positive	Positive	3.30	Positive	7166	25
Negative	BR114	Positive	Positive	35.29	Positive	76604	31
Negative	BR115	Positive	Positive	11.85	Positive	25732	14
Negative	BR116	Positive	Positive	13.48	Positive	29267	23
Negative	BR117	Positive	Positive	102.75	Positive	223065	15
Negative	SC01	Positive	Positive	6.94	Positive	15077	7
Negative	SC02	Positive	Positive	9.85	Positive	21382	12
Negative	SC07	Positive	Positive	30.62	Positive	66467	21
Negative	SC10	Positive	Positive	12.88	Positive	27962	5

Negative	SC16	Positive	Positive	17.96	Positive	38991	16
Negative	SC17	Positive	Positive	18.07	Positive	39230	24
Negative	SC18	Positive	Positive	21.91	Positive	47567	19
Negative	Z01	Positive	Positive	15.46	Positive	33567	7
Negative	Z06	Positive	Positive	10.78	Positive	23404	30
Negative	Z11	Positive	Positive	9.28	Positive	20157	14
Negative	Z13	Positive	Positive	22.72	Positive	49329	2
Negative	Z14	Positive	Positive	15.58	Positive	33823	20
Negative	Z20	Positive	Positive	9.50	Positive	20622	8
Negative	Z26	Positive	Positive	17.19	Positive	37323	7
Negative	Z27	Positive	Positive	19.34	Positive	41986	24
Negative	Z28	Positive	Positive	15.89	Positive	34490	14
Negative	Z29	Positive	Positive	10.31	Positive	22389	17
Negative	Z30	Positive	Positive	16.09	Positive	34934	20
Negative	Z31	Positive	Positive	19.15	Positive	41575	27
Negative	Z32	Positive	Positive	10.14	Positive	22014	10
Negative	Z34	Positive	Positive	10.91	Positive	23687	5
Negative	Z35	Positive	Positive	26.49	Positive	57506	23
Negative	Z36	Positive	Positive	8.24	Positive	17888	15
Negative	MT01	Positive	Positive	6.73	Positive	14616	9
Negative	MT21	Positive	Positive	15.73	Positive	34156	8
Positive	BR108	Positive	Positive	4.19	Positive	9104	30
Positive	BR118	Positive	Positive	0.69	Negative	1491	24
Positive	BR201	Positive	Positive	20.81	Positive	45186	8
Positive	BR206	Positive	Positive	6.59	Positive	14297	15
Positive	BR210	Positive	Positive	6.20	Positive	13467	16
Positive	BR212	Positive	Positive	7.65	Positive	16619	33
Positive	BR214	Positive	Positive	17.44	Positive	37871	29
Positive	BR216	Positive	Positive	12.51	Positive	27157	16
Positive	SC06	Positive	Positive	11.22	Positive	24364	34
Positive	SC08	Positive	Positive	1.11	Positive	2404	18
Positive	SC09	Positive	Positive	4.53	Positive	9832	28
Positive	SC12	Positive	Positive	3.53	Positive	7670	24
Positive	SC13	Positive	Positive	8.83	Positive	19164	14
Positive	SC14	Positive	Positive	63.40	Positive	137632	11
Positive	MT03	Positive	Negative	0.41	Negative	900	15
Positive	MT09	Positive	Positive	16.23	Positive	35244	34
Positive	MT11	Positive	Positive	0.93	Equivocal	2018	16
Positive	MT15	Positive	Positive	1.87	Positive	4062	15
Positive	MT25	Positive	Positive	11.30	Positive	24524	30
Positive	BR113	Negative	Positive	0.25	Negative	540	25
Positive	BR202	Negative	Equivocal	0.29	Negative	631	28
Positive	BR204	Negative	Equivocal	0.32	Negative	695	24
Positive	BR207	Negative	Positive	0.38	Negative	824	13
Positive	BR208	Negative	Positive	0.55	Negative	1202	15
Positive	BR209	Negative	Positive	0.90	Negative	1951	11
Positive	BR211	Negative	Equivocal	0.22	Negative	484	32
Positive	BR215	Negative	Equivocal	0.51	Negative	1117	21
Positive	SC03	Negative	Positive	0.31	Negative	668	28
Positive	SC04	Negative	Positive	0.35	Negative	768	15
Positive	SC05	Negative	Positive	0.19	Negative	406	14
Positive	SC11	Negative	Positive	0.25	Negative	545	13
Positive	SC15	Negative	Positive	0.27	Negative	594	19
Positive	Z16	Negative	Positive	0.24	Negative	515	34

Positive	Z22	Negative	Negative	0.30	Negative	652	34
Positive	Z24	Negative	Positive	0.22	Negative	472	32
Positive	Z38	Negative	Positive	0.16	Negative	354	1
Positive	Z40	Negative	Negative	0.56	Negative	1226	10
Positive	MT07	Negative	Positive	0.31	Negative	672	10
Positive	MT08	Negative	Positive	0.55	Negative	1202	18
Positive	MT13	Negative	Positive	0.27	Negative	579	21
Positive	MT16	Negative	Equivocal	0.35	Negative	750	23
Positive	MT24	Negative	Negative	0.29	Negative	625	21

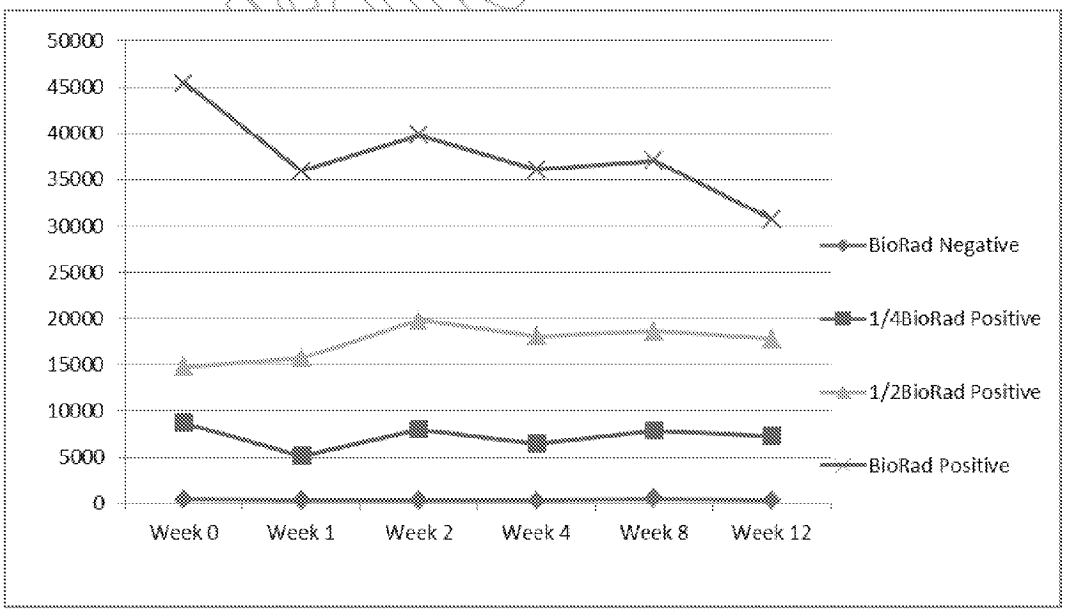
2.14 Stability over 12 weeks

The BioRad ToRCH negative and positive was used to test stability of the coated surface and the working solution of detection stored at 4°C at 0,1,2,4,8,and 12 weeks. The ToRCH positive control was diluted to 1/4X and 1/2X using the Surmodics Protein Free assay diluent before adding it to the sample well. After 12 weeks the reagents have shown to be stable.

Table [SEQ Table * ARABIC]: Commercial Kit Correlation

	Week 0	Week 1	Week 2	Week 4	Week 8	Week 12		Average	%CV
BioRad Negative	442	348	366	320	542	359		396	21
1/4BioRad Positive	8695	5136	8027	6448	7870	7282		7243	18
1/2BioRad Positive	14764	15718	19799	18066	18613	17768		17455	11
BioRad Positive	45443	35933	39833	36065	37106	30744		37520	13

Figure [SEQ Figure * ARABIC]: Reagent stability over 12 weeks



3 ASSAY SUMMARY

Table [SEQ Table * ARABIC]: Development Summary

Capture Antigen	HSV1 antigen @ 2.5 ug/mL
Wash Buffer	Tris Buffered Saline with Tween 20
Assay Buffer	Surmodics Assay Diluent (Protein Free)
Edison Protocol	10x_PSW_5-5-5
Detector Antibody	Novus clone 2C11 @ 100 ng/mL
Detector Stabilizer	Theranos AP Conjugate Stabilizer
Sample Dilution	10x

4 CLINICAL EVALUATION

To further validate the assay, more normal and positive samples need to be tested. The cutoff value needs to be verified by screening more normal patients. At least 100 or more patients are needed for clinical evaluation.