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Vancomycin
Assay Development Report

Theranos, Inc.

Sept 12, 2012

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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 detect Vancomycin in human serum, plasma or whole blood. The assay has a reportable range of 5ug/ml to 100ug/ml, and is calibrated to the ACS Centaur system.

1.1.1 Materials and methods

[TC "Materials and Methods" \f C \l "1"]
In this competitive assay format, the Vancomycin (Vanco) in the sample competes with alkaline phosphatase (AP) for binding to the anti-Vanco antibody. Briefly, an anti-sheep antibody serves as the capture surface for the competitive ELISA. Alkaline Phosphatase-labeled vancomycin (Vanco-AP) serves as the tracer. The mixture of sample, Sheep Anti-Vanco Antibody and Vanco-AP is incubated with the capture surface for 5 minutes followed by six wash steps. Then the alkaline phosphatase substrate is incubated with the capture surface for 5 minutes. The resulting chemiluminescence is read in Relative Light Units (RLU).

The key materials that were used for this assay are listed in Table 1.

Table [SEQ Table * ARABIC]: Materials

Name	Supplier	Catalog #
Vancomycin	Sigma	V8138-16
Rabbit Anti Sheep	Mybiosource	MBS620563
Vanco Alkaline Phosphatase Conjugate	Yj Bioproducts	K01702
Phospho Glo Substrate	In House	T-AlkP-SB01-004
Super Block Diluent	Pierce	37535
Carbonate-bicarbonate buffer	Sigma	C3041

Table [SEQ Table * ARABIC]: List of Antibodies-Set 1

Antibody#	Vendor	Catalog #	Clone /Info
1	MyBiosource	MBS315348	PAb-Rabbit
2	MyBiosource	MBS531707	MAb
3	MyBiosource	MBS620563	PAb-Sheep
4	MyBiosource	MBS535003	PAb-Sheep
5	MyBiosource	MBS623181	PAb-Rabbit
6	MyBiosource	MBS222323	PAb-Sheep
7	Fitzgerald	10-V10A	MAb
8	US Biological	V2050	PAb-Sheep

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

2.1 Antibody Screening (MTP) Set 1

The initial screens of antibodies were performed on Nunc 384 microtiter (MTP) plates. Antibodies were screened to determine ability to bind to Vanco-AP and to observe any modulation in the presence of the Vanco analyte (Table 2, Table 3). MTP plates were directly coated with antibodies in carbonate-bicarbonate buffer at the different dilutions/concentrations. A mixture containing Vanco-AP (1:10K final after dilution) and Vanco analyte (0 ug/mL or 50 ug/mL) in Low BSA buffer (0.03% BSA in TBS) was added to these wells. This mixture was incubated for 10 minutes followed by three wash steps. Alkaline phosphatase substrate was subsequently added for 10 minutes and the resulting chemiluminescence was read in Relative Light Units (RLU). Most of the antibodies screened showed strong ability to bind Vanco-AP. Most of the antibodies showed dose modulation in presence of 50 ug/mL of Vancomycin (comparing RLU data with no Vancomycin added). Antibody #2, #7 and #8 were eliminated for further testing on the Theranos system due to lack of binding to the Vanco-AP[TC "Detection Antibody Conjugate Verification" \f C \l "1"]. Antibodies #3, #4 and #6 showed the best modulation.

Table [SEQ Table * ARABIC]: Antibody Screen (MTP)

		0 µg/ml Vanco		50 µg/ml Vanco		
Antibody ID	[Ab](µg/ml or dil from stock)	Mean RLU	CV%	Mean RLU	CV%	Modulation b/w 0 µg/ml and 50 µg/ml Vanco
1	10	37380	2.5	4524	9.7	8.3
	1	1047	4.1	433	5.2	2.4
	0.1	493	3.8	347	14.2	1.4
2	10	630	18.3	504	11.9	1.2
	1	439	6.7	364	4.3	1.2
	0.1	404	9.2	372	6.2	1.1
3	1:1000	220701	3.7	1120	7	197.1
	1:10000	81243	3.2	604	12	134.6
	1:100000	3099	4	380	19.2	8.2
4	1:1000	213520	2.8	1134	12.9	188.2
	1:10000	65090	2.2	587	11	111.0
	1:100000	2176	0.9	501	19.2	5.2

Table 3: Antibody Screen (MTP) contd.

		0 µg/ml Vanco		50 µg/ml Vanco		
Antibody ID	[Ab](µg/ml or dil from stock)	Mean RLU	CV%	Mean RLU	CV%	Modulation b/w 0 µg/ml and 50 µg/ml Vanco
5	1:1000	17865	1.5	2850	7.3	6.3
	1:10000	1035	15	516	23.8	2.0
	1:100000	472	7.5	415	3.4	1.1
6	1: 1000	114371	2.2	771	6.9	148.4
	1:10000	32915	4.7	482	13.8	68.3
	1:100000	932	8.6	383	11.9	2.4
7	10	480	6.2	489	22.4	1.0
	1	410	4.9	417	4.7	1.0
	0.1	463	4.8	476	17.8	1.2
8	10	498	2.6	557	15.4	0.9
	1	431	17.9	429	12.4	1.0
	0.1	498	14.5	421	15.3	1.0

2.2 Competitive Assay Screen on the Theranos System

To determine the optimal capture antibody on the Theranos 3.0 system, screening was performed with antibodies #3, #4 and #6. The final conditions included a 10 fold sample dilution of the serum calibrators in Low BSA assay buffer (0.03% BSA in TBS). Both antibody and Vanco-AP were maintained at 1:10K final dilutions in final sample mixture. Out of all these antibodies screened Antibody #3 was selected as the best antibody based on modulation and sensitivity and was used for further optimization. Antibody #4 and #6 are tentative back-up antibodies.

Table [SEQ Table * ARABIC]: Competitive Assay Screen on the Theranos system

Antibody #	[Vanco], µg/ml	Mean RLU	CV%	Mod	Mod b/w pts
3	75.0	8251	5.6	42.7	1.2
	50	9632	9.9		1.3
	25	12661	15.7		1.8
	10	22733	14.5		15.5
	0	352541	8.1		
4	75.0	9810	2.1	16.4	1.0
	50	9525	8.6		1.3
	25	12845	9.1		1.4
	10	18524	15.2		1.7
	5	30603	14.5		5.2
	0	160401	17.3		

Table 4: Competitive Assay Screen on the Theranos system contd.

Antibody #	[Vanco], µg/ml	Mean RLU	CV%	Mod	Mod b/w pts
6	75	7041	12.4	18.8	1.2
	50	8546	6.6		0.9
	25	7980	21.2		1.3
	10	10769	20.5		1.3
	5	14041	8.6		9.4
	0	132303	9.1		

2.3 Training Set

Few clinical samples were evaluated with the selected antibody to make sure the results correlated well with the reported values. Final optimized loading levels of Vanco-AP were determined to be 1:1K dilution from stock, while the antibody loading concentration was 1:10K dilution from stock. Vanco-AP gets diluted an additional 10 fold while the sample gets diluted 50 fold in the final sample mixture. Correlation looked excellent. Optimizations were necessary to get good sensitivities for this antibody.

Table [SEQ Table * ARABIC]: Standard Curve-Training Set

	Signal (RLU)				Back-Calculated		
[Vanco], µg/ml	Mean RLU	CV %	Mod	Mod b/w pts	Mean Conc. µg/ml	CV%	% Recovery
100	12402	18.6	38.0	1.3	99.1	38.2	99
75	15715	9.1		0.9	58.3	16.6	78
50	14096	15.0		1.7	74.9	33.9	150
25	24289	12.6		1.8	27.3	24.9	109
10	44285	18.4		1.5	10.3	29.2	103
5	67491	13.4		7.0	5.2	19.9	103
0	471839	20.1			0.1		

Figure [SEQ Figure * ARABIC]: Standard Curve-Training Set

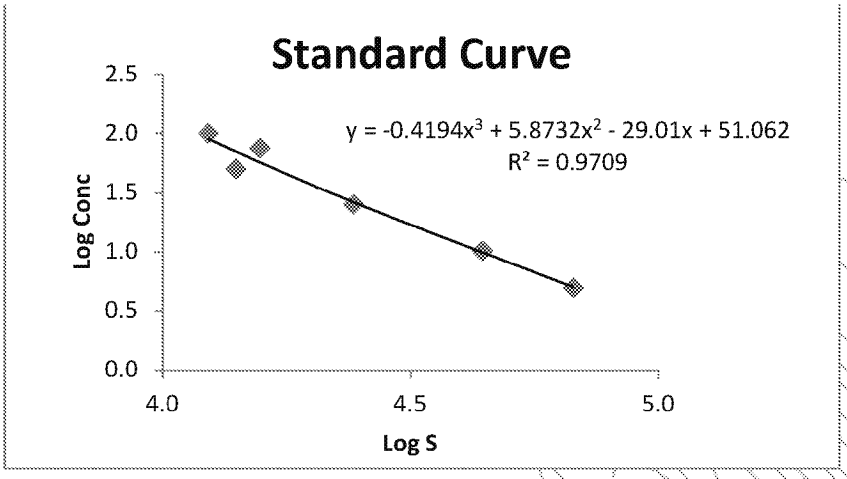
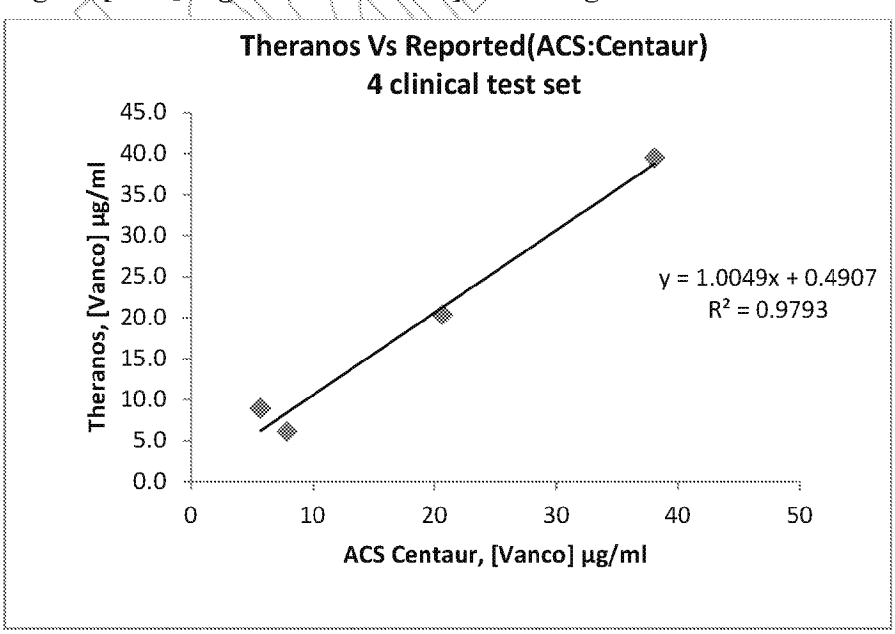


Table [SEQ Table * ARABIC]: Training Set

	Signal(RLU)		Back-Calculated		Reported-ACS Centaur
Sample ID	Mean RLU	CV%	Mean Conc. µg/ml	CV%	[Vanco] µg/ml
11	48419	14.3	8.9	27.0	5.7
13	29367	20.0	20.3	29.6	20.7
17	62582	18.7	6.1	34.3	7.9
33	19725	14.1	39.4	24.6	38.1

Figure [SEQ Figure * ARABIC]: Training Set- Correlation on the Theranos system



2.4 Titration of Reagents

To improve modulation and sensitivity of the assay with antibody #3, both antibody and Vanco-AP levels were titrated on the Theranos system. Final optimized loading levels of both Vanco-AP and antibody #3 were determined to be 1:1K dilution from stock. In the final mixture, both antibody and Vanco-AP gets further diluted 10 fold while sample gets diluted 50 fold.

Table [SEQ Table * ARABIC]: Titration of Reagents

Loading Condition: Dilution from stock		[Vanco], µg/ml	Mean RLU	CV%	Mod	Mod b/w pts
Ab 3	1:1K	100	12402	18.6	38.0	1.3
AP	1:1K	75	15715	9.1		0.9
		50	14096	15.0		1.7
		25	24289	12.6		1.8
		10	44285	18.4		1.5
		5	67491	13.4		7.0
		0	471839	20.1		
Ab 3	1:1K	100	7367	19.0	8.5	0.7
AP	1:10K	75	5204	15.4		1.2
		50	6274	9.8		1.2
		25	7720	16.7		1.3
		10	9677	16.4		6.5
		0	62931	15.3		
Ab 3	1:10K	100	1398	14.6	26.1	1.1
AP	1:10K	75	1471	14.9		1.3
		50	1940	28.4		0.9
		25	1685	20.0		1.6
		10	2748	16.5		13.3
		0	36437	22.8		

2.5 Sample Dilution

Final sample dilutions of 1:50, 1:100 were tested on the Theranos system. A final sample dilution of 1:50 was chosen for this assay as it has better modulation between the key ranges for this assay. In the final mixture, both antibody and Vanco-AP are maintained at 1:10K dilution from stock.

Table [SEQ Table * ARABIC]: Sample dilution

Sample Dilution	[Vanco], ug/ml	Mean RLU	CV%	Overall Modulation	Modulation between 50ug/ml and 10ug/ml Vanco
1:50	75	10502	12.2	38.6	
	50	12070	10.1		3.1
	25	22422	14.8		
	10	37615	13.2		
	0	405397	15.9		
1:100	75	11946	23.2	24.3	
	50	23180	12.1		2.7
	25	29763	10.8		
	10	63213	16.8		
	0	289774	15.8		

2.6 Alkaline Phosphatase Stabilizer

A commercial stabilizer and an in-house AP stabilizer was tested as the Vanco-AP conjugate diluent. The in-house AP stabilizer consisted of 0.1mM Zn^{2+} and 5mM Mg^{2+} in Low BSA buffer (0.03% BSA in TBS). Final optimized loading levels of both Vanco-AP and antibody #3 were determined to be 1:1K dilution from stock. In the final mixture, both antibody and Vanco-AP gets further diluted 10 fold while sample gets diluted 50 fold. The commercial stabilizer provided the best modulation and was chosen as the final condition.

Table [SEQ Table * ARABIC]: Alkaline Phosphatase Stabilizer

Stabilizer	[Vanco], µg/ml	Mean RLU	CV%	Mod
InHouse AP Stabilizer	100	726	19.1	65.4
	0	47487	25.9	
Stabilzyme AP	100	628	16.5	76.5
	0	48098	4.8	

2.7 Cross Reactivity and Interference

To test cross reactivity and interference, different substances that could potentially cross react or interfere with Vancomycin were tested. The test levels chosen for each test substance were based on the therapeutic drug levels and an excess of the clinical ranges. The recovery of Vancomycin was calculated on the control standard curve. No cross reactivity was observed with any of the substances tested. Substances such as sodium salicylate and methotrexate were also tested and no cross reactivity/interference were observed (data not shown).

Table [SEQ Table * ARABIC]: Standard Curve-Cross reactivity and Interference

	Signal (RLU)		Back-Calculated		
[Vanco] µg/ml	Mean RLU	CV%	Mean Conc. µg/ml	CV%	% Recovery
100	4716	19.1	96.9	21.9	97
75	5395	12.0	80.8	13.8	108
50	7402	15.4	55.0	19.4	110
25	14646	3.9	21.8	5.4	87
10	24079	6.8	11.2	8.4	112
5	44812	1.9	4.9	2.4	98
0	222739	8.0			

Figure [SEQ Figure * ARABIC]: Standard Curve-Cross reactivity and Interference

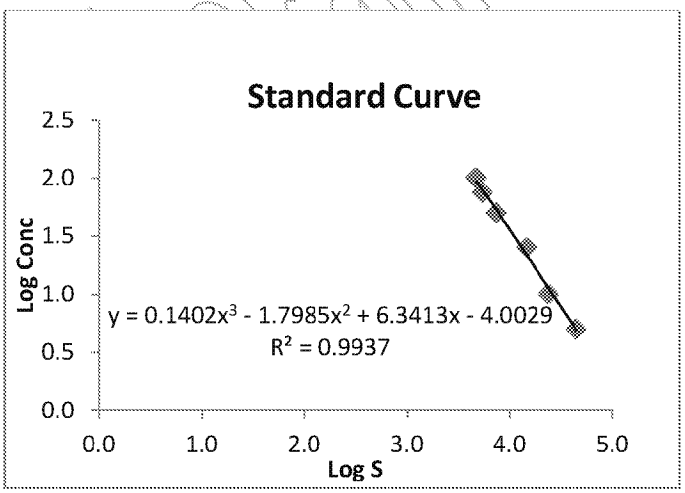


Table [SEQ Table * ARABIC]: Cross Reactivity and Interference

			Signal (RLU)		Back-calculated			
Analytes	Level - µg/ml	[Vanco] µg/ml	Mean RLU	CV%	Mean Conc. µg/ml	CV%	% Cross Reactivity	% Recovery
Control		0	293542	19.0	OORL			
		25	13745	11.0	24.1	15.0		
Teicoplanin	500	0	317390	19.9	OORL			
		25	13534	12.9	24.8	20.3	0.03	99
Tobramycin	500	0	330190	11.6	OORL			
		25	13603	10.9	24.4	12.6	0.12	98
Sisomycin	500	0	337700	15.9	OORL			
		25	13912	26.2	24.9	27.1	0.02	100
Kanamycin	500	0	285908	35.3	OORL			
		25	14273	8.2	22.8	11.2	0.45	91
Ampicilin	500	0	336249	9.5	OORL			
		25	14046	18.0	24.0	23.0	0.20	96

2.8 Optimizing Tip Coating Buffer

Super block (TBS) buffer is an albumin free buffer which has shown to be effective by increasing signal to background noise in some cases. Superblock showed a slight improvement in sensitivity of this assay in the key ranges on the Theranos system and was used as the coating buffer. Final optimized loading levels of both Vanco-AP and antibody #3 were determined to be 1:1K dilution from stock. In the final sample mixture, both antibody and Vanco-AP gets further diluted 10 fold while sample gets diluted 50 fold.

Table [SEQ Table * ARABIC]: Optimizing Tip Coating Buffer

Coating buffer	[Vanco], µg/ml	Mean RLU	CV%	Mod	Fold diff between 50 µg/ml and 5 µg/ml
Super block	50.0	15003	10.6	36.3	5.4
	5.0	81252	11.2		
	0.0	545030	6.5		
Regular 3% BSA	50.0	8986	16.5	39.9	4.6
	5.0	41166	14.9		
	0.0	358733	14.1		

2.9 Calibration Verification

Three level serum controls from BioRad (Liquichek Immunoassay Plus) were obtained and measured on the Theranos System. Results were compared to those reported by the clinical analyzers. The controls correlate very well with the Roche Cobas system. The serum calibrators were also run on the Siemens Advia 1800 in house. These also correlated well with the nominal values. Final optimized loading levels of both Vanco-AP and antibody #3 were determined to be 1:1K dilution from stock. In the final sample mixture, both antibody and Vanco-AP gets further diluted 10 fold while sample gets diluted 50 fold.

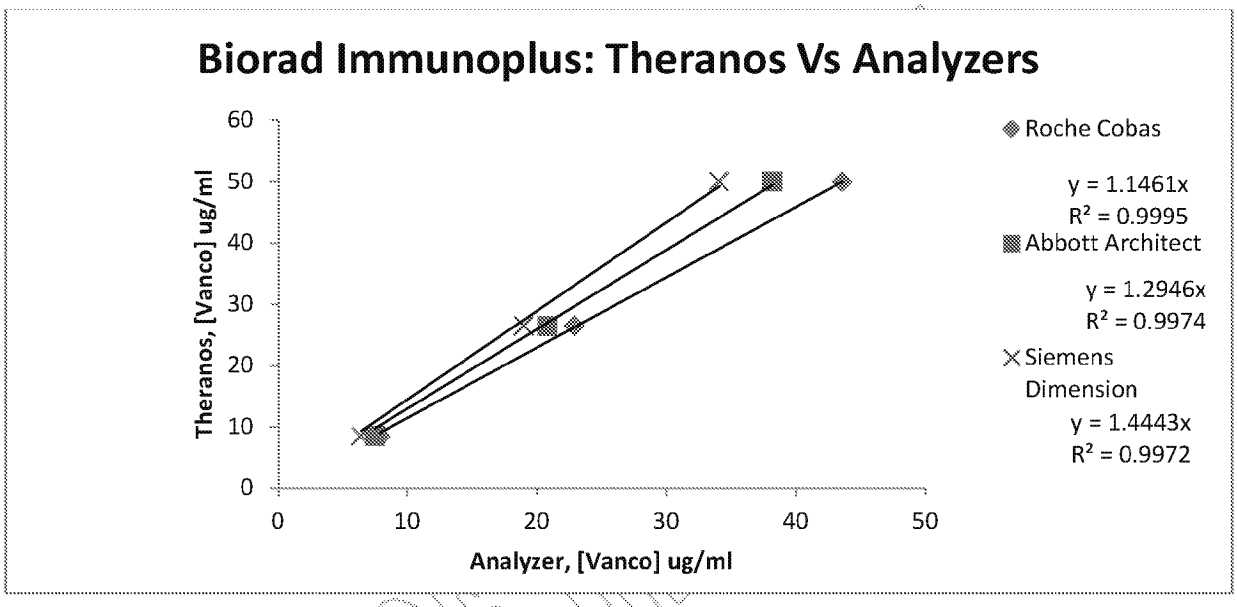
Table [SEQ Table * ARABIC]: Standard Curve-Calibration Verification

	Signal (RLU)		Back-calculated		
[Vanoc] µg/ml	Mean RLU	CV%	Mean Conc. µg/ml	CV%	% Recovery
100	10671	8.5	98.6	5.1	99
75	16036	11.6	68.0	13.7	91
50	17595	10.1	60.5	13.4	121
25	34541	12.3	21.8	21.5	87
10	50737	13.7	11.7	19.7	117
5	93800	12.4	5.0	14.4	100
0	642408	5.6			

Table [SEQ Table * ARABIC]: BioRad Controls evaluation on the Theranos system

Biorad	Mean [Vanco] µg/ml			
Control	Theranos 3.0	Roche Cobas Integra	Abbott Architect	Siemens Dimension
Level 1	8.4	7.87	7.5	6.44
Level 2	26.4	22.9	20.8	19
Level 3	50	43.6	38.2	34.1

Figure [SEQ Figure * ARABIC]: BioRad Controls evaluation on the Theranos system



2.10 Clinical Correlation

Serum samples from patients with different levels of Vancomycin were tested. A set of 21 clinicals samples across the Vancomycin range were selected to run on the Theranos system for the clinical correlation analysis. Final conditions in the sample mixture included a 50 fold sample dilution and a 1:10K dilution from stock for both antibody and Vanco-AP. A 5, 5 minute coincubation, substrate incubation time was used.

The Vancomycin values obtained correlated very well to the ACS centaur system.

	Signal (RLU)		Back-calculated		
[Vanco], µg/ml	Mean RLU	CV%	Mean Conc, µg/ml	CV%	% Recovery
100	9126	5.3	99.6	4.4	100
75	11220	6.2	80.7	7.2	108
50	15654	7.2	50.6	11.9	101
25	23342	9.3	25.7	17.4	103
10	38912	13.7	10.9	21.9	109
5	71635	8.5	5.2	5.7	104
0	551708	9.7			

Figure [SEQ Figure * ARABIC]: Standard curve-Clinical Correlation

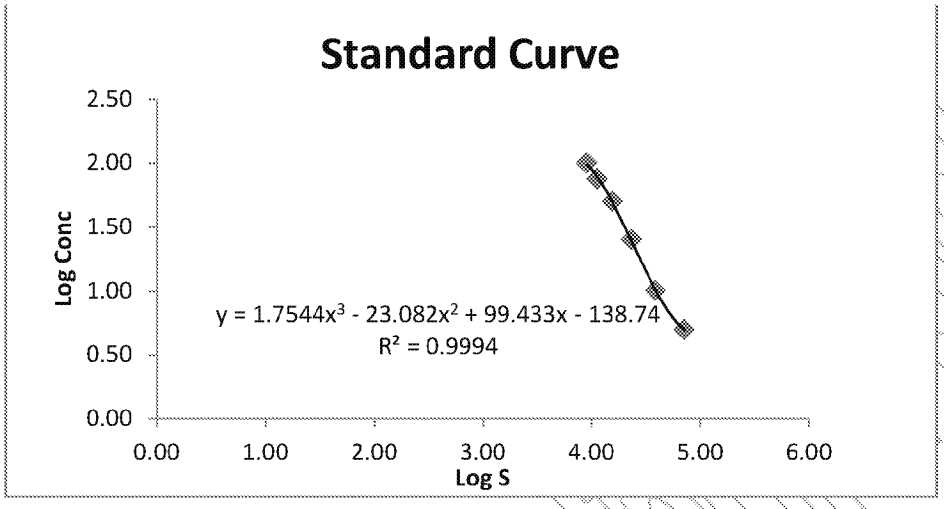


Table [SEQ Table * ARABIC]: Clinical Correlation

Sample ID	Mean RLU	CV%	Theranos Mean [Vanco], µg/ml	CV%	ACS:Centaur [Vanco], µg/ml	Siemens Advia 1800 [Vanco], µg/ml
1	64748	10.0	5.6	8.2	5.7	3.5
2	94594	15.5	4.8	2.8	7.8	4.8
3	53836	8.5	6.7	11.5	9	5.7
4	22173	11.1	28.2	17.1	27.1	15.6
5	14879	17.2	49.9	18.5	40.1	29.5
6	25546	17.3	22.6	28.7	20.7	14.1
7	38524	5.6	10.7	8.8	20.7	15.8
8	108621	11.2	4.9	4.8	5.2	2.8
9	30564	9.4	15.9	15.7	18.6	14
10	29740	8.7	16.6	15.9	20.7	11.7
11	35243	12.7	12.7	23.9	23.9	12.6
12	23472	3.7	25.0	6.3	30.4	24
13	20741	25.0	34.2	37.2	38.1	24.6
14	18999	18.6	32.2	17.3	36.1	27.6
15	18408	12.8	39.3	21.0	30.3	21.1
16	14895	16.7	48.1	7.4	40.2	25.4
17	17235	8.4	43.3	14.3	40.1	24.4
18	52656	23.4	7.4	27.0	10.9	7
19	57197	8.3	6.3	9.9	7.9	5.2
20	34448	10.5	13.0	16.0	15.1	10.5
21	35721	7.1	12.1	12.0	11.1	7.6

Calibration Equation:
Conc =10^(1.7544*(LOG(S))^3 -23.082*(LOG(S))^2 +99.433*(LOG(S)) -138.74)

Figure [SEQ Figure * ARABIC]: Clinical Correlation-Theranos Vs ACS Centaur

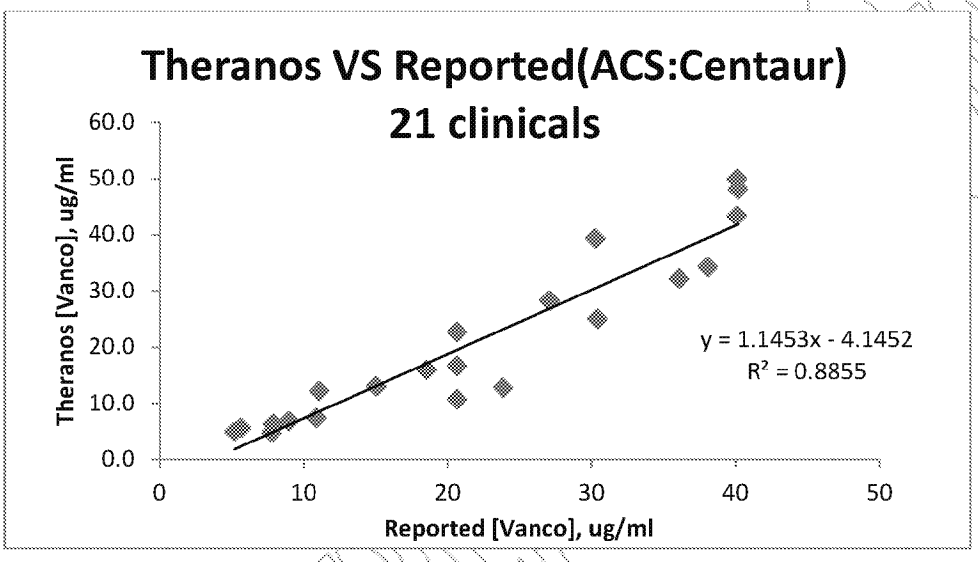
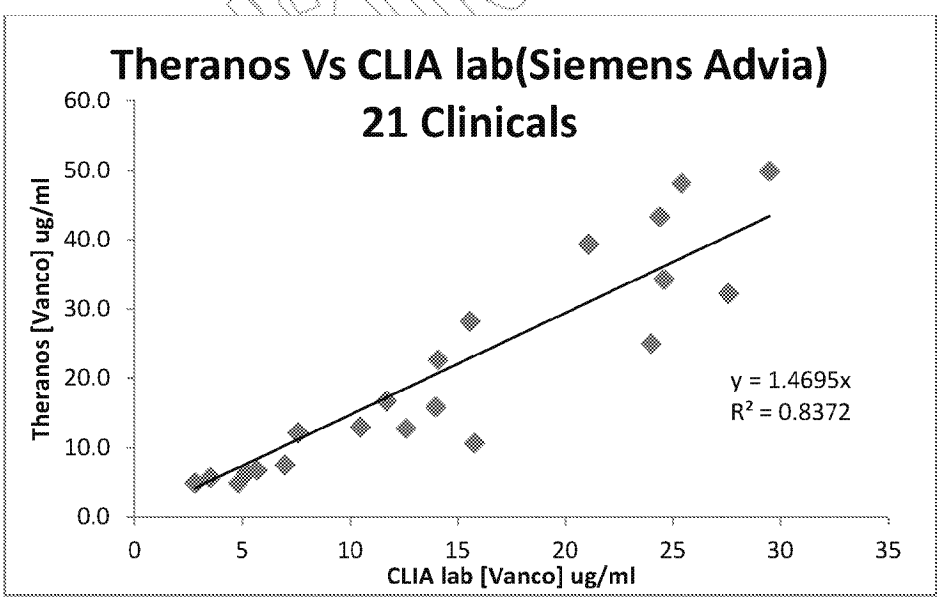


Figure [SEQ Figure * ARABIC]: Clinical Correlation- Theranos Vs Siemens Advia 1800



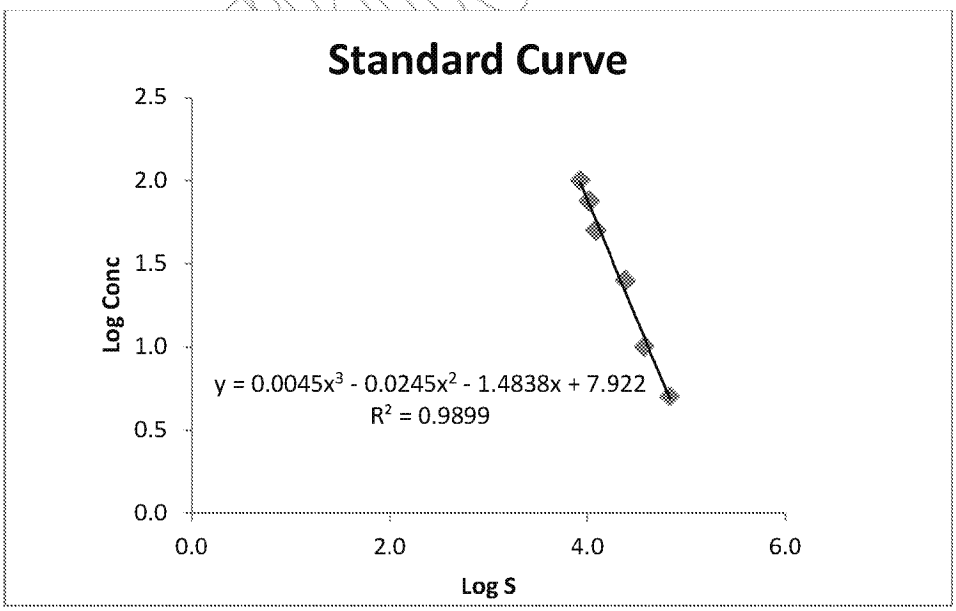
2.11 Standard Curve Data

The conditions on the Theranos system were finalized and the following standard curve data and calibration equation will be used for the rest of this study. Final conditions in the sample mixture included a 50 fold sample dilution and a 1:10K dilution from stock for both antibody and Vanco-AP. A 5, 5 minute coincubation, substrate incubation time was used. The assay has a reportable range of 5ug/ml to 100ug/ml.

Table [SEQ Table * ARABIC]: Standard Curve Data

	Signal(RLU)			Back-calculated		
Assigned- [Vanco], µg/ml	Mean RLU	CV%	Mod	Mean Conc. µg/ml	CV%	% Recovery
100	8531	7.2	47.5	97.1	10.6	97
75	10502	12.2		72.6	16.6	97
50	12070	10.1		58.9	15.3	118
25	22422	14.8		24.4	23.7	98
10	37615	13.2		11.5	17.8	115
5	68135	6.3		4.9	8.5	98
0	405397	15.9				

Figure [SEQ Figure * ARABIC]: Standard Curve Data



Calibration Equation:
Conc =10^(0.0045*(LOG(S))^3 -0.0245*(LOG(S))^2 -1.4838*(LOG(S)) +7.922)

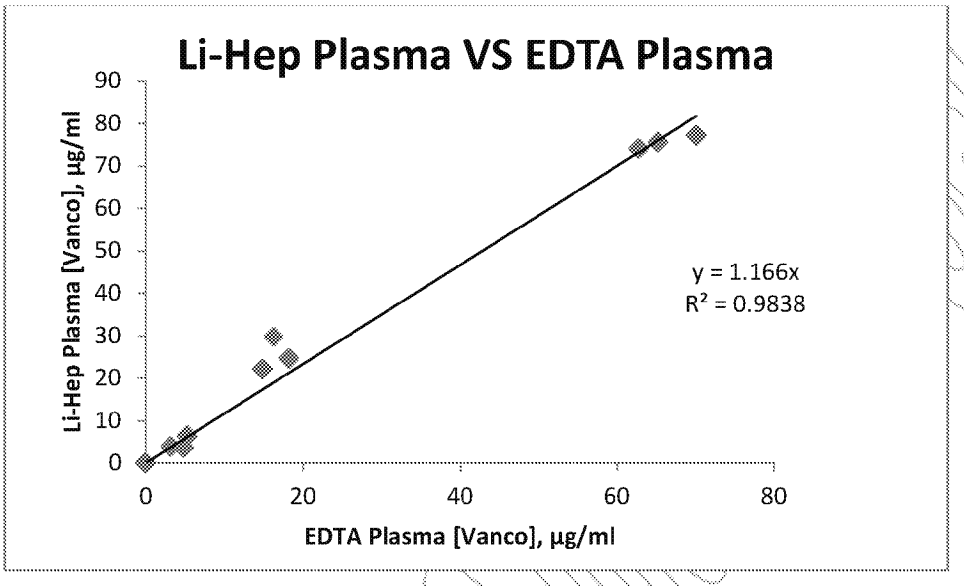
2.12 Effects of Anticoagulant

The Theranos System will be able to prepare plasma from both EDTA and lithium heparin (Li-Hep) treated blood. Matched plasma samples were prepared from both EDTA tubes and from Li-Hep tubes for normal donors. The correlation was good indicating either type of anticoagulant would work well for this assay in this system. Final conditions in the sample mixture included a 50 fold sample dilution and a 1:10K dilution from stock for both antibody and Vanco-AP. A 5, 5 minute coincubation, substrate incubation time was used.

Table [SEQ Table * ARABIC]: Effects of Anticoagulant-Lithium Heparin Vs EDTA plasma

		Spiked	Signal (RLU)		Back-Calculate		
Sample	Type	[Vanco] µg/ml	Mean RLU	CV %	Mean Conc µg/ml	CV %	% Recovery
Patient 1	Li-Hep	0.0	321118	11.1	0		
		5	66154	10.6	3.9	18.3	78
		25	18606	19.1	22.1	22.9	88
		75	9895	15.1	74.1	26.7	99
	EDTA	0.0	344263	8.3	0		
		5	73552	13.4	3.2	25.2	64
		25	25328	7.1	14.9	8.6	60
		75	12041	23.0	62.8	57.1	84
Patient 2	Li- Hep	0.0	258858	14.2	0		
		5	68597	11.6	4	24.0	73
		25	15945	10.7	30	17.0	119
		75	9462	17.5	76	27.1	101
	EDTA	0.0	229735	7.1	0		
		5	58564	14.2	5	21.2	97
		25	24036	14.7	16	18.2	65
		75	10573	15.3	65	24.1	87
Patient 3	Li- Hep	0	239103	4.3	0	22.9	
		5	45073	25.3	6	20.3	129
		25	18179	13.4	25	23.7	98
		75.0	10090	2.9	77	7.6	103
	EDTA	0	214828	14.3	0		
		5	56679	20.0	5	28.1	105
		25	22002	13.5	18	17.1	73
		75.0	11630	18.8	70	31.4	94

Figure [SEQ Figure * ARABIC]: Effect of Anticoagulant- Lithium Heparin Vs EDTA plasma correlation



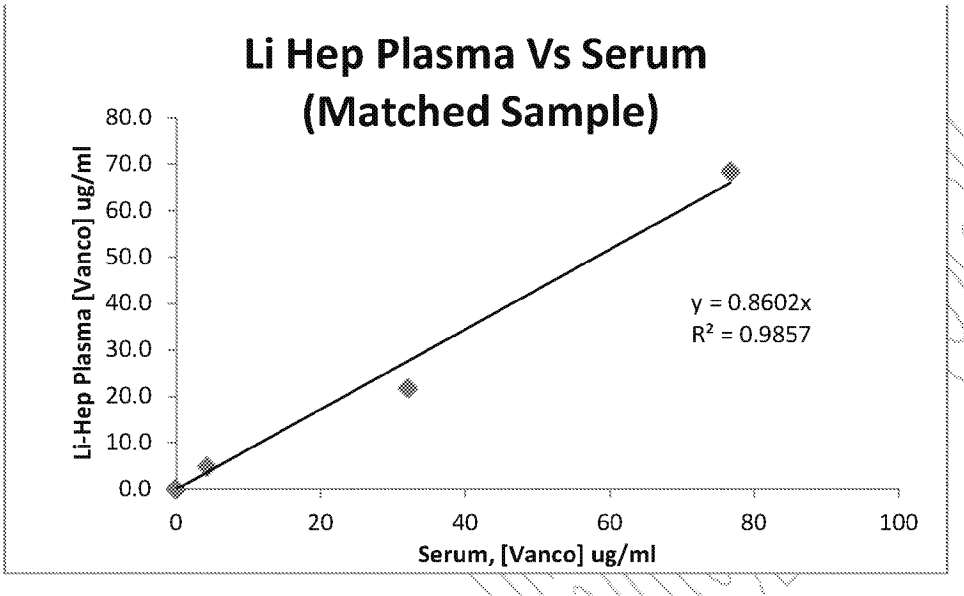
2.13 Matrix Effects: Lithium Heparin Plasma Vs Serum

Theranos system will be capable of testing either serum prepared in the lab or plasma prepared on board from a whole blood sample. Matched Serum and Lithium Heparin Plasma from a normal donor was analyzed. Correlation data was excellent indicating both plasma and serum samples can be used for the assay.

Table [SEQ Table * ARABIC]: Matrix Effects- Lithium Heparin Plasma Vs Serum

Sample	Type	Spiked [Vanco] µg/ml	Signal (RLU) Mean RLU	CV%	Back Calculate Mean Conc. µg/ml	CV%	% Recovery
Patient	Serum	0.0	399965	9.9	OORL		
		5	74004	2.0	4.3	2.7	86
		25	16689	45.4	32.1	39.2	128
		75	9990	4.9	76.7	6.9	102
	Li-Hep	0.0	425687	8.5	OORL		
		5	69762	11.4	4.8	17.5	96
		25	23865	5.2	21.7	7.9	87
		75	10836	7.6	68.4	10.6	91

Figure [SEQ Figure * ARABIC]: Matrix Effects- Lithium Heparin Plasma Vs Serum



2.14 Interfering Matrix Effects

Spike recovery was tested in lipemic, icteric and hemolyzed serum obtained from ProMedDx to ascertain whether there may be interference in the assay results when measuring these types of samples. Both hemolyzed and icteric samples do not seem to interfere for this assay. Lipemic might have slight interference.

Table [SEQ Table * ARABIC]: Interfering Matrix Effects- Hemolyzed, Lipemic and Icteric

	Spiked	Signal (RLU)		Back-Calculated		
Sample Type	[Vanco] µg/ml	Mean RLU	CV%	Mean Conc. µg/ml	CV%	% Recovery
Hemolyzed	0.0	353168	3.2	0.0		
	5	60005	10.1	5.9	15.2	118
	25	19175	10.1	30.1	16.4	120
	75	9060	10.2	89.7	15.5	120
Lipemic	0.0	318840	9.0	0.0		
	5	54235	5.5	6.7	7.7	134
	25	20447	6.9	27.1	9.9	109
	75	8162	8.2	98.8	6.4	132
Icteric	0.0	343491	6.6	0.0		
	5	59583	12.9	6.0	16.7	120
	25	19957	12.5	28.7	21.1	115

	75	9296	17.7	89.9	30.8	120

2.15 Normal Sample Screen- Whole Blood and Plasma

Normal whole blood and plasma clinical samples showed absence of Vancomycin.

Table [SEQ Table * ARABIC]: Normal Sample Screen-Whole Blood and Plasma

	Whole Blood			Plasma		
ID #	Mean RLU	CV%	Mean Conc. µg/ml	Mean RLU	CV%	Mean Conc. µg/ml
1	498604	7.2	OORL	508299	1.0	OORL
2	572430	7.5	OORL	460556	8.4	OORL
3	530647	3.5	OORL	470057	10.5	OORL
4	513477	19.7	OORL	512491	10.8	OORL
5	468641	14.8	OORL	516940	7.7	OORL
6	451952	10.9	OORL	540605	6.1	OORL
7	483830	7.2	OORL	425608	8.7	OORL
8	428183	9.5	OORL	409487	1.5	OORL

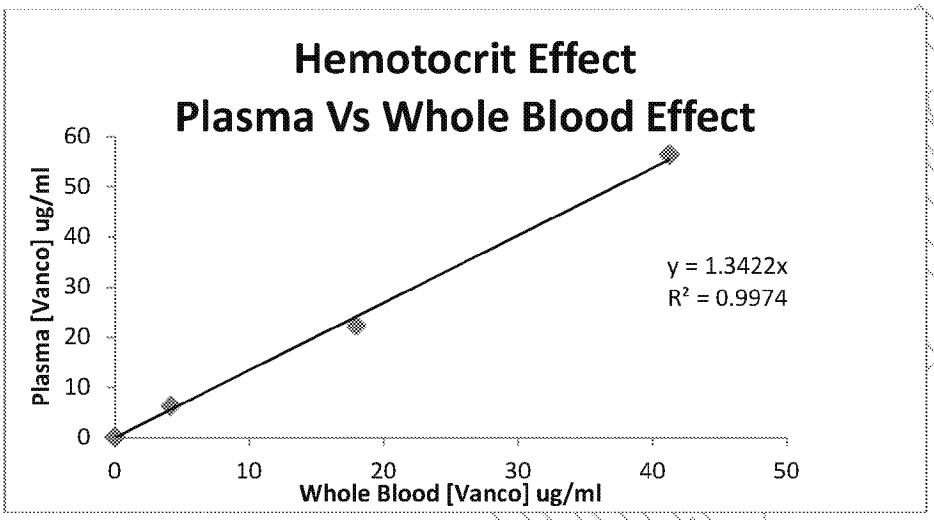
2.16 Hematocrit Effect

The hematocrit effect was tested in a whole blood sample collected in an EDTA tube and then compared to the plasma obtained from this same sample. Whole blood results could be calibrated on the serum standard curve with a hematocrit correction factor. Vancomycin levels were spiked at different levels and percent recovery is reported.

Table [SEQ Table * ARABIC]: Hematocrit Effect-Spike Recovery

		Signal(RLU)		Back-Calculated		
Sample Type	[Vanco], µg/ml	Mean RLU	CV%	Mean Conc. µg/ml	CV%	% Recovery
Whole Blood	0.0	440171	14.0	OORL		
	5	76285	8.9	4	10.7	84
	25	27395	10.6	18	15.6	72
	75	15596	15.4	41	21.6	55
Plasma	0.0	381562	11.5	OORL		
	5	49890	14.3	6	21.4	125
	25	19163	11.6	22	15.8	89
	75	11735	11.7	56	26.1	75

Figure [SEQ Figure * ARABIC]: Hematocrit Effect



2.17 Stability

Stability monitoring is ongoing for the the assay reagents stored at 4°C and protected from light. All reagents used for the standard curve will be evaluated at each time point.

Table [SEQ Table * ARABIC]: Stability- Standard Curve

	Signal(RLU)			Back-Calculated		
[Vanco], µg/ml	Mean RLU	CV%	Mod	Mean Conc. µg/ml	CV%	% Recovery
100	8531	7.2	47.5	97.1	10.6	97
75	10502	12.2		72.6	16.6	97
50	12070	10.1		58.9	15.3	118
25	22422	14.8		24.4	23.7	98
10	37615	13.2		11.5	17.8	115
5	68135	6.3		4.9	8.5	98
0	405397	15.9				

Figure [SEQ Figure * ARABIC]: Standard Curve-Stability