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Rheumatoid Factor IgM 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"]

Rheumatoid Factors (RF) are immunoglobulins of any isotype (IgA, IgG, IgM) in blood and synovial fluid with antibody activity against antigenic site on the Fc region of human or animal immunoglobulin G (IgG)[1]. Assays for RF are the most widely used serological tests as an aid for diagnosis of rheumatoid arthritis (RA). RF has been reported to occur in approximately 70-80% of patients with confirmed RA. RF IgM is the main isotype identified by clinically available diagnostic assays for RF detection[2].

This assay is designed to quantitatively determine the presence of Rheumatoid Factor IgM (RF IgM) in human serum.

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

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

- INOVA, QUANTA Lite™ RF IgM ELISA, Catalog # 708690, certified for diagnostic use.
- ALPCO, Rheumatoid Factor IgM ELISA, Catalog # GD23, certified for diagnostic use.
- DRG, Rheumatoid Factor IgM ELISA, Catalog # EIA-3585, certified for diagnostic use.

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

Rabbit IgG serves as the capture surface antigen for Rheumatoid Factor IgM in the sample. After incubation of the appropriately-diluted sample on the capture surface, the surface is washed and then the chicken anti-human IgM detection antibody conjugated to alkaline phosphatase is incubated on the surface. The surface is washed again. Finally, the alkaline phosphatase substrate is incubated on the surface, and then the resulting chemiluminescence is read in Relative Light Units (RLU).

Table [SEQ Table * ARABIC]: Materials and Reagents

Name	Supplier	Catalog #
IgG from Rabbit Serum (antigen)	Sigma	I5006
Chicken Anti-Human IgM Antibody	LifeSpan BioSciences	LS-C96131
Rheumatoid Factor, Control Serum, Human	USBiological	R1997-01B
Alkaline Phosphatase Labeling Kit (SH)	Dojindo	LK13
Phospho Glo Substrate	KPL	55-60-04
Blocking Buffer (3% BSA in TBS, 0.05% Sodium Azide)	Sigma (BSA, Fraction V, 99% Pure)	A3059-500G

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

2.1 Antigen Information

The rabbit IgG was used as the capture surface as it has been shown to be more specific than human IgG for RF IgA and RF IgG assays on Theranos 3.0 system. The rabbit IgG antigen was coated directly on the surface at 10 µg/mL in carbonate-biocarbonate buffer (pH= 9.6).

Table [SEQ Table * ARABIC]: Antigen Information

Antigen	Vendor	Cat #
IgG from Rabbit Serum	Sigma-Aldrich	I5006

2.2 Detection Antibody Screen

Various detection antibodies conjugated with alkaline phosphatase were screened on the Theranos 3.0 system using the protocol Generic 2_25x_PSW. First, the antibodies were screened at 100 ng/ml in 3% BSA in TBS, then they were screened at 10ng/ml in 3% BSA in TBS since the background at 100ng/ml was very high. DAb #6, #7 and #9 at 10 ng/ml were selected for further development.

Table [SEQ Table * ARABIC]: Anti-Human IgM Detection Antibody Information

Dab#	Discription	Vendor	Cat #
1	Rabbit Anti-human IgM	Fitzgerald	41R-1044
2	Goat Anti-human IgM	Fitzgeraldc	41C-CB0993
3	Mouse Anti-human IgM	Fitzgerald	10r-1130a
4	Goat Anti-IgM (Mu) Antibody	Genway	GWB-T00969
6	Chicken anti-human IgM	LifeSpan BioSciences	LS-C96131
7	Rabbit anti-human IgM	LifeSpan BioSciences	LS-C88648
8	Mouse anti-human IgM Fab'2	LifeSpan BioSciences	LS-C70370
9	Mouse anti-human IgM	LifeSpan BioSciences	LS-C70394
10	Anti-Human IgM (µ-chain specific)	Sigma	I0140-2ML

Table [SEQ Table * ARABIC]: Detection Antibody Screen on Theranos 3.0 system at 100 ng/ml

DAB #1

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	4680340	4618176	4649258	1	124
300	5064817	4881445	4973131	3	132
38	4147449	34895	2091172	139	56
0	40585	34663	37624	11	1

DAB #2

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	4487279	4490821	4489050	0	44
300	4781731	4809048	4795390	0	47
38	95281	109379	102330	10	1
0	95411	109606	102509	10	1

DAB #3

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	4528232	4526347	4527290	0	56
300	4791449	4851825	4821637	1	59
38	93102	98849	95976	4	1
0	83994	79045	81520	4	1

DAB #4

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	4054926	4175970	4115448	2	80
300	4220623	4233321	4226972	0	82
38	3525988	3200086	3363037	7	66
0	51986	50567	51277	2	1

DAB #6

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	208701	227851	218276	6	85
300	149581	141048	145315	4	57
38	3245	2467	2856	19	1
0	2637	2489	2563	4	1

DAB #7

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	2277392	2233038	2255215	1	321
300	1855183	1913469	1884326	2	268
38	7854	6983	7419	8	1
0	7900	6148	7024	18	1

DAB #8

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	4500506	4553174	4526840	1	102
300	4050858	4148772	4099815	2	92
38	45288	38257	41773	12	1
0	42957	45724	44341	4	1

DAB #9

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	4427453	4406061	4416757	0	270
300	3925466	4044079	3984773	2	244
38	19160	15075	17118	17	1
0	18074	14595	16335	15	1

DAB #10

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	4500136	4439099	4469618	1	237
300	4086763	4071966	4079365	0	216
38	20767	12810	16789	34	1
0	18810	18973	18892	1	1

Table [SEQ Table * ARABIC]: Detection Antibody Screen on Theranos 3.0 system at 10 ng/ml

DAb #1

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	2557604	2657020	2607312	3	244
300	2073762	2060582	2067172	0	193
38	1590654	1643376	1617015	2	151
0	11224	10144	10684	7	1

DAb #2

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	2041777	1936704	1989241	4	161
300	1296038	1272926	1284482	1	104
38	1313284	1172372	1242828	8	100
0	12673	12092	12383	3	1

DAb #3

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	2224241	2294734	2259488	2	132
300	1919418	1888596	1904007	1	111
38	1376901	1525809	1451355	7	85
0	21666	12495	17081	38	1

DAb #4

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	1349391	1463165	1406278	6	202
300	1356715	1282500	1319608	4	190
38	942499	975520	959010	2	138
0	9301	4604	6953	48	1

DAb #6

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	26751	21884	24318	14	87
300	11084	11411	11248	2	40
38	4359	4237	4298	2	15
0	252	310	281	15	1

DAb #7

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	413969	423078	418524	2	421
300	312569	316886	314728	1	316
38	103760	106521	105141	2	106
0	1026	963	995	4	1

DAb #8

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	1722503	1706281	1714392	1	324
300	1678238	1634856	1656547	2	313
38	1103015	1004472	1053744	7	199
0	4957	5642	5300	9	1

DAb #9

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	1686281	1692696	1689489	0	561
300	1573418	1580887	1577153	0	524
38	703352	714679	709016	1	235
0	2895	3127	3011	5	1

DAb #10

[RF IgM] IU/mL	Tip1	Tip2	Mean RLU	CV%	Modulation
1200	2042372	1780780	1911576	10	386
300	1512866	1664011	1588439	7	321
38	1208896	1199577	1204237	1	243
0	4833	5065	4949	3	1

Sample dilution at 25x, 50x and 100x has been tested on Theranos 3.0 system, and 100x dilution was selected due to lower background and higher modulation.

Table [SEQ Table * ARABIC]: Sample Dilution Test on Theranos

Generic2_25x_PSW			
DAB #6			
[RF IgM] IU/mL	Mean RLU	CV%	Modulation
1200	24318	14	87
300	11248	2	40
38	4298	2	15
0	281	15	1
DAB #7			
[RF IgM] IU/mL	Mean RLU	CV%	Modulation
1200	418524	2	421
300	314728	1	316
38	105141	2	106
0	995	4	1
DAB #9			
[RF IgM] IU/mL	Mean RLU	CV%	Modulation
1200	1689489	0	561
300	1577153	0	524
38	709016	1	235
0	3011	5	1

Generic2_50x_PSW			
DAB #6			
[RF IgM] IU/mL	Mean RLU	CV%	Modulation
1200	11709	10	34
300	7722	13	22
38	2736	6	8
0	348	2	1
DAB #7			
[RF IgM] IU/mL	Mean RLU	CV%	Modulation
1200	324506	3	446
300	210887	5	290
38	72295	2	99
0	727	17	1
DAB #9			
[RF IgM] IU/mL	Mean RLU	CV%	Modulation
1200	1666939	3	804
300	1369950	5	661
38	652786	4	315
0	2073	8	1

Generic2_100x_PSW			
DAB #6			
[RF IgM] IU/mL	Mean RLU	CV%	Modulation
1200	8913	9	31
300	4411	20	15
38	1279	3	4
0	289	3	1
DAB #7			
[RF IgM] IU/mL	Mean RLU	CV%	Modulation
1200	300534	8	501
300	152285	4	254
38	37034	1	62
0	600	10	1
DAB #9			
[RF IgM] IU/mL	Mean RLU	CV%	Modulation
1200	1632124	4	1136
300	1072137	10	746
38	289187	15	201
0	1437	8	1

Five Rheumatoid Factor positive samples from PromedDx and five negative clinical samples from Stanford Blood Bank had been tested on commercial ELISA kit to verify positive or negative first, then tested on Theranos 3.0 system using protocol Generic2_100x_PSW with the antibody concentration at 10ng/ml in 3%BSA in TBS. After comparison, DAb #6 was selected as final detection antibody since only DAb #6 shows clinical correlation between positive and negative samples.

Table [SEQ Table * ARABIC]: Screening of RF IgM positive and Negative Samples on Theranos (Training Set)

	Theranos (Back-Calculated)					
	DAB #6	DAB #7	DAB #9	ALPCO	INOVA	DRG
Sample #	Mean Conc (IU/mL)	Mean Conc (IU/mL)	Mean Conc (IU/mL)	Mean Conc (IU/mL)	Mean Conc(IU/mL)	Mean Conc(IU/mL)
38	1157	853	894	159.4	290.7	25.0
40	65	36	46	21.6	20.2	24.8
49	1081	968	791	1048.15	2982.2	942.4
50	878	711	598	580.07	1070.9	96.6
54	761	671	667	179.1	128.4	OORL
57	1074	691	535	101.08	1805.0	955.8
B2	16	203	85	0.9	0.2	OORL
B3	15	77	67	1.5	1.9	OORL
B6	18	149	94	1.0	0.6	OORL
B9	52	21	19	8.0	9.3	13.3
B11	22	66	46	1.4	1.1	OORL
B12	24	20	21	3.4	2.9	0.6
Positive	Negative					

Since the DAb #6 has been selected as the final detection antibody, we started with the protocol Generic2_25x_PSW for further development because DAb #6 showed best modulation under this condition.

2.3 Alkaline Phosphatase Stabilizers

Two commercial and one in-house alkaline phosphatase stabilizer were tested as detection antibody diluent with the DAb concentration at 10 ng/mL. In comparison with BioStab and Stabilzyme AP Stabilizers, In-house stabilizer consisting of 5 mM Mg^{2+}, 0.1 mM Zn^{2+}, and 3% BSA in TBS showed good signal modulation and low background.

Table [SEQ Table * ARABIC]: Alkaline Phosphatase Stabilizers

AP Stabilizer	[RF IgM] IU/mL	Signal, RLU Mean RLU	CV%	Modulation
3% BSA (Control)	1200	24318	14	87
	300	11248	2	40
	38	4298	2	15
	0	281	15	1
In-House AP Stabilizer*	1200	26525	3	47
	300	21761	26	38
	38	6787	11	12
	0	566	10	1
BioStab AP Satbilizer	1200	42184	26	40
	300	47158	26	45
	38	18105	31	17
	0	1046	22	1
Stabilzyme AP Stabilizer	1200	16361	20	28
	300	12303	9	21
	38	2860	10	5
	0	586	7	1

* 5 mM Mg^{2+}, 0.1 mM Zn^{2+}, and 3% BSA in TBS

2.4 Detection Antibody Titration

The DAb #6 was titrated in In-House AP Stabilizer (5 mM Mg^{2+}, 0.1 mM Zn^{2+}, and 3% BSA in TBS). The modulation was best with the DAb concentration at 50 ng/mL. Therefore, 50 ng/mL was selected as the final DAb concentration.

Table [SEQ Table * ARABIC]: Detection Antibody Titration

[Dab]	[RF IgM]	Signal, RLU		
ng/mL	IU/mL	Mean RLU	CV%	Modulation
10 ng/mL	1200	25980	7	61
	600	22871	14	54
	300	17561	29	41
	38	5175	21	12
	19	3958	11	9
	0	427	6	1
25 ng/mL	1200	52663	12	57
	600	52184	23	56
	300	43798	38	47
	38	11678	13	13
	19	7805	8	8
	0	931	27	1
50 ng/mL	1200	106239	11	86
	600	88099	19	71
	300	69465	7	56
	38	23948	6	19
	19	17174	10	14
	0	1240	10	1

2.5 Reagent Incubation Time

The effect of shorter reagent incubation time was tested with sample, detection conjugate and substrate incubation time respectively at 10, 10, 10 (original condition) and 5, 5, 5 minutes. With 5, 5, 5 minutes incubation, the modulation was reduced. The 10,10,10 minutes incubation protocol was selected as the final condition.

Table [SEQ Table * ARABIC]: Reagent Incubation Time

		Signal, RLU		
Incubation Time	**[RF IgM] IU/mL**	**Mean RLU**	**CV%**	**Modulation**
10, 10, 10 minutes	1200	106239	11	86
	600	88099	19	71
	300	69465	7	56
	38	23948	6	19
	19	17174	10	14
	0	1240	10	1
5, 5, 5 minutes	1200	36402	10	32
	600	33646	22	29
	300	20995	12	18
	38	6356	13	6
	19	3541	26	3
	0	1141	14	1

2.6 Sample Dilution

The effect of sample dilution was tested with final sample dilution factors of 1:25, 1:50, 1:100 and 1:500 into 3% BSA in TBS blocking buffer. When the sample dilution was up to 1:500, dose response was more linear across the projected range of the final assay (up to 2434 IU/mL). The other dilution shows saturation at the top level. Therefore 1:500 was selected as the final sample dilution.

Table [SEQ Table * ARABIC]: Effect of Sample Dilution

		Signal, RLU		
Sample Dilution	[RF IgM] IU/mL	Mean RLU	CV%	Modulation
25	1200	106239	11	86
	600	88099	19	71
	300	69465	7	56
	38	23948	6	19
	19	17174	10	14
	0	1240	10	1
50	1200	98554	12	78
	600	76106	10	60
	300	51899	9	41
	38	13098	10	10
	19	6893	23	5
	0	1268	9	1
100	1200	79914	13	50
	600	56667	6	36
	300	44282	6	28
	38	9939	12	6
	19	6100	5	4
	0	1591	16	1
500	2434	51546	19	31
	1200	38166	8	23
	600	21802	12	13
	300	13585	10	8
	38	3678	19	2
	19	2197	18	1
	0	1662	10	1

Figure [SEQ Figure * ARABIC]: Effect of Sample Dilution

[SHAPE * MERGEFORMAT]

2.7 Determination of Expected LLOQ and ULOQ

A lot of reagents were produced and a calibration was performed using the final assay conditions of 50 ng/mL DAb#6 in In-House AP Stabilizer, 10 ug/mL capture antibody with direct coat, and a 1:500 sample dilution with 3 cartridges per point. The protocol for Theranos 3.0 System is Generic2_500x_PSW (Incubation time is 10,10,10 minutes in this protocol).

A standard curve was used to calibrate the Theranos System from 19 to 2434 IU/mL, and Dexter had been run to decide LLOQ and ULOQ. The LLOQ was 19 IU/mL, and the ULOQ was 2434 IU/mL. The Serum Standard Curve from Dexter is applied for all the following tests.

Table [SEQ Table * ARABIC]: Calibrator and Calculation

Calibrator	Signal, RLU		Back-Calculated Conc., IU/mL		
[RF IgM] IU/mL	Mean RLU	CV%	Mean Conc.	CV%	%Recovery
2434	57264	12	2433	15	100
1200	34185	16	1230	22	103
600	18466	15	517	21	86
300	13907	13	341	18	114
150	8703	10	166	16	111
75	5076	16	71	26	94
38	3221	12	33	20	87
19	2519	12	22	21	114
0	1206	8	6	15	

=10^((-0.1651)*(LOG(S))^2+2.8607*(LOG(S))-6.4878)

Figure [SEQ Figure * ARABIC]: Standard Curve

[SHAPE * MERGEFORMAT]

Figure [SEQ Figure * ARABIC]: Standard Curve for Determination of LLOQ and ULOQ on Dexter

[SHAPE * MERGEFORMAT]

2.8 Clinical Samples

23 RF poistive clinical serum samples were obtained from PromedDx, and 12 RF negative clinical plasma samples was obtained from Stanforde Blood Bank. These clinical samples were tested on different commercial ELISA kits to verify positve or negative. These samples were then tested on Theranos 3.0 system using the final protocol Generic2_500x_PSW (Incubation time is 10,10,10 minutes in this protocol).

The Theranos RF IgM assay cutoff is 19IU/mL (RF IgM concentration ≥19 IU/mL is positive). In general, Theranos RF IgM assay correlated very well to the commercial ELISA kits.

Table [SEQ Table * ARABIC]: Results for Clinical Samples

Rheumatoid Factor IgM Concentration (IU/mL)

Set Type	Sample #	ALPCO	INOVA	DRG	Theranos (Dexter)
ProMedex	19	165.7	573.4	346.9	190
	27	741.27	2191.0	814.5	1397
	38	159.4	290.7	25.0	1885
	40	21.6	20.2	24.8	18
	41	602.48	765.6	1187.1	1063
	42	857.72	1210.3	838.9	1577
	43	232.2	380.9	777.8	354
	44	78.0	187.2	19.4	891
	45	596.80	992.7	5602.9	1941
	46	483.94	988.1	1216.6	987
	47	272.8	913.6	2110.1	708
	48	315.7	913.8	721.8	744
	49	1048.15	2982.2	942.4	1652
	50	580.07	1070.9	96.6	1266
	51	257.5	632.0	220.6	545
	52	276.7	314.4	1728.1	645
	53	912.38	1423.0	980.0	1304
	54	179.1	128.4	OORL	835
	55	171.2	277.1	134.7	365
	56	93.5	116.3	100.3	125
	58	552.11	947.5	347.8	1448
	59	412.68	783.0	862.8	900
	60	508.23	1560.8	327.9	1159
Stanford Blood Bank	B1	0.9	0.6	4.0	OORL
	B2	0.9	0.2	OORL	OORL
	B3	1.5	1.9	OORL	OORL
	B4	2.0	1.1	7.0	OORL
	B5	1.2	1.0	3.2	OORL
	B6	1.0	0.6	OORL	OORL
	B7	0.7	0.2	OORL	OORL
	B8	0.7	0.3	OORL	OORL
	B9	8.0	9.3	13.3	OORL
	B11	1.4	1.1	OORL	OORL
	B12	3.4	2.9	0.6	OORL
Positive					
Negative					

2.9 Specificity

Samples that may potentially cause false positives in RF IgM assays were tested for specificity.

Fourteen ANA positive samples were tested for RF IgM, there was no cross reactivity or false positives observed due to the presence of antinuclear antibodies.

One out of thirteen HAMA positive samples was positive for RF IgM. However, it was also positive on INOVA ELISA kit. There was no cross reactivity or false positives observed due to the presence of human anti-mouse antibody.

The HCV and HIV positive samples tested were all negative for RF IgM.

Table [SEQ Table * ARABIC]: Specificity test in ANA, HAMA, HCV and HIV positive samples

Rheumatoid Factor IgM Concentration (IU/mL)				
Sample #	**ALPCO**	**INOVA**	**DRG**	**Theranos (Dexter)**
ANA 61	1.785	5.235	4.155	18.0
ANA 62	1.438	3.608	0.703	OORL
ANA 63	2.822	4.765	0.436	OORL
ANA 64	2.644	2.775	5.334	OORL
ANA65	2.275	1.493	1.71	OORL
ANA66	0.592	7.424	0.635	OORL
ANA24	2.112	0.603	2.162	OORL
ANA25	1.331	1.298	4.007	OORL
ANA26	0.846	0.597	2.703	OORL
ANA27	2.467	0.811	61.643	OORL
ANA28	6.128	2.561	14.817	OORL
ANA29	3.234	14.29	8.79	OORL
ANA30	1.168	1.412	5.371	OORL
ANA31	3.716	3.294	4.999	OORL

HAMA 6	0.433	0.134	OORL	OORL
HAMA 7	1.058	0.571	OORL	OORL
HAMA 12	1.434	1.97	0.288	OORL
HAMA 15	0.534	0.641	0.193	OORL
HAMA 16	15.374	14.93	0.412	28
HAMA 17	0.683	0.148	OORL	OORL
HAMA-G	2.059	1.702	4.745	OORL
HAMA-H	4.715	3.991	5.872	OORL
HAMA-I	4.956	3.458	18.67	OORL
HAMA-J	0.386	0.615	1.486	OORL
HAMA-K	1.011	0.95	0.428	OORL
HAMA-L	3.075	5.89	8.154	OORL
HAMA-M	0.941	0.22	0.559	OORL
HCV	0.692	0.447	6.702	OORL
HIV-1 QC-2	1.201	0.952	2.8	OORL
HIV-2 QC-3	0.811	0.197	0.336	OORL
Positive				
Negative				

2.10 Hematocrit Effect

The hematocrit effect was determined with whole blood spiked (1:10) at three levels. The calibration with Dexter was applied. The spiked whole blood samples were measured on Theranos, then the remaining spiked whole blood was centrifuged and took the plasma to test on Theranos 3.0 system. The spiked RF IgM was found to fully concentrate into the plasma prepared from spiked whole blood. The RF IgM recovery from plasma was 1.7 fold of that from whole blood samples.

Table [SEQ Table * ARABIC]: Hematocrit Effect

	Whole Blood					Plasma				
Spiked RF IgM (IU/mL)	Signal (RLU)		RF IgM (IU/mL)			Signal (RLU)		RF IgM (IU/mL)		
	Mean	CV%	Mean	CV%	%Recovery	Mean	CV%	Mean	CV%	%Recovery
240	13481	22	306	32	128	19797	6	527	9	220
60	5439	8	74	14	124	6773	13	107	20	178
0	1910	16	OORL			2590	12	20	22	

Figure [SEQ Figure * ARABIC]: Whole Blood Spike Recovery

[SHAPE * MERGEFORMAT]

Figure [SEQ Figure * ARABIC]: Hematocrit Effect

[SHAPE * MERGEFORMAT]

2.11 Effect of Anticoagulant

EDTA and Lithium-Heparin plasma samples were obtained from Stanford Blood Bank. The recovery of RF IgM spiked into plasma (1:10) was evaluated on the Theranos 3.0 System. The calibration with Dexter was applied. There is no significant difference in recovery between EDTA and Lithium-Heparin plasma.

Table [SEQ Table * ARABIC]: Effect of Anticoagulant

Plasma Type	[RF IgM] IU/mL	Signal (RLU)		RF IgM Concentration (IU/mL)		
	Spiked	Mean	CV%	Mean	CV%	%Recovery
EDTA	240	10844	13	221	19	92
	60	4810	4	60	7	100
	0	1639	18	OORL		
Lithium-Heparin	240	10668	11	216	16	90
	60	4760	17	59	30	99
	0	1287	10	OORL		

Figure [SEQ Figure * ARABIC]: Effect of Anticoagulant

[SHAPE * MERGEFORMAT]

2.12 Interference Matrices

Hemolyzed, icteric, and lipemic serum samples were obtained from ProMedDx. The recovery of FR IgM spiked into these potentially interfering matrices (1:10) was evaluated on Theranos 3.0 System. The calibration with Dexter was applied. The assay did not show any interference from hemolysed, icteric, or lipemic serum samples for RF IgM assay.

Table [SEQ Table * ARABIC]: Interfering Matrices

Sample Type	[RF IgM] IU/mL	Signal (RLU)		RF IgM Concentration (IU/mL)		
	Spiked	Mean	CV%	Mean	CV%	%Recovery
Hemolyzed	240	11255	12	234	18	97
	60	4857	4	61	7	102
	0	1284	11	OORL		
Icteric	240	11724	25	251	37	104
	60	4803	17	60	29	101
	0	2270	19	17	4	
Lipemic	240	11324	10	235	15	98
	60	4592	14	55	24	92
	0	1549	14	OORL		

Figure [SEQ Figure * ARABIC]: Effect of Interference Matrices

[SHAPE * MERGEFORMAT]

2.13 Stability Studies

Stability was tested on day 0, week 2,4,8, and 12.

Day 0

			Back-Calculated Conc, IU/mL		
[RF IgM] IU/mL	Mean RLU	CV%	Mean Conc	CV%	%Recovery
2434	84470	13	2361	19	97
600	36468	25	691	35	115
19	3120	12	19	18	100
0	2010	22	11	27	

Week 2

			Back-Calculated Conc, IU/mL		
[RF IgM] IU/mL	Mean RLU	CV%	Mean Conc	CV%	%Recovery
2434	83276	5	2301	7	95
600	37852	5	717	8	119
19	2971	20	18	29	94
0	1853	10	9	15	

Week 4

			Back-Calculated Conc, IU/mL		
[RF IgM] IU/mL	Mean RLU	CV%	Mean Conc	CV%	%Recovery
2434	84752	12	2370	18	97
600	38128	16	730	23	122
19	3289	13	20	18	108
0	1863	6	9	8	

Week 8

			Back-Calculated Conc, IU/mL		
[RF IgM] IU/mL	Mean RLU	CV%	Mean Conc	CV%	%Recovery
2434	94846	17	2814	25	116
600	36212	34	694	47	116
19	3543	17	23	25	120
0	1735	26	8	34	

Week 12

			Back-Calculated Conc, IU/mL		
[RF IgM] IU/mL	Mean RLU	CV%	Mean Conc	CV%	%Recovery
2434	76905	9	2048	13	84
600	28683	15	479	22	80
19	2859	13	17	19	88
0	1675	19	8	27	

Figure [SEQ Figure * ARABIC]: Stability Test

[SHAPE * MERGEFORMAT]

References

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