

TARC (CCL17) Assay Development Report

Assay	Human Thymus and Activation-Regulated Chemokine (TARC) A.K.A. Chemokine (C-C motif) ligand 17 (CCL17)
Developer	Tina Noyes
Date released	04/01/09
Elog	E0547

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1. Analyte background

TARC (Thymus and Activation-Regulated Chemokine) or CCL17 is a basic protein with a predicted mass of 8 kDa. The cDNA encodes a highly basic 94 amino acid residue precursor protein with a 23 aa residue signal peptide that is cleaved to generate the 71 aa residue mature secreted protein. TARC has approximately 24 - 29% amino acid sequence identity with RANTES, MIP-1 α , MIP-1 β , MCP-1, MCP-2, MCP-3 and I-309 (13).

The gene for human TARC has been mapped to chromosome 16q13 - not chromosome 17 where the genes for many human CC chemokines are located. TARC is constitutively expressed in thymus, and at a lower level in lung, colon, and small intestine (14). TARC is also transiently expressed in stimulated peripheral blood mononuclear cells. Recombinant TARC has been shown to be chemotactic for T cell lines but not monocytes or neutrophils. TARC binds to chemokine receptors CCR-4 and CCR-8. This chemokine plays important roles in T cell

development in thymus as well as in trafficking and activation of mature T cells. TARC was recently identified to be a specific functional ligand for CCR-4, a receptor that is selectively expressed on T cells (15).

TARC acts on the chemokine receptor CCR-4, which is expressed on PBMCs and human T-cell lines but not on B cells, NK cells, or granulocytes (2, 3). MDC competes with TARC for binding to CCR-4 (4). TARC also binds to CCR-8 (5), which is expressed on lymphoid tissue. CCR-4 and CCR-8 expression is transiently up-regulated on activated T cells, preferentially on the Th2 subset (6). It thus appears that TARC is a chemokine specific for the trafficking of Th2 cells.

TARC has been demonstrated to be an inflammatory marker for a variety of conditions including; Asthma (10,11,12), Atopic Dermatitis (8), Acute Eosinophilic Pneumonia (7), and Primary Hodgkin's Disease (9).

2. Assay specifications

The Theranos assay for TARC (CCL17) is a sandwich ELISA specific to the recombinant and natural human TARC in whole blood, plasma and serum. Reportable ranges and limits of detection are:

Sample type	Low, pg/mL	High, pg/mL
Human plasma	10	2000
Human serum	10	2000
Human blood	15	2000

Whole blood and plasma samples spiked with TARC levels as high as 51,200 pg/mL have been tested in the assay and successfully recovered.

3. Reference Assays

The R&D Systems TARC ELISA catalogue # DDN00 has been used as the sole method of determining TARC in all literature reviewed.

The R&D Systems assay is a sandwich ELISA, Range: 7 pg/mL – 2000 pg/mL, 50 uL plasma sample size per replicate is required. Assay time is approximately 5 hours. This kit has been used in-house to validate the Theranos assay. Protocol can be found at the following link.

T:\Assay_Systems\Assay Development Reports\Completed Assays\Centocor Assays\TARC Assay Development Reports\TARC Literature\RnDSystems_TARC_ELISA.pdf

4. Antibody Screening

Antibody screening was carried out in 384 well Corning micro-titer plates, 10-10-10 minute incubation and coating times at room temperature and read on the M5. Initial testing was limited to monoclonal + polyclonal pairs, in order to reduce the potential for interference from substances such as Rheumatoid Factor. Since 11 working potential pairs were found in the initial round, further screening was not necessary.

Antibody Screening Set Summary

TARC Antibody Key				
Code	Vendor	Catalog #	Clone	Type
1	R&D Systems	MAB364	54026	Mouse Monoclonal; IgG
2	R&D Systems	MAB3641	54015	Mouse Monoclonal; IgG
3	R&D Systems	AF-364		Goat Polyclonal; IgG
4	abcam	ab9816		Rabbit Polyclonal; IgG
5	Zaris	PP1069B2		Rabbit Polyclonal; IgG
6	Cell Sciences	PA1232		Rabbit Polyclonal; IgG


Capture	Detection					
	1	2	3	4	5	6
1			X	X		X
2			X	X		X
3						
4	X	X				
5	X	X	X			
6						


Number of Capture antibody tested : 4


Number of Detection antibody tested: 5

Total Number of antibody pairs tested: 11

X Expected good pair

 No Modulation

 Modulation but background or other problem

 Modulation, good candidate pair

The antibody pair that was selected has R&D Systems MAB364 (monoclonal) as the capture antibody and Abcam ab9816 (rabbit polyclonal) as the detection antibody. The criteria were good dose response for the range of the assay in the desired matrix (whole blood, plasma) and highest sensitivity for the final assay conditions. The backup pair is: C-Ab: R&D MAB364 D-Ab: R&D AF-364 2.

Best Antibody Pairs on 384 well microtiter plates, 10, 10, 10 assay at room temperature,
 Plate chemiluminescence read on M5, Assay Buffer standards, C-Ab at 20 ug/mL, Dab at 100
 ng/mL in assay buffer. Standards were undiluted.

C-Ab	1	1
D-Ab	3	4
[TARC] pg/mL In Well	RLU	RLU
2000	244901	729820
1000	106809	416215
500	56744	216762
250	25673	107139
125	13737	60120
62.5	7410	30508
31.25	4509	16982
0	1349	545
<i>Low/BG</i>	<i>3.3</i>	<i>31.1</i>
<i>Slope</i>	<i>120</i>	<i>368</i>

Best Antibody Pairs on Edison

Protocol: Angiogenesis2_C svn 1956 (10-10-10, no post sample wash)

Samples: Fresh assay buffer calibrators, Hand Diluted 1:5

Tips: 10/23/08 Tomtec 20-30-10 1x PA washes, 20ug/mL Cab

D-Ab @ 250 ng/mL In Stabilzyme

Capture Ab Coated @ 20 ug/mL

C-Ab	1	1
D-Ab	3	4
[TARC] pg/mL In Sample	RLU	RLU
250	25417	27910
62.5	7980	8965
0	2442	1587
<i>Low/BG</i>	<i>3.3</i>	<i>5.7</i>
<i>Slope</i>	<i>92</i>	<i>104</i>

Best Antibody Pair with Spiked Plasma in Edison

Protocol: Angiogenesis2_C svn 1956 (10-10-10, no post sample wash)

Samples: Spiked Pooled Plasma (8 patients) Hand Diluted 1:5

Tips: 10/29/08 Tomtec 20-30-10 1x PA washes, 10ug/mL Cab1

Dab: DAb4 @ 350 ng/mL in Stabilzyme

[Spiked] pg/mL HD 1:5	RLU		
	Mean	StDev	CV %
2000	49721	2866	5.8
667	15920	1224	7.7
222	7173	353	4.9
74	3673	279	7.6
25	2197	160	7.3
0	1445	5	0.3
		<i>Avg CV%</i>	<i>5.6</i>
	<i>Bottom 3</i>	<i>Slope</i>	<i>30.1</i>
	<i>Bottom 3</i>	<i>Avg StDev</i>	<i>148</i>
	<i>LOD in Sample pg/mL</i>		<i>9.8</i>

5. Assay Reagents Assay Reagents

A. Capture Antibody

Vendor	R&D Systems
Catalog #	MAB364
Current lot #	B110025111
Clone #	54026.11
Type	Mouse monoclonal, IgG1
Specificity	No significant cross reactivity with rh6Ckine, rm6Ckine, rhBLC/BCA-1, rrCINC-1, rrCINC-2 α , rrCINC-2 β , rmCRG-2, rhENA-78, rhEotaxin, rmEotaxin, rhFractalkine, rhGCP-2, rmGCP-2, rhGRO α , rhGRO β , rhGRO γ , rhHCC-4, rhIL-8, rhIP-10, rmJE, rmKC, rmMARC, rhMCP-1, rhMCP-2, rhMCP-3, rhMCP-4, rmMCP-5, rhMDC, rmMDC, rhMIG, rmMIG, rhMIP-1 α , rmMIP-1 α , rhMIP-1 β , rmMIP-1 β , rmMIP-1 γ , rmMIP-2, rhMIP-3 α , rrMIP-3 α , rhMIP-3 β , rhMPIF-1, rhMPIF-2, rhNAP-2, rhParc, rhRANTES, rmRANTES, rhSDF-1 α , rhSDF-1 β , rhTeck, rmTeck
Stock Conc.	Unlabeled: lyophilized, 500ug. Reconstitute with sterile PBS to 1mg/mL and incubate at RT on rotisserie for 15 minutes before conjugating
Storage	Store lyophilized at -20, use entire vial up for conjugation.
Working Concentration	10 ug/mL of SH-labeled Biotin conjugate in Blocking Buffer

B. Detection Antibody

Vendor	Abcam
Catalog #	ab9816
Current lot #	478668
Type	Rabbit Polyclonal
Specificity	TBD
Stock Conc.	Unlabeled: 0.2 mg/mL, liquid
Storage	Store at 4C for up to 1 year
Working Concentration	75 ng/mL of SH-labeled AP conjugate in Stabilzyme

C. Analyte

Vendor	R&D Systems
Catalog #	364-DN/CF
Current lot #	
Peptide	71 amino acid residue recombinant protein
Mol Wt	Approx. 8 kDa.
Stock Conc.	Reconstitute to 100 ug/mL in sterile PBS, and incubate at RT on rotisserie for 15 minutes before conjugating , then dilute to 50 ug/mL in assay buffer (3% BSA in TBS w/ Thimerosal) and aliquot.
Storage	Aliquot stock and store at -20C, Avoid freeze thaw cycles

D. Analyte Diluent

Composition	50 mM TBS, 3% BSA, and 0.05% Thimerosal
Storage	4C for 3 months

E. Antibody Conjugation Kits

	Antibody	Cat #	Dojindo kit	Dojindo Cat #	Antibody conjugated/rxn
1	Capture	ABS 33-10	Biotin labeling kit -SH	LK10-10	100 ug
2	Detection	SPR174B	Alkaline Phosphatase kit – SH	LK13-10	100 ug

6. Protocols

Reagent preparation protocols are provided to MFG released 04/01/09.

Edison Protocol is Centocor_Multiplex_1 svn 2237, a copy of the code is located at T:\Assay_Systems\Assay Development Reports\Completed Assays\Centocor Assays\TARC Assay Development Reports\Protocol_Centocor_Multiplex_1.txt

7. Capture Antibody Titration

Capture antibody was tested at 2.5, 5, 10 and 20 ug/mL. A concentration of 10 ug/mL was determined to be optimum.

Date: 11/18/2008

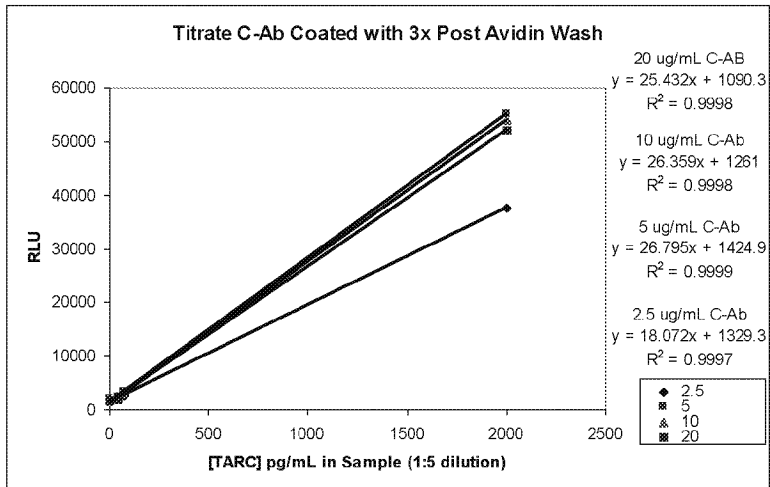
Protocol: Generic_ND svn 2090

Samples: Assay Buffer Calibrators, Hand Diluted 1:5

Tips: 11/14/08 Tomtec 30-30-10 3x PA washes, Titrate CAb

Dab: DAB 350 ng/mL in Stabilzyme

Chart 1: Capture Antibody Titration



Capture Antibody Titration

	2.5 ug/mL C-Ab			5 ug/mL C-Ab			10 ug/mL C-Ab			20 ug/mL C-Ab		
[In Sample] pg/mL	Mean	StDev	CV %	Mean	StDev	CV %	Mean	StDev	CV %	Mean	StDev	CV %
2000	37487	2752	7.3	55023	5313	9.7	53988	1484	2.7	51963	3093	6.0
74	2681	332	12.4	3331	227	6.8	3149	213	6.8	3370	78	2.3
44	2044	251	12.3	2249	221	9.8	2036	131	6.4	1756	111	6.3
0	1723	177	10.3	1776	174	9.8	1637	145	8.8	1533	161	10.5
Avg CV %						9.0			6.2			6.3
Slope			18.0			26.8			26.4			25.4

8. Detection Antibody Titration

Experiment: Titrate D-Ab for new 1:5 sample dilution and new tip MFG process

Assay: TARC

Date: 3/1/09

Reagents: Pre-Made Dry Generic cartridges (TN wash buffer & substrate)

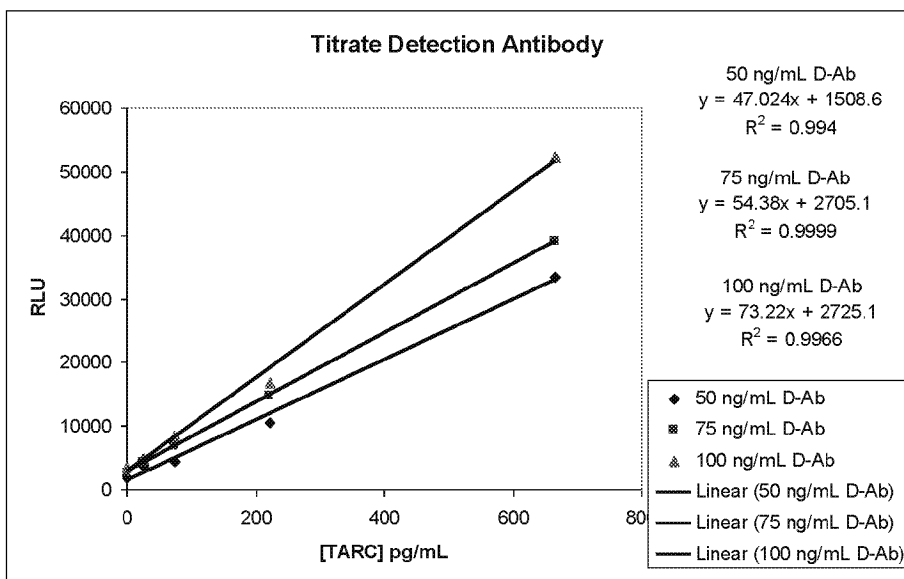
Protocol: Metabolic2_C svn 2231 - Tips 3-4, 1:5 edison dilution

Samples: Frozen Assay Buffer Calibrators Batch 1

D-Ab: Titrate in Stabilzyme (ab9816 conj 2/03/08)

Tips: 2/19/09 MFG HT TARC

Substrate: Lot 080870 open 2/12/09



Detection Antibody Titration

[TARC] pg/mL	50 ng/mL D-Ab			75 ng/mL D-Ab			100 ng/mL D-Ab		
	Mean RLU	StDev	CV %	Mean RLU	StDev	CV %	Mean RLU	StDev	CV %
2000	73469	17907	24.4	127267	16498	13.0	134492	16093	12.0
667	33398	4635	13.9	39008	2344	6.0	52207	3849	7.4
222	10413	628	6.0	14646	827	5.6	16917	3170	18.7
74	4522	670	14.8	6771	383	5.7	8404	679	8.1
25	3708	270	7.3	4281	318	7.4	4908	438	8.9
0	1962	267	13.6	2547	465	18.2	3531	57	1.6

9. Whole Blood and Plasma Screen

Blood was obtained from the Stanford blood bank and the whole blood and plasma derived from the blood was measured in the Theranos System.

Measured TARC in whole blood and plasma samples from Stanford blood bank

<i>Patient Info</i>		Whole Blood [Calc.] (pg/mL)	Plasma [Calc.] (pg/mL)	% Recovery
Date	Tube #			
2/12/09	1	96	17	18
2/12/09	2	68	47	68
2/12/09	3	26	21	81
2/12/09	4	65	6	9
2/12/09	5	117	42	36
2/12/09	6	48	11	22
2/12/09	7	126	18	14
2/13/09	1	234	29	13
2/13/09	2	37	8	20
2/13/09	3	24	22	90
2/13/09	4	74	35	48
2/13/09	5	44	30	68
2/13/09	6	57	66	116
2/13/09	7	38	31	82
3/19/09	1	191	77	40
3/19/09	2	47	37	79
3/19/09	4	266	279	105
3/19/09	5	70	23	32
3/19/09	6	125	74	60
3/19/09	7	25	8	31
3/19/09	8	86	23	26
3/19/09	9	34	8	23
3/19/09	10	105	53	50
3/19/09	11	132	75	56
3/19/09	12	41	19	47
3/19/09	13	215	31	14
3/19/09	14	92	67	73
3/19/09	15	249	32	13
3/19/09	16	82	18	22

10. Optimize Protocol

The effect of post-sample wash on assay response and sensitivity was tested. The assay appears to be little affected by the use of a post sample wash versus no post sample wash – using either assay buffer standards or spiked whole blood. Either protocol will provide good results for the Theranos TARC assay.

Date: 11/04/2008

Protocol: Generic_ND_svn 2090 versus Generic_ND_PSW_svn 2091

Samples: Spiked Whole Blood (Stanford 11/04/08) & Assay Buffer Calibrators, HD 1:5

Tips: 11/04/08 Tomtec 30-30-10 1x PA washes, 10ug/mL C-Ab

Dab: 350 ng/mL in Stabilzyme

No Post Sample Wash versus Post Sample Wash with Assay Buffer Standards

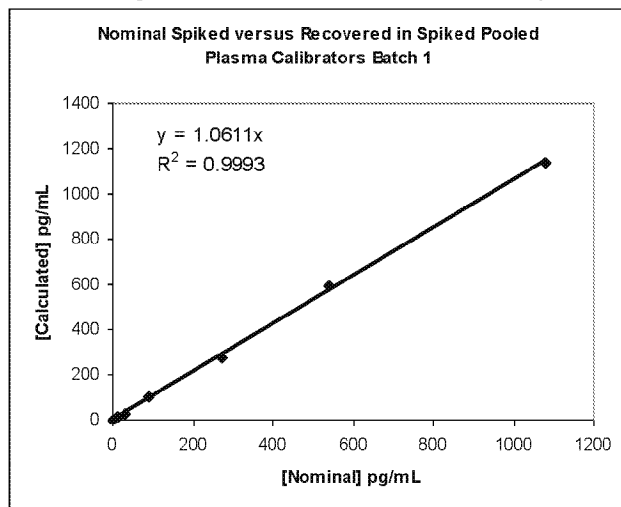
[In Sample] pg/mL	No post Sample Wash			Post Sample Wash		
	Mean	StDev	CV %	Mean	StDev	CV %
0	2947	474	16.1	2885	37	1.3
25	4056	481	11.9	3719	217	5.8
74	6106	4	0.1	5425	553	10.2
222	10679	746	7.0	9656	45	0.5
667	25485	1617	6.3	24435	2959	12.1
2000	81603	17206	21.1	79817	353	0.4
<i>Avg CV %</i>			<i>10.4</i>			<i>5.1</i>
<i>Slope Bottom 3</i>			<i>42.6</i>			<i>34.4</i>
<i>Avg StDev Bottom 3</i>			<i>319</i>			<i>269</i>
<i>LOD in Sample pg/mL</i>			<i>15.0</i>			<i>15.6</i>

No Post Sample Wash versus Post Sample Wash with Spiked Whole Blood

[In Sample] pg/mL	No post Sample Wash			Post Sample Wash		
	Mean	StDev	CV %	Mean	StDev	CV %
0	3751	560	14.9	3382	113	3.3
25	4149	148	3.6	3890	NA	NA
74	5200	9	0.2	4873	200	4.1
222	8904	974	10.9	8671	126	1.5
667	23728	2398	10.1	16892	1178	7.0
2000	69542	2053	3.0	52497	5049	9.6
<i>Avg CV %</i>			<i>7.1</i>			<i>5.1</i>
<i>Slope Bottom 3</i>			<i>19.8</i>			<i>20.1</i>
<i>Avg StDev Bottom 3</i>			<i>239</i>			<i>157</i>
<i>LOD in Sample pg/mL</i>			<i>24.1</i>			<i>15.6</i>

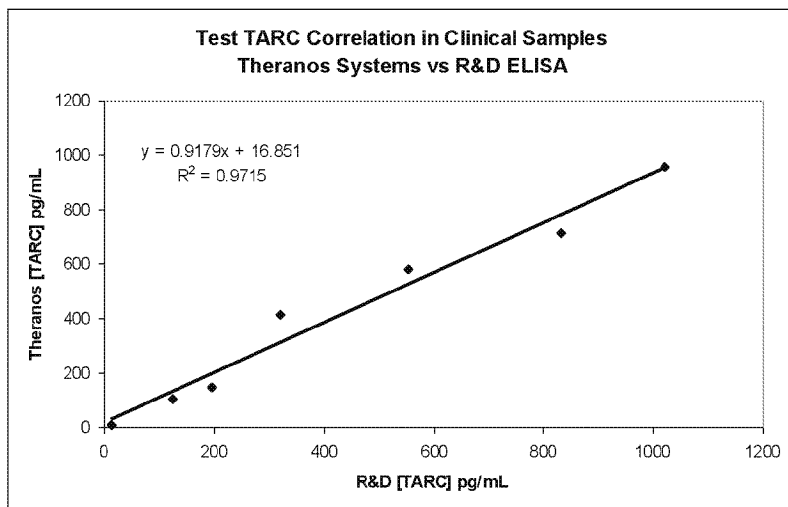
11. Confirming Assay Standards.

The analyte used for the Theranos assay is R&D recombinant human TARC, the same catalogue number as used in the R&D ELISA kit. Spiked pooled plasma calibrators were tested in R&D Quantikine kit. Average recovery was 100% and slope of Nominal versus Calculated was ~ 1. There is no need to reassign calibrators for this lot of analyte.



12. Preliminary Testing for Clinical Correlation and Confirming Clinical Range.

A set of clinical samples from 20 patients diagnosed with asthma (all patients are on at least one asthma medication) were tested in the R&D ELISA kit to confirm the assay range and to conduct a preliminary test for clinical correlation in the Theranos assay. The lowest result was 14 pg/mL and the highest 1020 pg/mL in the R&D kit. This confirms the clinical range of 10 – 2000 pg/mL (healthy to asthma patients) determined from the literature. Seven clinical samples spanning the range were selected and run on the Theranos assay to check for clinical correlation. Theranos (y) = 0.9179*Reference method (x) + 16.851; $R^2 = 0.9179$.



13. Precision

Three lots of reagents were used to create a 6 point standard curve and the Coefficients of Variation (CV) were determined. Samples were assayed in replicate (N = 2) on multiple instruments (N = 3) across the entire range of the assay.

Summary

Inter-run (on one instrument) CV: 7.8 %
 Inter-instrument CV: 10.2 %
 Inter-Lot System CV 6.0 %
 Total CV (any cartridge, any instrument): 13.0 %

Total CVs for 3 Reagent Lots

[TARC] pg/mL	Mean RLU	StDev	CV %
0	462	72	15.7
25	887	107	12.1
74	2027	261	12.9
222	5277	673	12.8
667	20945	2977	14.2
2000	79722	8194	10.3
Avg Total CV %			13.0

System CVs by Reagent Lot

[TARC] pg/mL	Mean RLU			System CVs		
	Lot 1	Lot 2	Lot 3	Mean	StDev	CV %
0	449	476	461	462	14	3.0
25	918	859	885	887	29	3.3
74	2021	2147	1912	2027	117	5.8
222	4687	5864	5279	5277	588	11.1
667	21924	19368	21542	20945	1379	6.6
2000	76183	85302	77681	79722	4890	6.1
Avg System CV %						6.0

One sample in the mid range of the assay (222 pg/mL) was assayed in a total of 24 cartridges on 24 different instruments to determine the mid-range Coefficients of Variation (CV).

Summary

Total CV (any cartridge, any instrument): 11.7 %
 Inter-Cartridge CV 10.8 %
 Intra-Cartridge CV 5.5 %

Inter and Intra-Cartridge CVs at 222 pg/mL

Cartridge #	Mean RLU	StDev	CV %
1	5170	90	1.7
2	5241	1008	19.2
3	5520	112	2
4	5694	21	0.4
5	5074	720	14.2
6	4993	167	3.3
7	5558	187	3.4
8	6070	407	6.7
9	5669	377	6.6
10	5186	143	2.8
11	5753	562	9.8
12	5854	25	0.4
13	6019	278	4.6
14	6777	363	5.4
15	4937	561	11.4
16	5946	56	0.9
17	5264	587	11.2
18	5630	408	7.2
19	4752	106	2.2
20	6531	322	4.9
21	5154	71	1.4
22	5143	375	7.3
23	4444	123	2.8
24	4475	64	1.4

14. Sensitivity

Limit of detection in plasma (95% confidence) = 10.6 pg/mL

Date: 3/12/2009

Protocol: Centocor_Multiplex_1 svn 2237

Samples: Frozen Assay Buffer Calibrators Batch 2, Frozen Spiked Plasma Calibrators Batch 1

D-Ab: 75 ng/mL in Stabilzyme (ab9816 conj 2/13/08)

Tips: 3/5/09 MFG HT TARC (dried in McDry)

<i>Standard Curve: Assay Buffer Calibrators Batch 2</i>			
[TARC]	Mean	StDev	CV %
2000	115739	12399	10.7
667	29222	1681	5.8
222	11132	262	2.3
74	6810	601	8.8
25	4297	52	1.2
0	2980	171	5.7
Avg CV %			5.8
Bottom 3 Slope			51.7
Bottom 3 Avg StDev			274
LOD in Sample pg/mL			10.6

15. Dilution Linearity

Two high-endogenous TARC clinical samples obtained from Bioreclamation were serially diluted into 2 low-endogenous samples with the results shown below. The result from the Theranos system agree within < 10 % of the target. The percentage recovery was calculated by the following formula: $[\text{Recovered S2}] / ([\text{Recovered S1}] * [\text{Nominal S2}] / [\text{Nominal S1}])$

<i>Sample # 6 into Sample #3</i>			<i>Sample #5 into Sample #2</i>		
[Nominal] (pg/mL)	[Recovered] (pg/mL)	% Recovery	[Recovered] (pg/mL)	[Nominal] (pg/mL)	% Recovery
802	802	NA	594	594	NA
408	403	99%	332	304	109%
211	180	86%	130	159	75%
113	97	101%	63	87	88%
64	57	105%	42	51	115%
15	15	NA	28	33	103%

16. Whole Blood and Plasma Spike Recovery

Samples of plasma and whole blood were spiked with known concentrations of rhTARC across the range of the assay.

rhTARC Spike Recovery in Plasma

[Nominal] pg/mL	[Calc.] pg/mL	% Recovery
2160	1877	86%
1080	1249	115%
540	521	94%
270	228	80%
90	66	60%
30	33	70%
0	12	NA

Avg % Recovery

84%

rhTARC Spike Recovery in Whole Blood

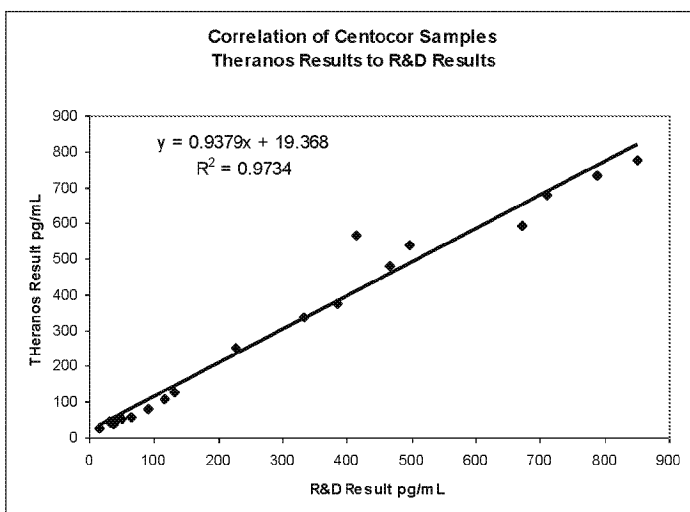
[Nominal] pg/mL	[Calc.] (pg/mL)	% Recovery
2000	1793	88%
667	545	77%
222	185	70%
74	72	56%
0	31	NA

Avg % Recovery

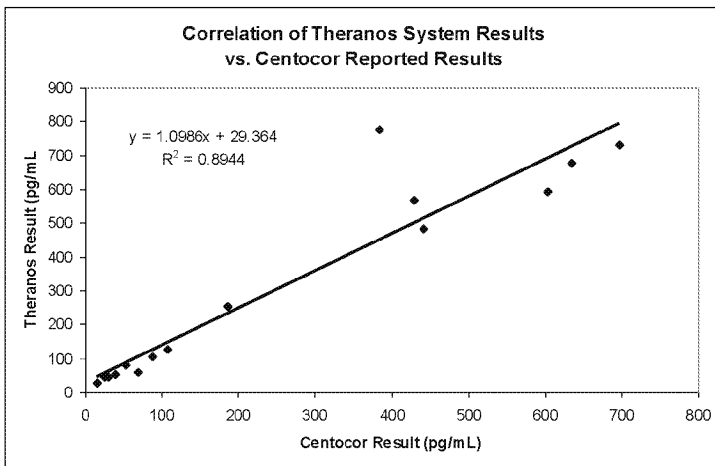
73%

17. Clinical Samples

Clinical asthma samples provided by Centocor were run in the Theranos system and the R&D ELISA. Correlation was Theranos (y) = 0.9379*Reference method (x) + 19.368; R² = 0.9734.



Centocor reported their measured values for 16 of these samples. Correlation was Theranos (y) = 1.0965 *Centocor (x) + 29.364; R² = 0.8944. With sample #11 acting as an outlier. Without Sample #11, the correlation is Theranos (y) = 1.043*Centocor (x) + 20.432; R² = 0.9837.



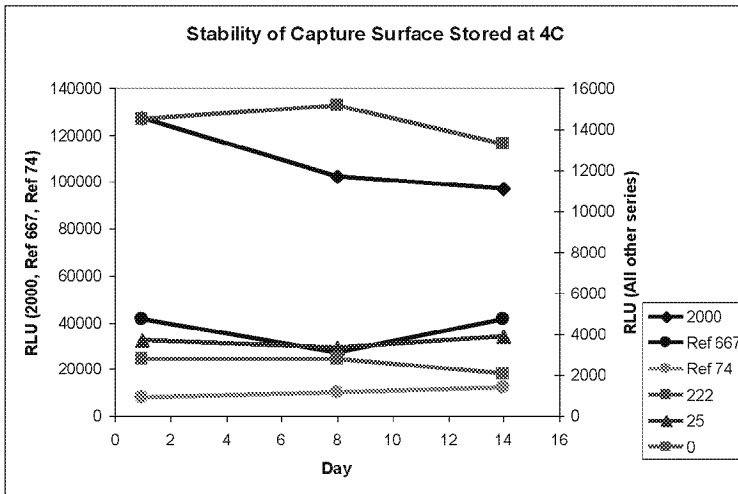
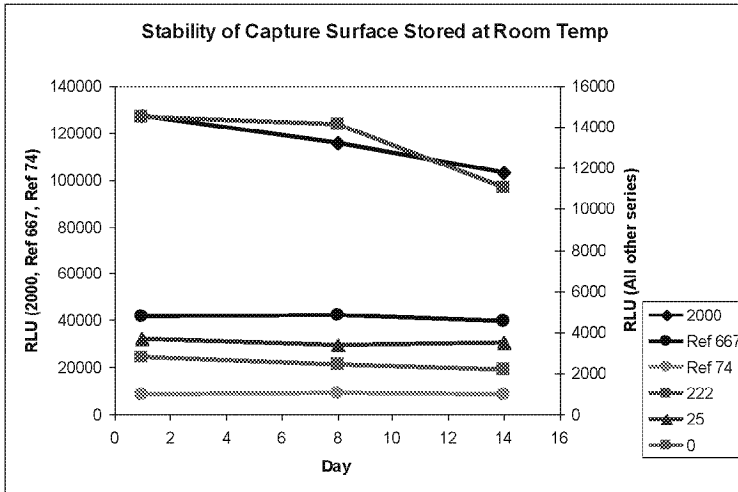
Results for 20 Centocor Clinical Asthma Samples

Sample#	Theranos Result (pg/mL)	R&D Result (pg/mL)	Centocor Reported Result (pg/mL)
1	45	31	24
2	337	333	Missing
3	594	671	603
4	45	33	30
5	41	36	Missing
6	48	40	29
7	81	90	54
8	59	65	69
9	376	383	Missing
10	53	49	39
11	777	851	384
12	107	117	88
13	28	15	16
14	677	711	635
15	128	131	108
16	253	227	185
17	537	497	Missing
18	732	789	697
19	482	467	441
20	568	415	429

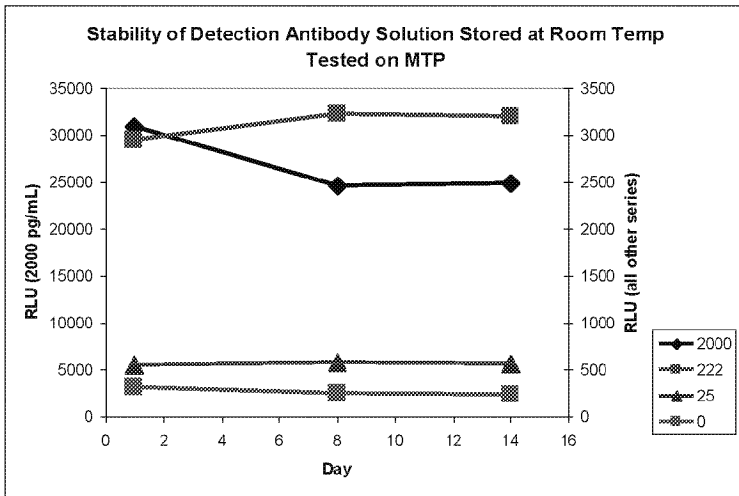
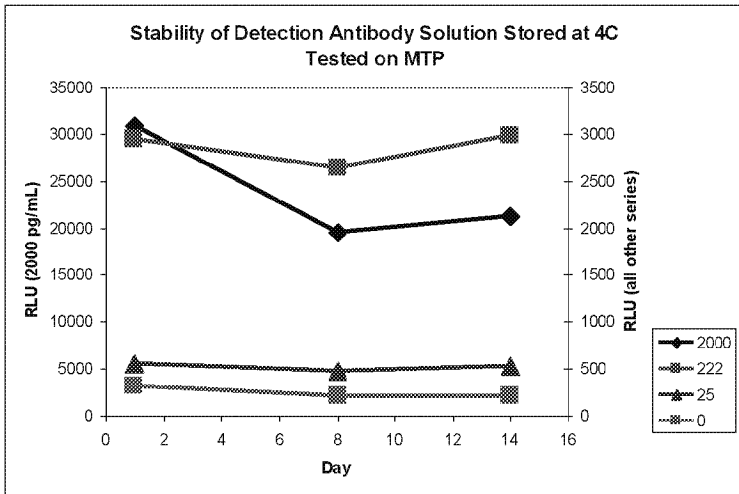
18. Stability

The stability of the capture surface and the working solution of detection antibody in stabilizer is in an ongoing stability study. The results to date are shown.

Stability of the Capture Surface and Dried References Stored at 4°C and at Room Temperature.



Stability of the Detection Antibody Solution Stored at 4°C and at Room Temperature.



19. Interfering Substances and Matrix Effects

The assay was tested for matrix effects with lipemic and hemolyzed plasma – no adverse effects occurred. No evidence of interfering substances has been found.

Spiked Normal Plasma

[Spiked] pg/mL	Mean RLU	StDev	CV%	[Conc.] pg/mL	% Recovery
1080	11995	838	7.0	1193	107%
270	2352	537	22.8	258	80%
0	461	18	3.9	42	NA
Avg % Recovery					93%

Spiked Lipemic Plasma

[Spiked] pg/mL	Mean RLU	StDev	CV%	[Conc.] pg/mL	% Recovery
1080	15983	3699	23.1	1499	135%
270	3685	623	16.9	404	134%
0	1023	101	9.8	108	NA
Avg % Recovery					119%

Spiked Hemolyzed Plasma - ProMedDx #11213871

[Spiked In]	Mean RLU	StDev	CV%	[Conc.] pg/mL	% Recovery
1080	17741	1684	9.5	1233	96%
270	4749	961	20.2	479	103%
0	1929	118	6.1	200	NA
Avg % Recovery					100%

20. Maximum Assay Range

Very high levels of rhTARC were spiked into assay buffer and whole blood, and the recovery in whole blood and the resulting plasma was measured to determine the upper range of the assay. Recovery of spikes up to 51,200 pg/mL were consistent with the normal assay range.

Standard Curve in Assay Buffer

[Conc.] pg/mL	Mean	StDev	CV%
51200	1862524	56526	3.0
12800	1051220	35621	3.4
3200	237375	34142	14.4
800	53688	1526	2.8
200	14286	756	5.3
0	2365	222	9.4

Spiked Whole Blood

[Spiked] pg/mL	Whole Blood		Plasma from Spiked Whole Blood	
	[Calc]. pg/mL	% Recovery	[Calc]. pg/mL	% Recovery
51200	41173	80%	34030	66%
12800	7528	59%	6971	54%
3200	2190	68%	2068	64%
800	612	73%	611	72%
200	186	79%	122	45%
0	27		32	
<i>Avg % Recovery</i>		72%		60%

21. Literature and References

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