Assay Development Report

Assay	RANTES (CCL5)
Developer	Elaine Than

1. Analyte background

RANTES (Regulated upon Activation, Normal T cell Expressed and presumably Secreted), also known as CCL5, is a member of the "CC" subfamily of chemokines. It plays a primary role in the inflammatory immune response via its ability to chemoattract leukocytes and modulate their function. Human RANTES cDNA encodes a highly basic 91 amino acid (aa) residue precursor polypeptide with a 23 aa hydrophobic signal peptide that is cleaved to generate the 68 aa mature protein.

RANTES is a potent chemoattractant for a number of different cell types including unstimulated CD4+/CD45RO+ memory T cells and stimulated CD4+ and CD8+ T cells with naive and memory phenotypes, NK cells, basophils, eosinophils, dendritic cells, mast cells, monocytes, and microglia. In addition to its effects on migration, RANTES can activate a number of cell types including T cells, monocytes, neutrophils NK cells, dendritic cells, and astrocytes.

RANTES, is known to interact with four identified seven transmembrane G-protein coupled receptors: CCR1, CCR3, CCR4, and CCR5. RANTES stimulation can initiate a variety of signaling cascades that are cell context dependent.

2. Assay specifications

The assay is designed to measure human and recombinant human RANTES in human blood and plasma, which has 68 aa mature protein. Human and mouse RANTES share approximately 85% sequence homology at the amino acid level and exhibit cross-species activities.

Reportable ranges are:

Sample type	Low, ng/ml	High, ng/ml
Human plasma	1	500
Human blood	12	500

3. Reference assays

R&D Systems: cat# DRN00B

This is a Quantikine ELISA formatted as a Sandwich ELISA, Range: 2000 – 31.25 pg/ml, 100 uL plasma sample size, Assay time is approximately 3.5 hours. This kit has been used in-house to validate the Theranos assay. Protocol can be found at the following link.

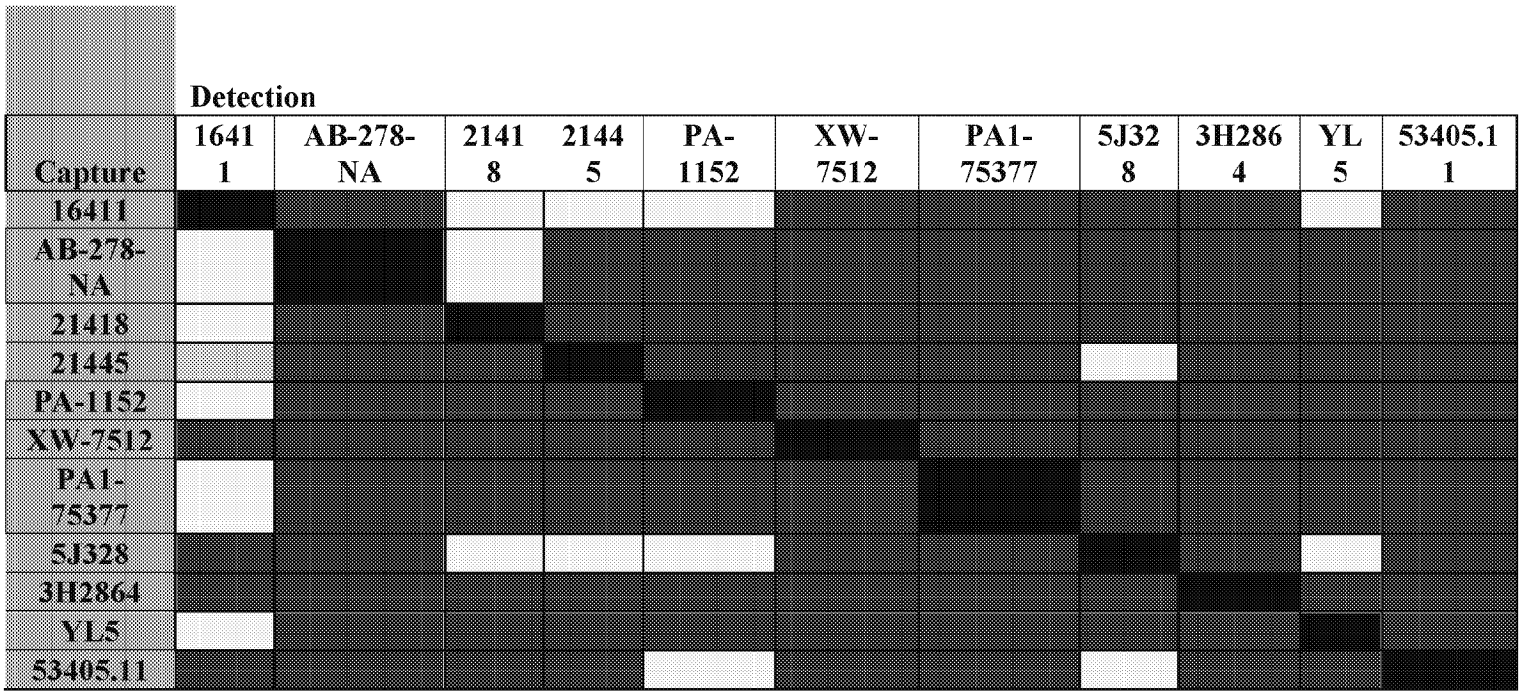
[HYPERLINK "http://www.rndsystems.com/pdf/dy201.pdf"]

4. Antibody screening for matched pair

Antibody screening was carried out in 384 well micro-titer plates, 10/10/10 assay time at room temperature and read on the M5.

Table 1: Antibody screen summary

Clone #	Vender	Catalog#	Lot#
16411	R&D	MAB2781	JFV026101
	R&D	AB-278-NA	DP07031
21418	R&D	MAB678	JA0908041
21445	R&D	MAB278	BHD016061
	Cell Sciences	PA-1152	2113601
	ProSCi	XW-7512	
	Affinity BioReagents (ABR)	PA1-75377	ABC-P1256
5J328	US Bio	R1100-01H	
3H2864	US Bio	R1100-01	L8050867
YL5	Santa Cruz Biotechnology	sc-74263	G1008
53405.11	Santa Cruz Biotechnology	sc-57422	H2807



Number of Capture antibody tested : 11
Number of Detection antibody tested: 11
Total Number of antibody pairs tested: 121

No Modulation
Modulation; good pairs
Modulation, chosen

Capture Ab: 21445 with detection Ab: 16411 met the criteria of good dose response for the range of the assay in the desired matrix (whole blood, plasma).

5. Assay Reagents

A. Capture Antibody

Vendor	R&D
Catalog #	MAB278
Current lot #	BHD016061
Type	Mouse monoclonal, IgG1
Specificity	Human RANTES.
Stock Conc.	In lyophilized from a 0.2um filtered solution in phosphate-buffered saline (PBS) with 5% trehalose; when reconstituted with 0.5ml of sterile PBS- the Ab concentration would be 1mg/mL of unlabeled Ab.
Data Sheet	[HYPERLINK "http://www.rndsystems.com/pdf/mab278.pdf"]

B. Detection Antibody

Vendor	R&D Systems
Catalog #	MAB2781
Current lot #	JFV026101
Type	Mouse monoclonal, IgG1
Specificity	Human RANTES.
Stock Conc.	In lyophilized from a 0.2um filtered solution in phosphate-buffered saline (PBS) with 5% trehalose; when reconstituted with 0.5ml of sterile PBS- the Ab concentration would be 1mg/mL of unlabeled Ab.
Data Sheet	[HYPERLINK "http://www.rndsystems.com/pdf/mab2781.pdf"]

C. Analyte

Vendor	R&D Systems
Catalog #	278-RN
Current lot #	DO1408091
Mol Wt	68 amino acid residue; 7.8kDa
Stock Conc.	Lyophilized from a 0.2 μm filtered solution in 30% acetonitrile and 0.1% TFA, containing 50 μg of bovine serum albumin per 1 μg of cytokine
Storage	Reconstituted in 1%BSA in PBS @100ug/ml. Aliquoted 5ul/tube. Flash frozen and stored at –20C. This is stable for 3 months. Lyophilized samples are stable up to 12 months at -20C to -70C. Avoid freeze thaw cycles
Data sheet	[HYPERLINK "http://www.rndsystems.com/pdf/278-rn.pdf"]

6. Reagent Handling and Storage

For the lyophilized detection and capture antibody, R&D Systems suggested storage at -20 to -70°C; stable up 12 months. After reconstitution, samples can be aliquotted, flashed frozen, and stored at -20 to -70C up to 6 months. Avoid repeated freeze thaw cycles.

Lyophilized analyte samples are stable for up to twelve months from date of receipt at -20° C to -70° C. Upon reconstitution, sample can be aliquotted in smaller volume, flashed frozen and stored at 2 - 8° C for one month or at -20° C to -70° Cfor three months without detectable loss of activity.Avoid repeated freeze-thaw cycles.

Biotin and Alkaline Phosphatase conjugates were stored at 4°C in Dojindo storage buffers. Since Dojindo specifies that the conjugates are stable for at least two months at 0-5°C; the conjugates were qualified at the end of three months either against a new conjugate or compared to historical data.

7. Protocols

Tip coating and assay protocols can be found at:

[HYPERLINK "RANTES%20(CCL5).%20Assay_%20Protocol_%20Reaction%20Tips.doc"]

Recommended Theranos system protocol is:

	Protocol name	Svn #	Sample dilutions	Tip positions	Prewash/ Post sample wash
1	Generic_200_PSW	2155	200x	200x to all six tips	2/2
2	Centocor Multiplex 1	2237	200x	Tips 5,6	2/2
3	Centocor Multiplex 3	2234	200x	Tip5 only	2/2

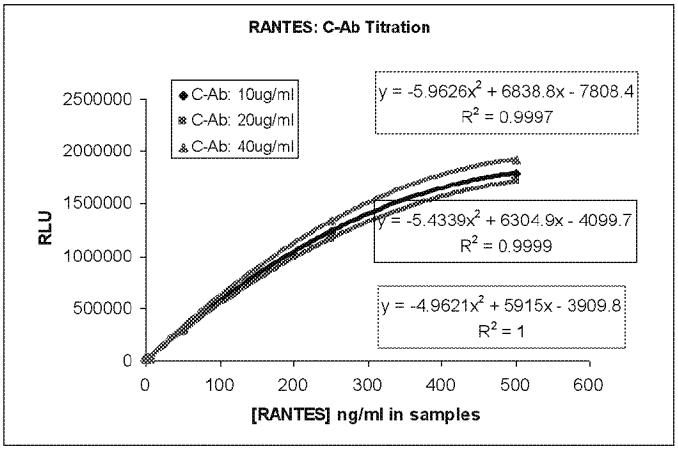
Links to Theranos instrument assay protocol steps:

[HYPERLINK "T:\\Assay_Systems\\Assay Development Reports\\Completed Assays\\Centocor Assays\\RANTES (CCL5)\\Edison Protocols\\Generic_200_PSW.py"]
[HYPERLINK "T:\\Assay_Systems\\Assay Development Reports\\Completed Assays\\Centocor Assays\\RANTES (CCL5)\\Edison Protocols\\Centocor_Multiplex_2.py"]
[HYPERLINK "T:\\Assay_Systems\\Assay Development Reports\\Completed Assays\\Centocor Assays\\RANTES (CCL5)\\Edison Protocols\\Centocor_Multiplex_3_svn 2234.py"]

8. Capture Antibody Titration

Capture antibody was tested at 10, 20, and 40 ug/mL on reactions tips and read on M5 reader. A concentration of 10 ug/mL was determined to be optimum.

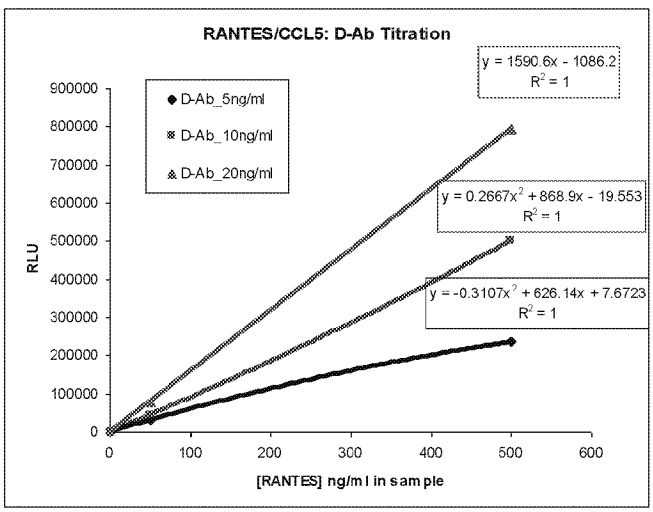
Tips Coat protocol	30'UA/3x wash/30'Cab/3x wash/ 10' fixative-Tomtec
UA	35 ug/mL in carbonate bi carbonate buffer, 10 mg/mL stock reconstitiuted, aliquoted, and stored @-20C
Cab	C-Ab @10, 20, 40ug/ml (clone:21445.1 (R&D; cat: MAB278))
Dab	50 ng/ml in StabilZyme AP Stabilizer. (clone: 16411; R&D; cat:MAB2781)
Analyte	rhCCL5/RANTES (R&D System, cat: 278-RN; lot: DO108051; recon: 11/03/08 in 1%BSA in PBS stock: 100ug/ml in -20C)
Edison	Generic_ND_PSW svn 2091 (10.10.10); 200 fold hand dilution



C-Ab Titration	10ug/ml	20ug/ml	40ug/ml
Analyte	**Values**	**Values**	**Values**
ng/mL in sample			
500	1788757	1712589	1918969
250	1237517	1166819	1338185
50	283837	274046	294440
5	24644	20702	24480
0.5	2630	3399	2709
0	361	324	421

9. Detection Antibody Titration

Tips Coat	
protocol	30'UA/3x wash/30'Cab/3x wash/ 10' fixative-Tomtec
UA	35 ug/mL in carbonate bi carbonate buffer, 10 mg/mL stock reconstitiuted, aliquoted, and stored @-20C
Cab	10ug/ml (clone: 21445 (R&D; cat:MAB278)) conj. On 12/02/08
Dab	5, 10, or 20 ng/ml in StabilZyme AP Stabilizer. (clone: 16411; R&D; cat:MAB2781)
Analyte	rhCCL5/RANTES (R&D System, cat: 278-RN; lot: DO108051; recon: 11/03/08 in 1%BSA in PBS stock: 100ug/ml in -20C)
Edison	Generic_ND_PSW svn 2091 (10.10.10); 200 fold hand dilution



D-Ab Titration	5ng/ml	10ng/ml	20ng/ml
Analyte	**Values**	**Values**	**Values**
ng/mL in sample			
500	235392	501094	794423
50	30539	44093	76217
0.5	215	289	512
0	112	105	115

10. UltraAvidin Titration

Tips Coat	
protocol	30'UA/3x wash/30'Cab/3x wash/ 10' fixative-Tomtec
UA	20 & 35 ug/mL in carbonate bi carbonate buffer, 10 mg/mL stock reconstitiuted, aliquoted, and stored @-20C
Cab	10ug/ml (clone: 21445 (R&D; cat:MAB278)) conj. On 12/02/08
Dab	5, 10, or 20 ng/ml in StabilZyme AP Stabilizer. (clone: 16411; R&D; cat:MAB2781)

Analyte	rhCCL5/RANTES (R&D System, cat: 278-RN; lot: DO108051; recon: 11/03/08 in 1%BSA in PBS stock: 100ug/ml in -20C)
Edison	Generic_200_PSW svn 2155

UA_20ug/ml

	[RANTES] ng/ml in sample	TOTAL Avg.
1	500	658475
2	50	12781
3	0.5	217
4	0	166
	Slope	253
	average Stdev	227
	LOD ng/ml in sample	1.8
	S/B	3971
	avg. CV%	7

UA_35ug/ml

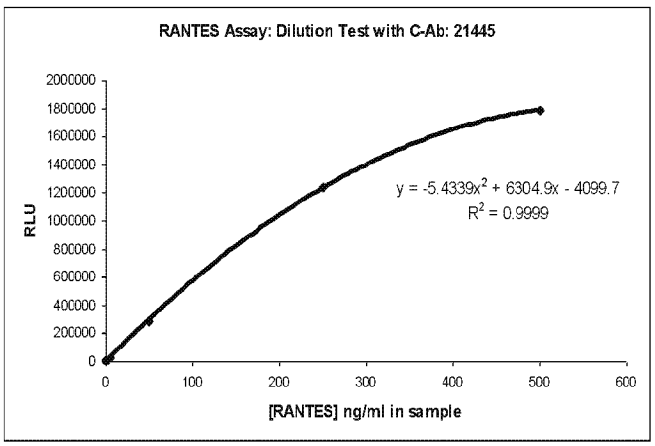
	[RANTES] ng/ml in sample	TOTAL Avg.
1	500	680909
2	50	14491
3	0.5	278
4	0	178
	Slope	287
	average Stdev	715
	LOD ng/ml in sample	5.0
	S/B	3833
	avg. CV%	14

35ug/ml will be use for further studies even though UltraAvidin is saturated at 20ug/ml.

11. Sample Dilution

Robot Coated tips: [30.30.10 coating protocol with 3 washes after both NA and Cab steps.] Dry in 37C O/N then in McDry
C-Ab @10, 20, 40ug/ml (clone:21445.1 (R&D; cat: MAB278))
UltraVidin @35ug/ml in Carb-bi-Carb.
Analyte: rhCCL5/RANTES (R&D System, cat: 278-RN; lot: DO108051; recon: 11/03/08 in 1%BSA in PBS stock: 100ug/ml in -20C)
D-Ab @ 50 ng/ml in StabilZyme AP Stabilizer. (clone: 16411; R&D; cat:MAB2781)
Protocol: Generic_ND_PSW svn 2091 (10.10.10); hand dilution

The assay was optimized at 200x on Theranos system



[RANTES] ng/ml in sample	Avg.	Stdev	%CV
500	1788757	127803	7
250	1237517	89963	7
50	283837	2519	1
5	24644	4853	20
0.5	7061	6267	89
0	361	46	13

12. Dilution Linearity

Two clinical asthma samples- sample#1 with RANTES concentration of 148.16 ng/ml and sample # 2 with a RANTES concentration of 0.48 ng/ml were used to test for dilution linearity. Both concentrations were validated on reference ELISA kit (R&D Systems; cat# DRN00B).
Sample #1 was diluted serially 4 fold into sample # 2, to the specified target concentrations. The results are shown below.
The following equation was used to determine the recovery percentage: 100*(Calculated Conc/(Calculated Conc of previous sample *Nominal Conc. / Nominal Conc of previous sample)

The experiment was repeated to see reproducibility.

Experiment #1

Nominal [RANTES] ng/ml in sample	**Calculated [RANTES] ng/ml in sample**	**% Recovery**
168.1	168.1	NA
42.2	53.1	126
10.7	9.4	70
2.8	2.8	110
0.9	1.1	133
0.4	0.9	183
0.7	0.7	NA
	Avg % Recovery	124.30

Experiment# 2

Nominal [RANTES] ng/ml in sample	**Calculated [RANTES] ng/ml in sample**	**% Recovery**
188.80	188.8	NA
37.16	61.1	129
9.41	10.7	69
2.47	2.8	100
0.74	1.2	143
0.30	0.7	168
0.60	0.6	NA
	Avg Recovery%	121.88

13. Whole Blood screen

Nineteen human blood samples were screened to check for samples with low endogenous RANTES level to do spike-in experiments to generate percentage recovery in whole blood vs. plasma.
An assay buffer standard curve was used to calculate sample concentrations.

Blood sample#	**Avg**	**Stdev**	**CV%**	**Calculated [RANTES] ng/ml in sample**
1	55189	7965	14	56.3
2	77398	5140	7	73.2
3	190319	20148	11	132.8
4	28908	7743	27	32.6
5	83620	17812	21	77.5
6	124476	6762	5	102.0
7	116571	10927	9	97.7

8	190827	14518	8	133.1
9	194761	63745	33	134.8
10	123721	26522	21	101.6
11	97966	5832	6	86.8
12	24186	2855	12	27.9
13	9628	383	4	12.3
14	192597	12331	6	62.9
15	201104	15717	8	65.2
16	969290	23065	2	464.5
17	447476	26075	6	132.8
18	324233	7390	2	97.7
19	29326	12052	41	33.0

14. Precision

A 8 point assay buffer standard curve was assayed on replicate cartridges for 3 lots to determine precision. The cartridge used a 200x fold dilution of the sample.

Intra lot CV%: 8.7%
Inter lot CV%: 13.7%

Inter and Intra CV values							
[RANTES] ng/ml in sample	**Lot 1**	**Lot 2**	**Lot 3**	**TOTAL INTER CV%**			**Intra Lot CV%**
				Avg. Value	**Stdev**	**CV%**	
500	493947	593253	572965	553388	52468	9	10.5
250	352087	340484	419368	370646	42591	11	13.5
50	45863	43431	52831	47375	4879	10	5.7
10	5924	7014	7714	6884	902	13	5.6
5	1807	2924	3012	2581	672	26	9.9
2.5	1563	1684	1623	1623	61	4	5.0
0.5	499	358	445	434	71	16	10.8
0	210	184	141	178	35	19	8.3

15. CV%

One sample in the mid range of the assay (50 ng/mL) was assayed in a total of 24 cartridges on 24 different instruments to determine the mid-range Total CV.
Total CV (any cartridge, any instrument) at mid range: 27%

16. Whole Blood Spike In

RANTES was spiked at 8 levels ranging from 500 – 0.5 ng/mL into human whole blood. The recovery average is 76%. Recovery was calculated against an assay buffer standard curve. Plasma spun down from the spiked blood. Recovery for this plasma has an average recovery of 20%.
Percent recovery = observed ÷ expected × 100%.

Blood sample#1

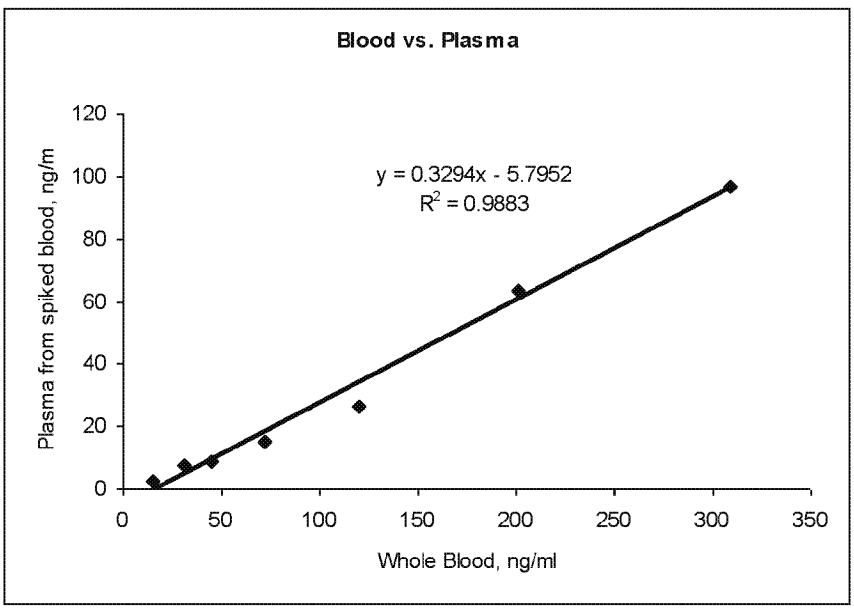
Spike Into Whole Blood

	Nominal [RANTES] ng/ml in sample	**TOTAL Avg.**	**Calculated [RANTES] ng/ml in sample**	**% Recovery**

1	**515**	818473	309	60
2	**265**	596883	201	76
3	**140**	351785	120	86
4	**90**	192938	72	80
5	**53**	112869	45	86
6	**34**	76226	31	93
7	**15**	35086	15	NA
	avg recovery %	**80**		

Plasma (retrieved from spiked whole blood sample)

	Nominal [RANTES] ng/ml in sample	**TOTAL Avg.**	**Calculated [RANTES] ng/ml in sample**	**% Recovery**
1	**515**	273566	97	19
2	**265**	166361	63	24
3	**140**	64056	27	19
4	**90**	35502	15	17
5	**53**	20046	9	17
6	**34**	16759	7	22
7	**15**	5597	3	17
	avg recovery %	**19**		



Blood sample#2

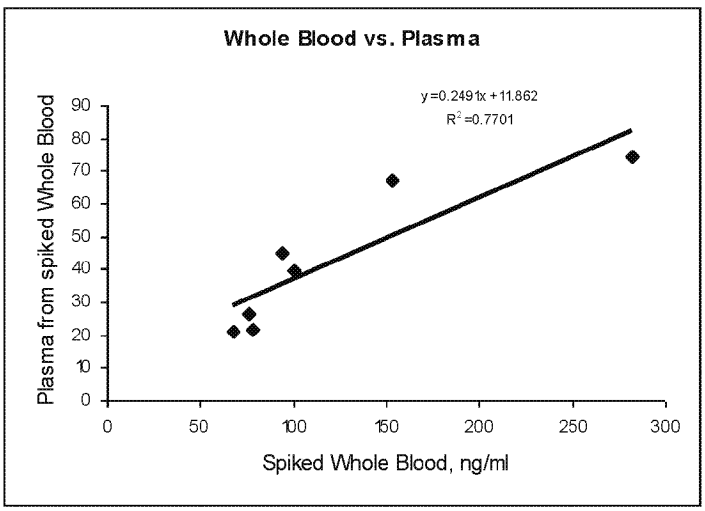
Spike Into Whole Blood

	Nominal [RANTES] ng/ml in sample	**TOTAL Avg.**	**Calculated [RANTES] ng/ml in sample**	**% Recovery**
1	**567**	867060	282	50
2	**317**	608169	153	48
3	**192**	377852	94	49
4	**142**	408089	100	70
5	**105**	302249	78	75

6	**86**	289094	76	88
7	**67**	247585	67	NA
	avg recovery %	**63**		

Plasma (retrieved from spike in whole blood sample)

	Nominal [RANTES] ng/ml in sample	TOTAL Avg.	Calculated [RANTES] ng/ml in sample	% Recovery
1	**567**	281359	74	13
2	**317**	248314	67	21
3	**192**	147509	45	23
4	**142**	126218	39	28
5	**105**	63729	22	21
6	**86**	79305	26	31
7	**67**	61184	21	31
	avg recovery %	**24**		



17. Plasma Spike In

RANTES was spiked at 8 levels ranging from 500 – 0.5 ng/mL into human plasma. The recovery ranged from 52 -81%, with an average of 67%. Recovery was calculated against an assay buffer standard curve.
Percent recovery = observed ÷ expected × 100%.

Plasma Sample #1

Avg Recovery %		**56**	
[RANTES] ng/ml in sample	**TOTAL Avg.**	**Calculated [RANTES] ng/ml in sample**	**% Recovery**
501	297768	208	43
251	163271	110	44
51	16446	23	27
11	3363	6	46
6	1931	3	59
4	1534	2	75
2	1038	1	94
1	779	1	NA

Plasma Sample #2

avg recovery %		78	
Nominal [RANTES] ng/ml in sample	**TOTAL Avg.**	**Calculated [RANTES] ng/ml in sample**	**% Recovery**
504	838629	322	64
254	589990	198	78
129	295590	104	80
79	139488	54	69
41	87037	35	86
23	47637	20	89
4	8582	4	NA

Plasma Sample #3

avg recovery %		52	
Nominal [RANTES] ng/ml in sample	**TOTAL Avg.**	**Calculated [RANTES] ng/ml in sample**	**% Recovery**
515	674174	177	34
265	452014	110	41
140	214719	60	43
90	169583	50	55
53	107640	34	65
34	77553	26	76
15	44125	15	NA

Plasma Sample #4

avg recovery %		81	
Nominal [RANTES] ng/ml in sample	**TOTAL Avg.**	**Calculated [RANTES] ng/ml in sample**	**% Recovery**
511	944033	342	67
261	627131	159	61
136	387246	96	70
86	194234	56	65
48	132869	41	85
29	79221	26	90
20	66126	22	112
11	29718	11	100

18. Extended Assay Range

Assay response at 4-fold higher concentration than highest level (500 ng/mL)of the Theranos standard curve was tested by spiking 2000 ng/mL into whole blood and plasma to check assay response for out of range high samples. It resulted in good modulation at the top end.

Spiked Whole Blood

avg recovery %		86	
Nominal [RANTES] ng/ml in sample	**TOTAL Avg.**	**Calculated [RANTES] ng/ml in sample**	**% Recovery**

2044	1858967	1168	57
1044	1583335	753	72
544	1470174	620	114
294	1119791	320	109
169	757327	148	88
107	508332	84	79
76	424787	69	91
44	266816	44	NA

Plasma recovered from spiked whole blood

avg recovery %	**15**		
Nominal [RANTES] ng/ml in sample	**TOTAL Avg.**	**Calculated [RANTES] ng/ml in sample**	**% Recovery**
2044	1096859	305	15
1044	670195	122	12
544	312638	51	9
294	265237	44	15
169	107950	22	13
107	74819	17	16
76	45905	13	17
44	27933	10	23

Spiked in plasma

avg recovery %	**79**		
Nominal [RANTES] ng/ml in sample	**TOTAL Avg.**	**Calculated [RANTES] ng/ml in sample**	**% Recovery**
2009	1927461	1294	64.4
1009	1615485	795	78.8
509	1378043	525	103.1
134	491339	81	60.3
41	149238	28	68.8
9	21794	9	100.0

19. Validation

A. Clinical Serum Samples

Bioreclamation Asthma Serum Samples (commercially available) on Reference (R&D ELISA kit) vs. Theranos system

i. Edison Systems:
Edison Protocol: Generic_200_PSW svn 2155
Coating: 30.30.10 with Cab: 10ug/ml
Dab: 20ng/ml in Stabilzyme

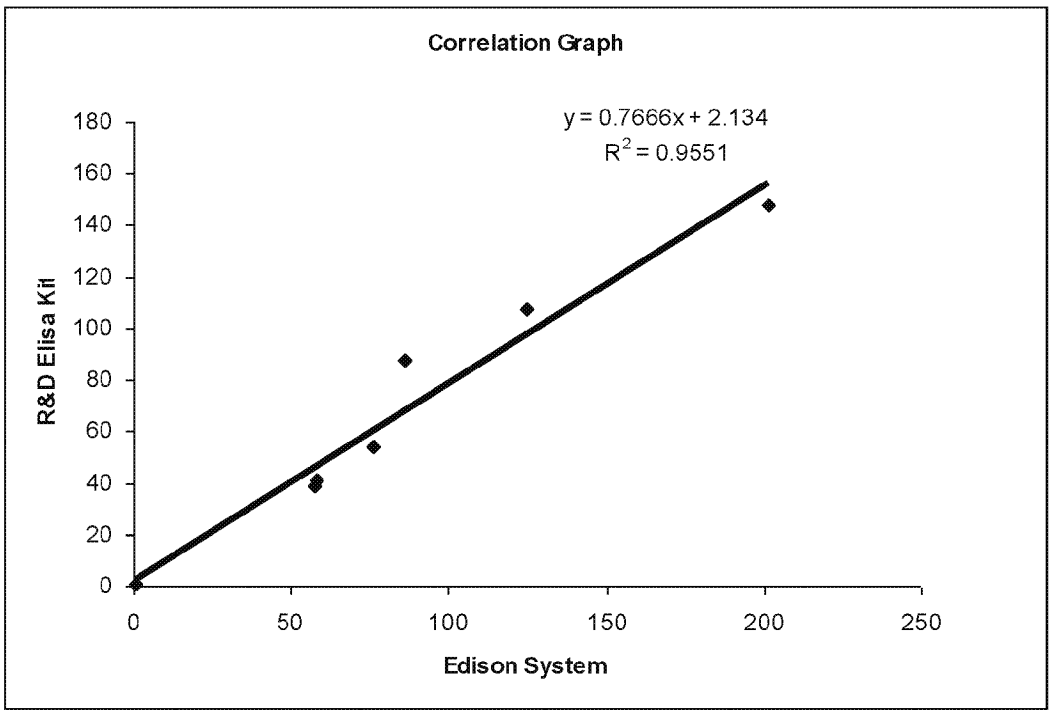
ii. R&D Systems ELISA Kit: cat: DRN00B; lot# 256262; exp: 24JULY-09

HUMAN SERUM (ASTHMA)
CURRENT INVENTORY 11/3/08

Theranos #	ID	Lot	in stock VOLUME (ML)	AGE	GENDER	MEDICATIONS
2	955	BRH236142	3	83	Female	Advair, Micardis, Singulair, Zopenex, Oscal, Combivent Albuterol
3	1247	BRH236143	3	46	Male	Advair, Lisinopril, Zocor
4	1436	BRH236144	8	34	Female	Albuterol
7	1446	BRH236147	3	10	Male	Dexamethasone
10	1900	BRH236150	8	58	Male	Albuterol, Advair, Amaryl, Novolog
15	2229	BRH236155	7	46	Female	Albuterol, Singulair, Azmacort
20	2437	BRH236160	12	51	Female	Prednisone, Advair, Singulair

Table 1: Clinical serum samples in Theranos Edison system vs. R&D Commercial Elisa Kit

	R&D Elisa	Edison System
Sample#	**Calculated [RANTES] ng/ml in sample**	**Cal. Conc. ng/ml in samples**
2	148.16	201.5
3	54.44	76.3
4	41.23	57.9
7	0.48	0.7
10	39.21	57.4
15	107.69	125.0
20	87.53	86.2



20. Matrix Effects

RANTES was spiked in into both lipemic (ProMedDx 11121165) and hemolyzed (Stanford Blood 7/8/08) plasma samples. Theranos protocol used: Centocor_Multiplex_1 svn 2237

A) In lipemic (data below), RANTES' average recovery was 58%.

Nominal [RANTES] ng/ml in sample	TOTAL Avg.	Calculated [RANTES] ng/ml in sample	% Recovery
502	1284059	261	52.1
127	576483	63	49.4
52	235664	38	73.0
2	10559	2	NA

B) In hemolyzed sample (data below), RANTES' average recovery was 65%.

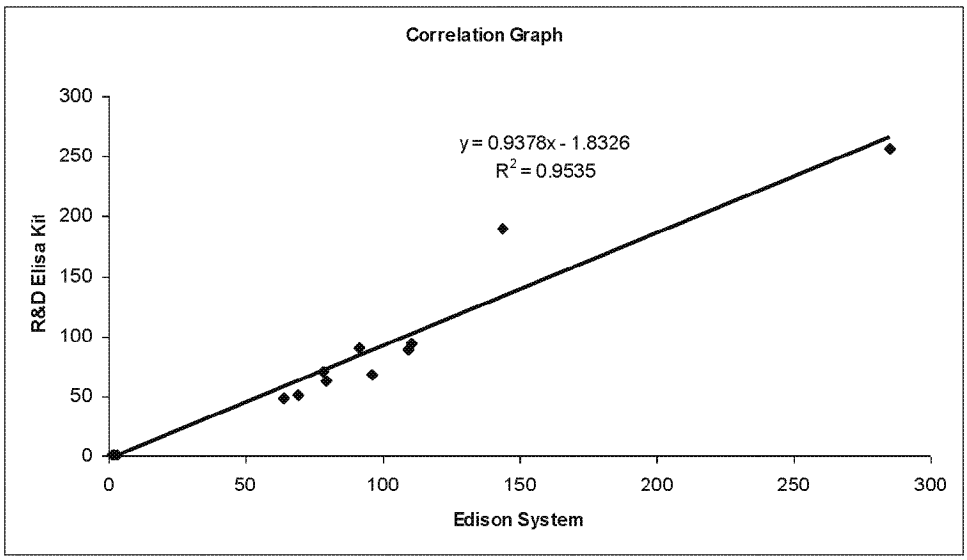
Nominal [RANTES] ng/ml in sample	TOTAL Avg.	Calculated [RANTES] ng/ml in sample	% Recovery
547	1516049	447	81.8
172	802822	87	50.9
97	542955	60	62.3
47	336927	47	NA

21. Centocor's Samples

The following are calculated RANTES value for Centocor's samples using Theranos system vs. R&D Elisa Kit. The dilution factor of samples on Theranos system is 200x. On R&D system, all except 10 samples were 4 fold dilutions. 10 samples (# 2, 3, 9, 11, 14, 16, 17, 18, 19, and 20) were diluted 100 fold.

Protocol used: **Generic_200_PSW svn 2155**

Centocor Samples	Edison System (Assay Buffer)	R&D Elisa
Sample	Calculated [RANTES] ng/ml in sample	Cal. Conc. ng/ml in samples
1	1.3	1.14
2	143.6	189.6
3	63.8	48.6
4	2.8	1.88
5	1.5	1.11
6	1.5	1.35
7	1.5	1.21
8	1.5	1.23
9	110.4	94.5
10	1.2	0.85
11	69.0	51.7
12	2.7	1.73
13	1.3	0.82
14	79.4	63.0
15	1.6	1.22
16	109.3	88.7
17	78.0	71.3
18	91.4	90.8
19	285.2	256.2
20	95.8	68.3



22. Stability

An on going 3 months stability test set up for both D-Ab and C-Ab.

1. **Tips Stability**

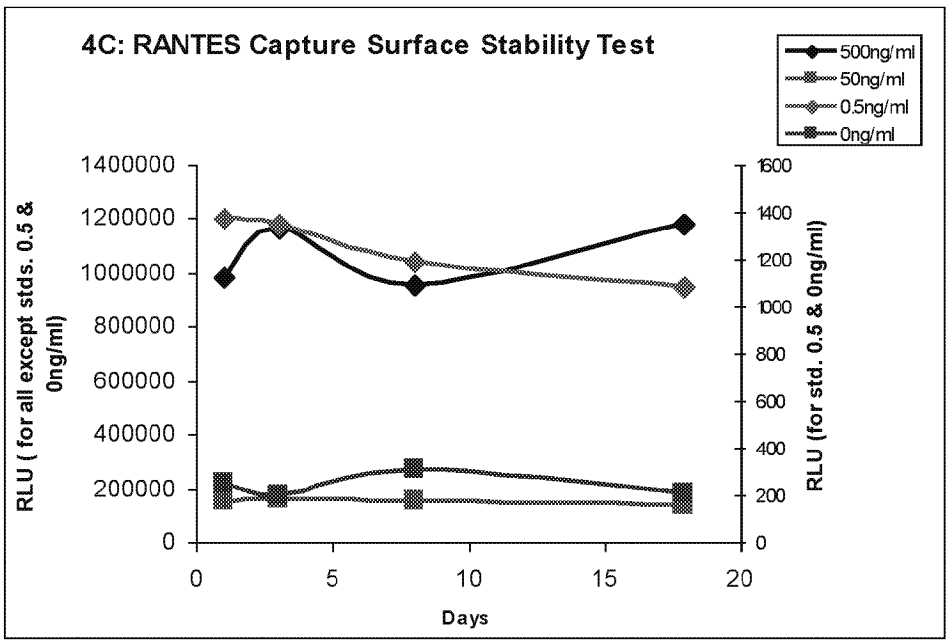
Robot Coated Test tips dated 2/27/09 by Mfg on large Tom Tec
UltraVidin @35ug/ml in Carb-bi-Carb. (pre-coated)
Cab_21445 @10ug/ml conj. 2/25/09
Analyte: rhCCL5/RANTES (R&D System, cat: 278-RN; lot: DO108051) frozen calibrators made on 03/03/09 stored at -80C
D-Ab @ 20 ng/ml in StabilZyme AP Stabilizer. (clone: 16411; R&D; cat:MAB2781) conj. On 12/11/08

Analyte- Frozen Calibrators prepared on 03/03/05
200x hand dilution from frozen calibrator with Blocking buffer (3%BSA in TBS).
20ul/well
Dab_16411 [conj. 12/11/08] @ 20ng/ml in Stabilzyme AP

Protocol: Generic_200_PSW svn 2155

Four standard point: 500. 50, 0.5, 0 ng/ml
Reference Capture surfaces: 100, 10 ng/ml

Figure 1: Progression Graph of Tips Stored at 4C.



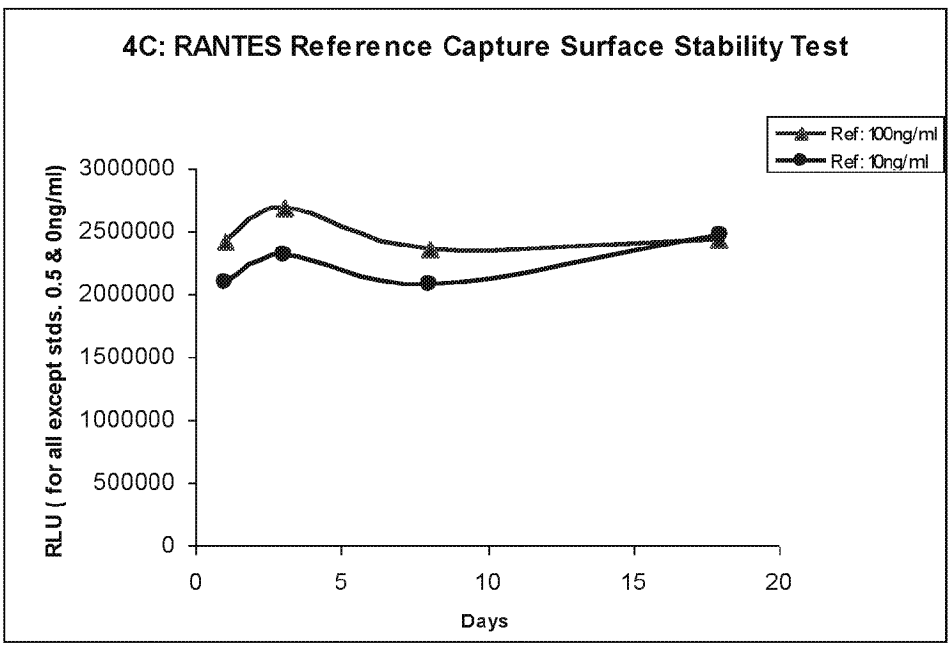
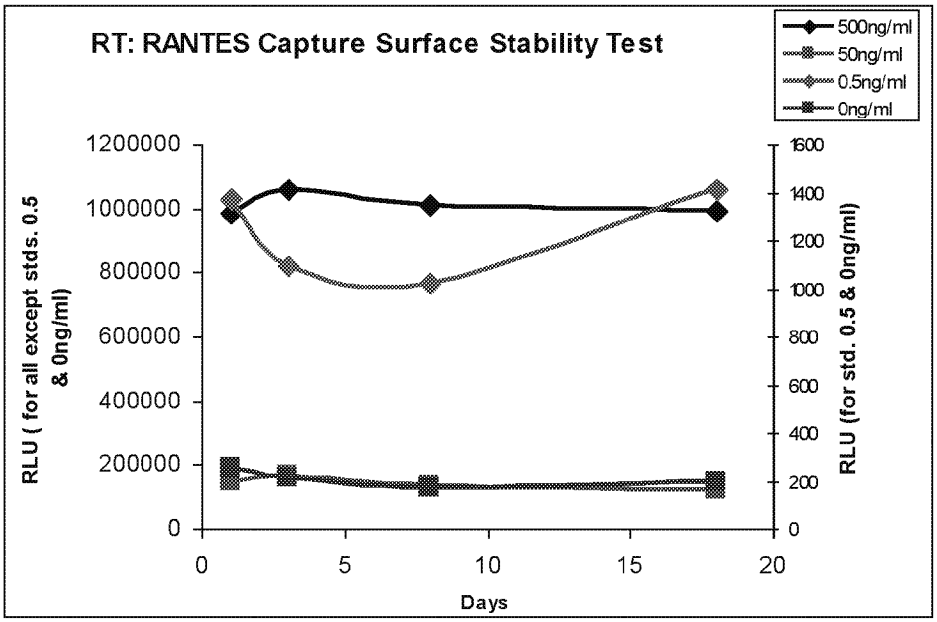
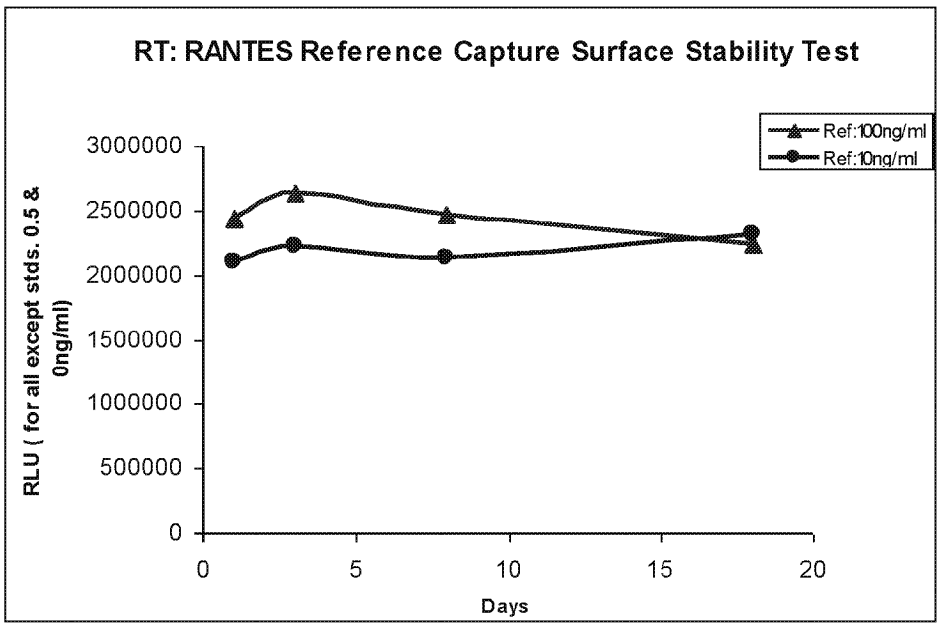


Figure 2: Progression Graph of Tips Stored at Room Temperature (25C-27C).





2. **D-Ab Stability Test**

384 Plate is coated one day prior to the test date. Dry at McDry O/N
[30.30.10 coating protocol with 3 washes after both NA and Cab steps (Wash buffer: 105ul/well).]
Cab_21445.1 @10ug/ml conj. 01/26/09 (stock @500 ug/ml stored @ 4C) 15ul/well
UltraVidin @35ug/ml in Carb-bi-Carb. 15ul/well
Fixative: 105ul/well
Analyte:
Analyte- Frozen Calibrators prepared on 03/03/05
200x hand dilution from frozen calibrator with Blocking buffer (3%BSA in TBS).
20ul/well

Dab_16411 [conj. 12/11/08] @ 20ng/ml in Stabilzyme AP

Control: Freshly diluted working concentration: 20ng/ml in StabilZyme from stock: 500 ug/ml

Figure 1: Progression Graph of Tips Stored at 4C.

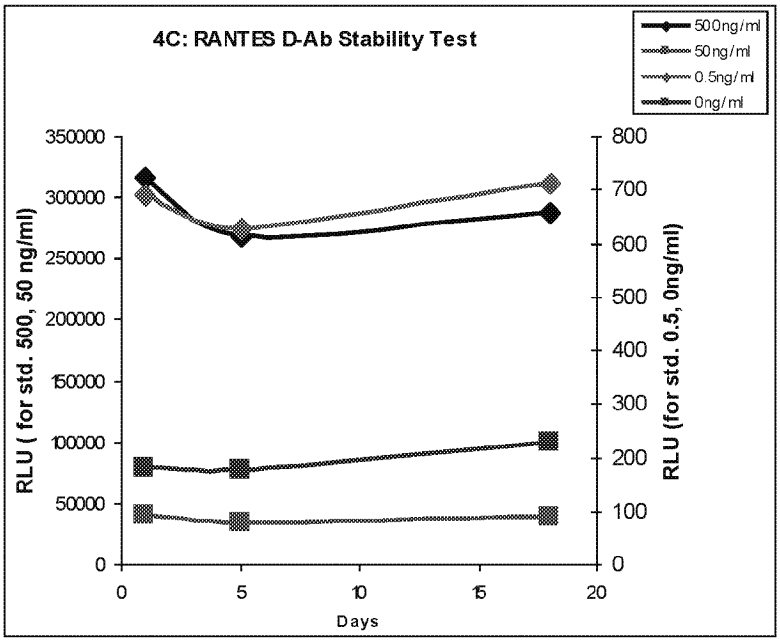
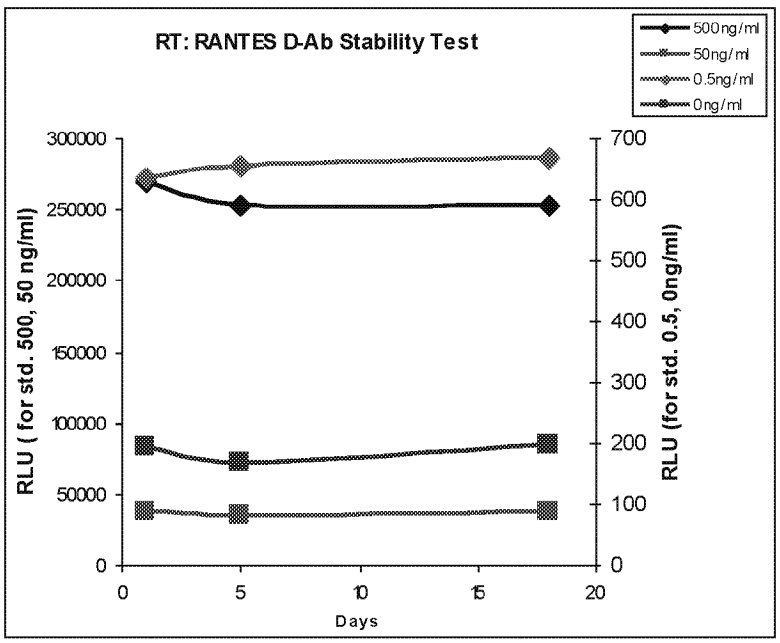


Figure 2: Progression Graph of Tips Stored at Room Temperature (25C-27C).



23. Literature

1. Decreased T-bet expression and changes in chemokine levels in adults with asthma

[download: [HYPERLINK "http://www3.interscience.wiley.com/cgi-bin/fulltext/117996213/PDFSTART"]]

2. Expression of the inflammatory chemokines CCL5, CCL3 and CXCL10 in juvenile idiopathic arthritis, and demonstration of CCL5 production by an atypical subset of CD8+ T cells.

[download: [HYPERLINK "http://arthritis-research.com/content/8/2/R50"]]