



# Group A Streptococcus 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" ]

An enzyme linked immunosorbent assay (ELISA) was developed for the quantitative detection of Group A Streptococcus from throat swabs. The Group A Streptococcus is a class of bacterium containing A class Streptococcus antigens and is a major contributor to pharyngitis and tonsillitis. This report describes the assay development and performance of the Group A Streptococcus assay as aid to diagnosing Strep A infections. Clinical data is required to further validate the assay and will be generated once a biosafety level 3 laboratory is available.

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

The following commercial kit has been used in house as predicate methods:

- Bioquant, Strep A Rapid Test Strip (BQ 058-RTIF)

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

The Group A Streptococcus assay is a sandwich ELISA utilizing a biotin labeled antibody coated on an avidin surface and detection with alkaline phosphatase labeled antibodies. The capture antibody is a rabbit polyclonal (MyBiosource, MBS53463) specific to Group A Streptococcus. The detection antibody is also a rabbit polyclonal antibody (MyBiosource, MBS220642) labeled with alkaline phosphatase. An antigen extraction step is required before performing the assay. Samples are tested undiluted and co-incubated with the detector antibody on the capture surface for 5 minutes. After incubation, a wash cycle is performed and the alkaline phosphatase substrate is incubated on the surface for 5 minutes, and the resulting chemiluminescence is read in Relative Light Units (RLU) on the Edison readers.

**Table [ SEQ Table \\* ARABIC ]:** Materials

Name	Supplier	Catalog #
Rabbit anti-Streptococcus Group A	MyBiosource	MBS534636
Rabbit anti-Streptococcus Group A	MyBiosource	MBS220642
Heat inactivated Streptococcus Group A	MyBiosource	MBS537397
Stabilzyme AP	Surmodics	SA01-2000
Alkaline Phosphatase Labeling Kit (SH)	Dojindo	LK13-10
Biotin Labeling Kit (SH)	Dojindo	LK10
Sodium Nitrite	J.T. Baker	3780-01
Acetic Acid	EMD	AX0073-9
Tris	VWR	VW1500-01
Phospho Glo Substrate	KPL	

Blocking Buffer (3% BSA in TBS, 0.05% Sodium Azide)	Sigma (BSA, Fraction V, 99% Pure)	A3059-500G
10X PBS	Sigma	D1408-500ML
Theranos Cartridge	Theranos	
Edison Readers	Theranos	

### 1.1.3 Labeling of Capture Antibody

The rabbit anti-Streptococcus Group A antibody (MyBiosource, MBS534636) is labeled with biotin according to kit instructions (Dojindo, LK10)

### 1.1.4 Labeling of Detector Antibody

The rabbit anti-Streptococcus Group A antibody (MyBiosource, MBS220642) is labeled with alkaline phosphatase according to kit instructions (Dojindo, LK13-10).

### 1.1.5 Preparation of 2M Sodium Nitrite

Weigh the appropriate amount of sodium nitrite to make 2M Nitrite (for example, add 2.76g sodium nitrite to water up to 20 mL).

### 1.1.6 Preparation of 1M Acetic Acid

Add 2.9 mL of 17.4 M acetic acid to 47.1 mL water.

### 1.1.7 Preparation of 3M Tris

Weigh the appropriate amount of Tris to make 3M Tris (for example, add 7.5g Tris to water up to 20 mL).

### 1.1.8 Preparation of Assay Buffer

The assay buffer is prepared by dissolving 1 packet of TBS into water and adding 10 mL of 10% azide, and 30 g of BSA to a final volume of 1000mL. The final composition of the assay buffer is 3% BSA, 50mM Tris, 138mM NaCl, 2.7mM KCl pH 8.0 in water. The assay buffer is filtered before use.

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

### 2.1 Antibody Screen 1

A total of 20 antibodies were screened using a direct assay format. In the assay, heat inactivated cells were immobilized on the surface of a microtiter plate, blocked, and washed before adding alkaline phosphatase labeled antibodies to the wells. Alkaline phosphatase substrate was added to the wells, and the resulting chemiluminescence was read on the M5 reader. Based on signal and modulation, antibodies 1, 2, 3, 4, 5, 6, and 8 were potentially good antibodies.

**Table [ SEQ Table \\* ARABIC ]:** Direct Assay Antibody Screen

Antibody	Strep A [cells]	AVG	CV	Modulation
1	1000000	55159	13%	101.62
	200000	18248	1%	33.62
	40000	7327	0%	13.50
	0	543	3%	1.00
2	1000000	308984	19%	405.14
	200000	108781	5%	142.63
	40000	44394	9%	58.21
	0	763	25%	1.00
3	1000000	368676	4%	398.10
	200000	114970	9%	124.14
	40000	41731	3%	45.06
	0	926	33%	1.00
4	1000000	322325	2%	147.13
	200000	100351	4%	45.81
	40000	31943	0%	14.58
	0	2191	1%	1.00
5	1000000	434612	7%	394.67
	200000	147386	7%	133.84
	40000	53371	13%	48.47
	0	1101	62%	1.00

Antibody	Strep A [cells]	AVG	CV	Modulation
6	1000000	119902	2%	188.46
	200000	39849	3%	62.64
	40000	18553	2%	29.16
	0	636	37%	1.00
7	1000000	251	8%	1.08
	200000	267	7%	1.15
	40000	344	30%	1.49
	0	232	15%	1.00
8	1000000	10028	15%	10.23
	200000	3350	6%	3.42
	40000	1440	11%	1.47
	0	981	6%	1.00
9	1000000	704	27%	0.87
	200000	1072	2%	1.32
	40000	1558	52%	1.92
	0	813	18%	1.00
10	1000000	195	42%	0.69
	200000	270	36%	0.97
	40000	243	60%	0.87
	0	280	37%	1.00

Antibody	Strep A [cells]	AVG	CV	Modulation
11	1000000	580	48%	1.82
	200000	648	85%	2.03
	40000	243	3%	0.76
	0	319	2%	1.00
12	1000000	1233	17%	4.23
	200000	636	36%	2.18
	40000	340	9%	1.17
	0	292	13%	1.00
13	1000000	825	8%	1.56
	200000	564	15%	1.07
	40000	700	52%	1.33
	0	527	5%	1.00
14	1000000	488	32%	1.81
	200000	302	1%	1.12
	40000	212	1%	0.78
	0	270	5%	1.00
15	1000000	568	34%	1.74
	200000	311	0%	0.95
	40000	465	34%	1.42
	0	327	40%	1.00



Antibody	Strep A [cells]	AVG	CV	Modulation
16	1000000	476	36%	0.96
	200000	346	25%	0.70
	40000	290	16%	0.58
	0	498	38%	1.00
17	1000000	424	61%	1.66
	200000	270	23%	1.06
	40000	255	14%	1.00
	0	255	14%	1.00
18	1000000	245	16%	1.16
	200000	171	16%	0.81
	40000	159	0%	0.75
	0	212	48%	1.00
19	1000000	138	34%	1.51
	200000	105	10%	1.15
	40000	138	2%	1.51
	0	91	3%	1.00
20	1000000	328	6%	1.00
	200000	515	41%	1.57
	40000	358	18%	1.09
	0	328	38%	1.00

## 2.2 Antibody Screen 2 [ TC "Plasma Screening" \f C \l "1" ]

Antibodies 1, 2, 3, 4, 5, 6, and 8 were further tested using a sandwich format and antigen extraction step. In this format, Streptococcus Group A antigens were extracted for 10 minutes using 2M nitrite, 1M acetic acid and neutralized with 3M tris. This extraction procedure has been shown to work and is commonly used in commercial Rapid Strep A tests. Extracts were added to wells coated with capture antibodies, blocked, washed, and detector antibodies added. Alkaline phosphatase substrate were added to wells, and the resulting chemiluminescence read on the M5 reader. Antibodies 1, 2, 3, 4, 5, and 6 gave modulations.

**Table [ SEQ Table \\* ARABIC ]:** Capture Antibody 1 (Novus Biologicals, clone 3401, NB200-644)

DAB	Extract [Dilution]	RLU-1	RLU-2	AVG	CV	Modulation
1	1	1808	1614	1711	8%	1.45
	5	1203	1191	1197	1%	1.01
	25	1529	931	1230	34%	1.04
	0	1269	1098	1184	10%	1.00
2	1	7466	9015	8240	13%	30.34
	5	5068	4680	4874	6%	17.94
	25	2650	2390	2520	7%	9.28
	0	272	272	272	0%	1.00
3	1	11882	10062	10972	12%	4.78
	5	5949	6465	6207	6%	2.70
	25	4160	3380	3770	15%	1.64
	0	2255	2336	2295	3%	1.00
4	1	4397	4044	4220	6%	7.74
	5	2076	1773	1925	11%	3.53
	25	861	974	918	9%	1.68
	0	768	322	545	58%	1.00
5	1	9077	8630	8853	4%	4.05
	5	4765	4878	4822	2%	2.20
	25	2480	2701	2590	6%	1.18
	0	1754	2619	2187	28%	1.00
6	1	2569	2542	2555	1%	5.70
	5	1211	1087	1149	8%	2.56
	25	563	648	605	10%	1.35
	0	473	423	448	8%	1.00
8	1	400	380	390	4%	1.39
	5	407	334	371	14%	1.32
	25	194	244	219	16%	0.78
	0	264	299	281	9%	1.00

**Table 4:** Capture Antibody 2 (MyBiosource, MBS220642)

DAB	Extract [Dilution]	RLU-1	RLU-2	AVG	CV	Modulation
1	1	89939	78968	84454	9%	83.98
	5	45032	51225	48128	9%	47.86
	25	4344	6712	5528	30%	5.50
	0	841	1170	1006	23%	1.00
2	1	1194914	1194906	1194910	0%	3.74
	5	1000402	1040132	1020267	3%	3.20
	25	952146	975049	963597	2%	3.02
	0	333595	304677	319136	6%	1.00
3	1	1058238	1172867	1115552	7%	5.47
	5	1029079	1117569	1073324	6%	5.27
	25	909362	953064	931213	3%	4.57
	0	190331	217284	203807	9%	1.00
4	1	694054	762532	728293	7%	9.62
	5	693737	663784	678760	3%	8.96
	25	461393	442919	452156	3%	5.97
	0	89273	62180	75727	25%	1.00
5	1	1002277	1091655	1046966	6%	5.30
	5	912323	986047	949185	5%	4.80
	25	717221	781913	749567	6%	3.79
	0	144365	250996	197680	38%	1.00
6	1	514381	508277	511329	1%	16.18
	5	503855	471903	487879	5%	15.44
	25	266718	259855	263286	2%	8.33
	0	51070	12122	31596	87%	1.00
8	1	6728	6270	6499	5%	5.01
	5	5953	3333	4643	40%	3.58
	25	911	767	839	12%	0.65
	0	1868	725	1296	62%	1.00

**Table 5: Capture Antibody 3 (MyBiosource, MBS534252)**

DAB	Extract [Dilution]	RLU-1	RLU-2	AVG	CV	Modulation
1	1	280734	253241	266988	7%	97.74
	5	114900	94684	104792	14%	38.36
	25	10754	8715	9734	15%	3.56
	0	3382	2082	2732	34%	1.00
2	1	2428497	2400317	2414407	1%	24.41
	5	2182184	2265132	2223658	3%	22.48
	25	1463078	1455782	1459430	0%	14.75
	0	116380	81449	98914	25%	1.00
3	1	2225826	2106475	2166150	4%	13.76
	5	2034983	2020191	2027587	1%	12.88
	25	1431092	1379042	1405067	3%	8.92
	0	155191	159727	157459	2%	1.00
4	1	1390931	1343961	1367446	2%	46.65
	5	1162270	1064465	1113367	6%	37.98
	25	568715	578725	573720	1%	19.57
	0	33206	25423	29314	19%	1.00
5	1	2203585	2239912	2221749	1%	18.03
	5	2027879	1905452	1966665	4%	15.96
	25	1352384	1266285	1309334	5%	10.62
	0	127127	119390	123258	4%	1.00
6	1	1037765	947801	992783	6%	90.64
	5	773138	807471	790304	3%	72.15
	25	343619	330012	336816	3%	30.75
	0	14949	6959	10954	52%	1.00
8	1	621	606	613	2%	6.81
	5	464	280	372	35%	4.13
	25	222	142	182	31%	2.02
	0	81	100	90	15%	1.00

**Table 6: Capture Antibody 4 (MyBiosource, MBS534636)**

DAB	Extract [Dilution]	RLU-1	RLU-2	AVG	CV	Modulation
1	1	19717	18126	18921	6%	14.22
	5	10928	16741	13834	30%	10.39
	25	1629	4334	2981	64%	2.24
	0	1037	1626	1331	31%	1.00
2	1	866191	943262	904726	6%	72.95
	5	853087	890337	871712	3%	70.29
	25	676881	634322	655601	5%	52.86
	0	9822	14982	12402	29%	1.00
3	1	850528	949810	900169	8%	55.36
	5	821467	819455	820461	0%	50.45
	25	634651	664489	649570	3%	39.95
	0	21373	11149	16261	44%	1.00
4	1	445199	508236	476718	9%	48.11
	5	380946	463761	422354	14%	42.63
	25	276570	284728	280649	2%	28.32
	0	7948	11868	9908	28%	1.00
5	1	806038	855394	830716	4%	22.83
	5	806998	804600	805799	0%	22.15
	25	540782	625743	583262	10%	16.03
	0	30530	42234	36382	23%	1.00
6	1	279194	266476	272835	3%	33.12
	5	272994	259752	266373	4%	32.33
	25	176015	150649	163332	11%	19.82
	0	10358	6120	8239	36%	1.00
8	1	145	157	151	5%	0.31
	5	84	122	103	26%	0.21
	25	153	134	143	9%	0.29
	0	593	386	490	30%	1.00

**Table 7: Capture Antibody 5 (MyBiosource, MBS535227)**

DAB	Extract [Dilution]	RLU-1	RLU-2	AVG	CV	Modulation
1	1	64502	60608	62555	4%	26.65
	5	27755	24170	25962	10%	11.06
	25	4039	3008	3523	21%	1.50
	0	2558	2137	2347	13%	1.00
2	1	1096767	1024402	1060584	5%	7.52
	5	1094064	1050685	1072374	3%	7.61
	25	779211	724677	751944	5%	5.33
	0	226541	55444	140992	86%	1.00
3	1	1084022	1097907	1090964	1%	16.35
	5	1073917	1164104	1119011	6%	16.77
	25	681712	658984	670348	2%	10.04
	0	39875	93604	66740	57%	1.00
4	1	726606	703827	715217	2%	66.39
	5	625382	593486	609434	4%	56.57
	25	333160	307498	320329	6%	29.74
	0	7152	14393	10772	48%	1.00
5	1	989398	1050416	1019907	4%	15.12
	5	969521	1014228	991874	3%	14.71
	25	599470	594725	597097	1%	8.85
	0	79278	55604	67441	25%	1.00
6	1	480907	388576	434742	15%	20.48
	5	437790	369660	403725	12%	19.02
	25	173753	166652	170203	3%	8.02
	0	32563	9897	21230	75%	1.00
8	1	6288	6074	6181	2%	11.55
	5	1742	2336	2039	21%	3.81
	25	562	664	613	12%	1.15
	0	574	496	535	10%	1.00

**Table 8:** Capture Antibody 6 (MyBiosource, MBS534589)

DAB	Extract [Dilution]	RLU-1	RLU-2	AVG	CV	Modulation
1	1	30268	28545	29406	4%	11.44
	5	21433	17578	19506	14%	7.59
	25	4500	2493	3496	41%	1.36
	0	3258	1881	2569	38%	1.00
2	1	1379991	1370451	1375221	0%	16.37
	5	1201344	1205487	1203415	0%	14.33
	25	1060170	1133765	1096968	5%	13.06
	0	118303	49670	83986	58%	1.00
3	1	1274039	1254453	1264246	1%	11.24
	5	1232555	1319524	1276039	5%	11.35
	25	971747	1104462	1038105	9%	9.23
	0	64213	160718	112466	61%	1.00
4	1	628644	582370	605507	5%	26.16
	5	552891	530712	541801	3%	23.41
	25	449243	442877	446060	1%	19.27
	0	16648	29641	23144	40%	1.00
5	1	1029679	1070721	1050200	3%	22.74
	5	1032445	1002834	1017639	2%	22.04
	25	772195	820261	796228	4%	17.24
	0	37573	54774	46173	26%	1.00
6	1	456432	422241	439337	6%	20.38
	5	445908	428438	437173	3%	20.28
	25	268724	282198	275461	3%	12.78
	0	31630	11486	21558	66%	1.00
8	1	3254	3031	3143	5%	0.46
	5	3146	2458	2802	17%	0.41
	25	1958	2450	2204	16%	0.32
	0	11447	2266	6856	95%	1.00

**Table 9:** Capture Antibody 8 (Lifespan Biosciences, LS-C58832)

DAB	Extract [Dilution]	RLU-1	RLU-2	AVG	CV	Modulation
1	1	2335	2751	2543	12%	1.13
	5	4257	2884	3571	27%	1.58
	25	1339	3405	2372	62%	1.05
	0	2339	2168	2253	5%	1.00
2	1	8406	8869	8637	4%	7.41
	5	5075	4106	4590	15%	3.94
	25	3596	1973	2784	41%	2.39
	0	1751	580	1166	71%	1.00
3	1	9566	12204	10885	17%	2.95
	5	6873	6982	6927	1%	1.88
	25	3580	5289	4435	27%	1.20
	0	3744	3639	3691	2%	1.00
4	1	4238	4736	4487	8%	0.91
	5	2187	2389	2288	6%	0.46
	25	4102	7635	5869	43%	1.19
	0	3670	6184	4927	36%	1.00
5	1	11227	10686	10957	3%	3.61
	5	4888	4631	4759	4%	1.57
	25	2631	4082	3357	31%	1.11
	0	2810	3265	3037	11%	1.00
6	1	3308	4989	4148	29%	1.07
	5	2066	3144	2605	29%	0.67
	25	1199	2888	2043	58%	0.53
	0	1269	6483	3876	95%	1.00
8	1	724	1708	1216	57%	0.32
	5	977	1775	1376	41%	0.37
	25	3397	5347	4372	32%	1.16
	0	4043	3491	3767	10%	1.00



## 2.3 Antibody Screen 3

The best modulating antibody pairs were further tested in an extended standard curve to determine preliminary sensitivity levels. The most sensitive antibody combination was capture antibody 4 with detection antibody 2.

**Table 10:** Sensitivity (Top AVG RLU, Bottom Modulations)

	CAB1/DAB2	CAB2/DAB1	CAB3/DAB1	CAB4/DAB2	CAB5/DAB4	CAB6/DAB4
Strep A	AVG RLU	AVG RLU	AVG RLU	AVG RLU	AVG RLU	AVG RLU
1000000	23578	129371	233836	1225204	998187	869988
500000	13902	100464	186555	1176581	924645	898556
250000	8240	63333	115063	1204901	857782	850778
125000	4566	27288	52469	1124942	641063	744887
62500	3374	7345	18554	963150	427895	649938
31250	2419	3443	8030	810998	294468	381789
15625	1429	2709	4374	621521	192047	361754
7813	1258	2002	3200	418782	100593	162427
3906	1055	2055	2668	266137	58705	90457
1953	1100	1840	2519	155732	35262	48700
0	943	1961	2494	7481	5711	5916

### [ TC "Capture Surface Titration on the Therasos System" \f C \ "1" ]

	CAB1/DAB2	CAB2/DAB1	CAB3/DAB1	CAB4/DAB2	CAB5/DAB4	CAB6/DAB4
Strep A	Modulation	Modulation	Modulation	Modulation	Modulation	Modulation
1000000	25.0	66.0	93.7	163.8	174.8	147.0
500000	14.7	51.2	74.8	157.3	161.9	151.9
250000	8.7	32.3	46.1	161.1	150.2	143.8
125000	4.8	13.9	21.0	150.4	112.3	125.9
62500	3.6	3.7	7.4	128.7	74.9	109.9
31250	2.6	1.8	3.2	108.4	51.6	64.5
15625	1.5	1.4	1.8	83.1	33.6	61.1
7813	1.3	1.0	1.3	56.0	17.6	27.5
3906	1.1	1.0	1.1	17.9	10.3	15.3
1953	1.2	0.9	1.0	20.8	6.2	8.2
0	1.0	1.0	1.0	1.0	1.0	1.0

## 2.4 Specificity

The most sensitive antibody pair Cab4/Dab2 was tested for cross reactivity to a number of potential cross reactants in a sandwich assay. None of the cross reactants at 5ug/mL appear to significantly interfere with the assay.

Group B Streptococcus

Group C Streptococcus

Candida albicans

Haemophilus influenzae b polysaccharide polyribosyl ribitol phosphate

Neisseria meningitis serogroup A

Neisseria meningitis serogroup C

Corynebacterium diphtheriae 20kD antigen (a.a.26-216)

Streptococcus mutans major cell-surface adhesin PAc (Antigen I/II)  
(a.a.61-460)

Streptococcus pneumoniae pneumococcal vaccine antigen A (a.a.18-204)

Streptococcus salivarius SK126 Surface antigen (a.a.38-338)

Streptococcus sanguinis Cell surface SD repeat antigen, (a.a.421-770)

Staphylococcus aureus penicillin-binding protein 1 (a.a.61-361)

Bordetella Pertusis Vaccine (whole cell)

**Table 11: Cross Reactivity**

Conditions	Cells	AVG RLU	% Signal Difference	Back-Calculated	Recovery
Control-No Cross Reactants	100000	69428	0%	100269	100%
	50000	35032	0%	47789	96%
	25000	22280	0%	28332	113%
	0	2797	0%		
Group B Streptococcus, Group C Streptococcus, Candida albicans, Haemophilus influenzae b polysaccharide polyribosyl ribitol phosphate	100000	69131	0%	99817	100%
	50000	38607	10%	53243	106%
	25000	21957	-1%	27839	111%
	0	2282	-18%		
Neisseria meningitis serogroup A, Neisseria meningitis serogroup C, Corynebacterium diphtheriae 20kD antigen (a.a.26-216), Streptococcus mutans major cell-surface adhesin PAc (Antigen I/II) (a.a.61-460)	100000	89764	29%	131299	131%
	50000	45811	31%	64235	128%
	25000	24772	11%	32134	129%
	0	2400	-14%		
Streptococcus pneumoniae pneumococcal vaccine antigen A (a.a.18-204), Streptococcus salivarius SK126 Surface antigen (a.a.38-338), Streptococcus sanguinis Cell surface SD repeat antigen, (a.a.421-770), Staphylococcus aureus penicillin-binding protein 1 (a.a.61-361)	100000	94415	36%	138394	138%
	50000	35396	1%	48345	97%
	25000	19591	-12%	24229	97%
	0	2748	-2%		
Bordetella Pertusis Vaccine (whole cell)	100000	77395	11%	112426	112%
	50000	44105	26%	61633	123%
	25000	23147	4%	29655	119%
	0	2733	-2%		

## 2.5 Effect of Detector Diluents

The optimum detector antibody diluent was determined by comparing 3% BSA to Stabilzyme AP and Biostab. Stabilzyme AP gave the lowest background and highest modulations.

**Table 12:** Dab Diluents

Dab Diluent	Cells	AVG	CV	Modulation
3% BSA	100000	51920	21%	38.68
3% BSA	50000	25186	16%	18.76
3% BSA	25000	14176	24%	10.56
3% BSA	0	1342	30%	1.00
Stabilzyme AP	100000	75038	9%	72.69
Stabilzyme AP	50000	31822	21%	30.83
Stabilzyme AP	25000	16635	19%	16.12
Stabilzyme AP	0	1032	22%	1.00
Biostab	100000	86677	19%	19.85
Biostab	50000	54506	16%	12.48
Biostab	25000	34223	24%	7.84
Biostab	0	4367	20%	1.00

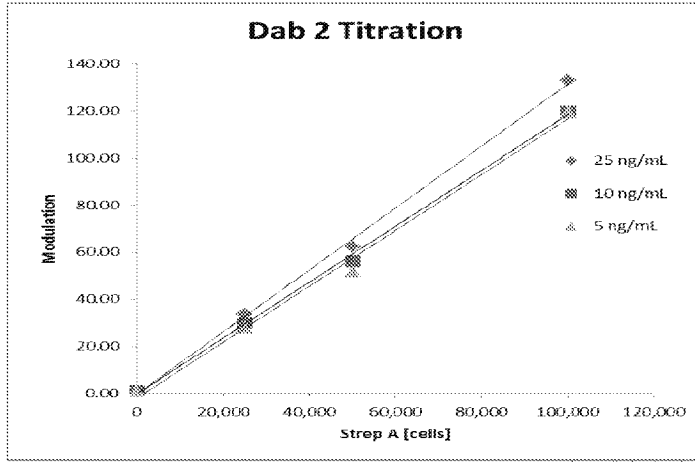
## 2.6 Detector Antibody Titration

The optimum concentration of detector antibody was determined by titrating the antibody from 5 ng/mL to 25 ng/mL. Detector antibody at 5 ng/mL gave the lowest background and acceptable modulations.

**Table 13:** Dab 2 Titration

Dab 2	Cells	AVG	CV	Modulation
25 ng/mL	100000	225904	15%	133.10
25 ng/mL	50000	105727	9%	62.29
25 ng/mL	25000	57140	30%	33.67
25 ng/mL	0	1697	9%	1.00
10 ng/mL	100000	102063	14%	119.86
10 ng/mL	50000	47853	23%	56.20
10 ng/mL	25000	25478	22%	29.92
10 ng/mL	0	851	18%	1.00
5 ng/mL	100000	58712	8%	119.57
5 ng/mL	50000	25415	17%	51.76
5 ng/mL	25000	13665	8%	27.83
5 ng/mL	0	491	12%	1.00

**Figure 1: Dab 2 Titration**



## 2.7 Effect of Sample Dilutions

An increase in sample dilution is required to fully extract the antigen from throat swabs. To test the effect of sample dilutions, extracts were diluted 1:1, 1:2, 1:4, and 1:13. The sensitivity of the assay decreased with larger sample dilutions as expected but was within acceptable levels.

**Table 14: Effect of Sample Dilutions**

Sample Dilution	Cells	AVG	CV	Modulation
1	100000	306030	4%	56.18
1	50000	215664	6%	39.59
1	25000	102977	14%	18.90
1	0	5447	8%	1.00
2	100000	147377	10%	71.98
2	50000	92320	14%	45.09
2	25000	50463	11%	24.65
2	0	2047	7%	1.00
4	100000	103138	17%	51.69
4	50000	42984	3%	21.54
4	25000	29357	8%	14.71
4	0	1995	11%	1.00
13	100000	30716	22%	14.70
13	50000	19471	20%	9.32
13	25000	9232	14%	4.42
13	0	2090	21%	1.00

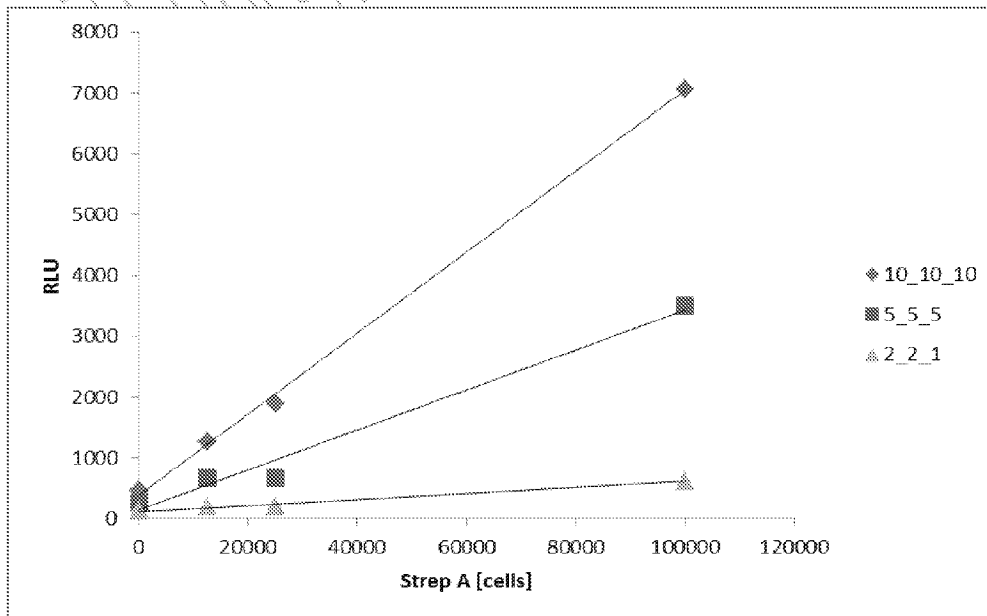
## 2.8 Effect of Assay Protocols

Several Edison assay protocols were tested to determine the optimum protocol for sensitivity, background, and time. The results show that the co-incubation protocols performed the best in terms of having better sensitivity and requiring less time to run the assay. The protocol Generic2\_10X\_Coincubation\_5-5 was selected for the assay.

**Table 15:** Effect of Assay Protocols

Protocol	Cells	AVG	CV	Modulation
10_10_10	100000	7076	9%	15.18
10_10_10	25000	1889	8%	4.05
10_10_10	12500	1258	14%	2.70
10_10_10	0	466	14%	1.00
5_5_5	100000	3500	10%	12.59
5_5_5	25000	672	8%	2.42
5_5_5	12500	677	33%	2.44
5_5_5	0	278	11%	1.00
2_2_1	100000	623	12%	4.54
2_2_1	25000	200	11%	1.46
2_2_1	12500	199	20%	1.45
2_2_1	0	137	3%	1.00

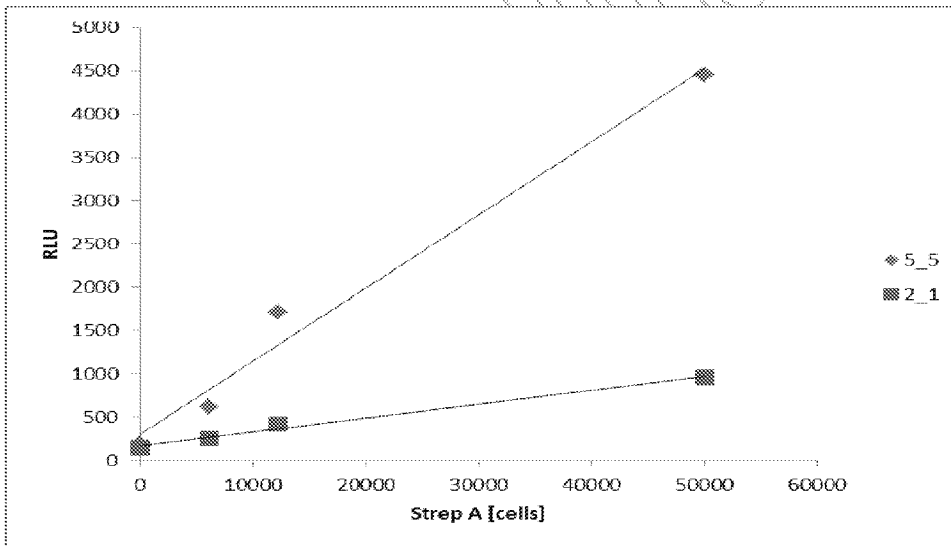
**Figure 2:** Assay Protocol Comparison



**Table 16:** Coincubation Protocols

Protocol	Cells	AVG	CV	Modulation
Generic2_10X_Coincubatin_5-5	50000	4455	19%	22.86
Generic2_10X_Coincubatin_5-5	12250	1716	19%	8.80
Generic2_10X_Coincubatin_5-5	6125	620	16%	3.18
Generic2_10X_Coincubatin_5-5	0	195	14%	1.00
Generic2_10X_Coincubatin_2-1	50000	960	19%	6.31
Generic2_10X_Coincubatin_2-1	12250	417	18%	2.74
Generic2_10X_Coincubatin_2-1	6125	253	8%	1.66
Generic2_10X_Coincubatin_2-1	0	152	8%	1.00

**Figure 3:** Coincubation Protocols



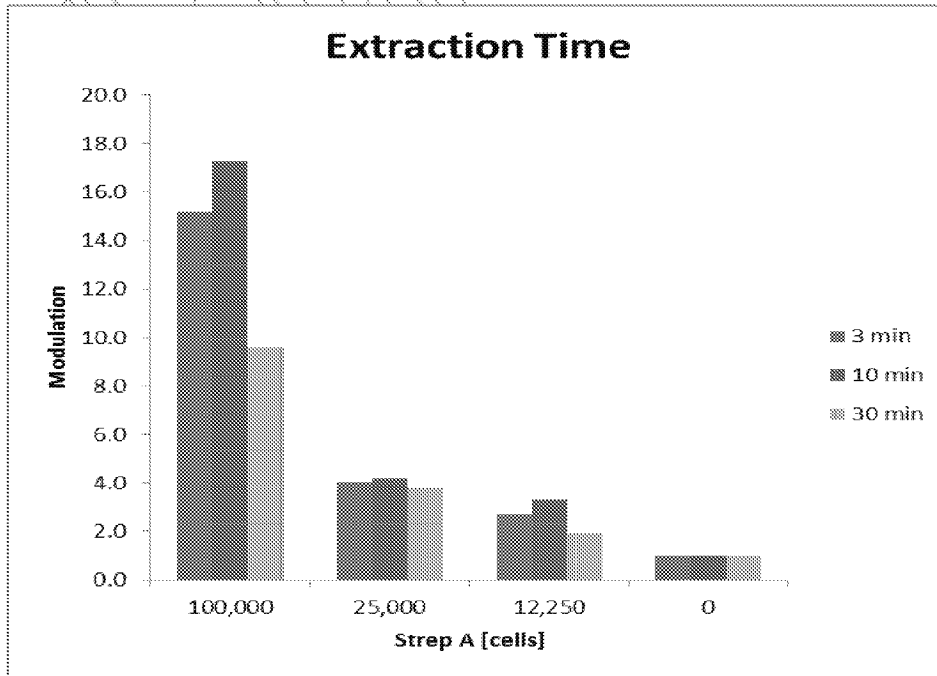
## 2.9 Effect of Extraction Time

The effect of extraction time was tested by comparing assay performance at 3 extraction times (3 min, 10 min, 30 min). The data suggests an extraction time of 3 to 10 minutes is sufficient for antigen extraction from throat swabs under current extraction conditions.

**Table 17:** Extraction Time

Time [min]	Cells	AVG	CV	Modulation
3	100000	7076	9%	15.18
3	25000	1889	8%	4.05
3	12250	1258	14%	2.70
3	0	466	14%	1.00
10	100000	7383	11%	17.27
10	25000	1800	13%	4.21
10	12250	1422	15%	3.33
10	0	427	4%	1.00
30	100000	6278	12%	9.59
30	25000	2475	10%	3.78
30	12250	1245	9%	1.90
30	0	655	15%	1.00

**Figure 4:** Extraction Time





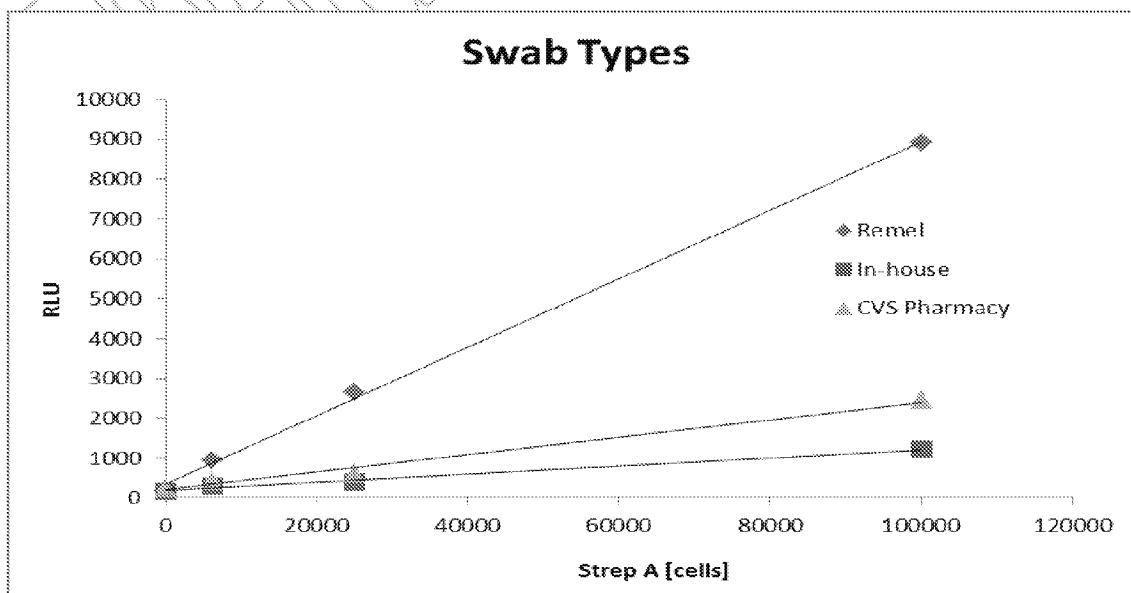
## 2.10 Effect of Swab Types

Swab type and composition is another variable that could affect assay performance. Polyester and cotton tipped swabs were tested and assay performance evaluated. The best performing swab was polyester tipped. Cotton tipped swabs did not give acceptable sensitivity. More swab types will be tested in the future.

**Table 18:** Swab type

Swab Type	Cells	AVG	CV	Modulation
Remel, Polyester, Plastic Shaft	100000	8912	22%	47.5
Remel, Polyester, Plastic Shaft	25000	2651	24%	14.1
Remel, Polyester, Plastic Shaft	6250	957	5%	5.1
Remel, Polyester, Plastic Shaft	0	188	10%	1.0
In-house, Cotton, Wood Shaft	100000	1221	14%	6.9
In-house, Cotton, Wood Shaft	25000	397	35%	2.2
In-house, Cotton, Wood Shaft	6250	303	3%	1.7
In-house, Cotton, Wood Shaft	0	177	6%	1.0
CVS Pharmacy, Cotton, Plastic Shaft	100000	2436	12%	9.5
CVS Pharmacy, Cotton, Plastic Shaft	25000	610	10%	2.4
CVS Pharmacy, Cotton, Plastic Shaft	6250	417	17%	1.6
CVS Pharmacy, Cotton, Plastic Shaft	0	256	21%	1.0

**Figure 5:** Swab types



### 3. ASSAY SUMMARY

**Table 19:** Development Summary

Capture Antibody	MBS534636 @ 10 ug/mL
Coating	UA @ 20 ug/mL
Wash Buffer	1X Enzo from 20X
Assay Buffer	3% BSA in TBS
Edison Protocol	Generic2 10X Coincubation 5-5
Detector Antibody	MBS220642 @ 50 ng/mL (coincubation protocol)
Sample Dilution	none
Antigen Extraction	3 to 10 minutes
2M Nitrite	150 uL
1M Acetic Acid	150 uL
3M Tris	100 uL (after nitrite/acid extraction)
Range	3000 – 100,000 cells
Sensitivity Cutoff	6000 cells (RLU ~ 650)

#### 4. CLINICAL EVALUATION

**Table 20:** Clinical Study Plan

Patients	Age Group	Theranos	Clearview Advanced Strep A Test or equivalent	Culture
200	<= 17 yrs	Yes	Yes	Yes
100	>= 18 yrs	Yes	Yes	Yes

References:

1. Clearview Advanced Strep A Tests: [ [HYPERLINK](http://www.accessdata.fda.gov/cdrh_docs/reviews/K091489.pdf) "http://www.accessdata.fda.gov/cdrh\_docs/reviews/K091489.pdf" ]
2. Inverness Medical Tests Pack + Plus Strep A with OBC: [ [HYPERLINK](http://www.accessdata.fda.gov/cdrh_docs/reviews/K051638.pdf) "http://www.accessdata.fda.gov/cdrh\_docs/reviews/K051638.pdf" ]
3. Status First Strep A: [ [HYPERLINK](http://www.accessdata.fda.gov/cdrh_docs/reviews/K040708.pdf) "http://www.accessdata.fda.gov/cdrh\_docs/reviews/K040708.pdf" ]