theranos™
redefining healthcare

Flu A
Assay Development Report

Theranos, Inc.

November 28, 2012

Prepared by: Sheena Menezes

theranos™
redefining healthcare

TABLE OF CONTENTS

[TOC \o "1-3" \h \z \u]**LIST OF TABLES**

[TOC \h \z \c "Table"]

LIST OF FIGURES

[TOC \h \z \c "Figure"]

1. ASSAY INFORMATION[TC "ASSAY INFORMATION" \F C \L "2"]

1.1 Assay Specifications[TC "Assay Specifications" \f C \l "3"]

This assay is designed to qualitatively determine the presence of Influenza A nucleoprotein antigens in a nasal swab. The test is intended to aid in the diagnosis of Influenza A (Flu A) viral infections. Negative tests should be confirmed by cell culture.

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

The following commercial rapid test kit has been used in house as predicate methods:
Remel X/pect Flu A & B

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

A biotin-labeled anti-Influenza A antibody coated on an avidin surface serves as the capture surface for the sandwich ELISA. The nasal swab is subjected to an extraction process. This extracted material is then mixed with alkaline phosphatase-labeled anti-influenza A antibody. This mixture is then incubated with capture surface for 5 minutes. After the incubation, the surface is washed and the alkaline phosphatase substrate is incubated on the surface for 5 minutes, and then the resulting chemiluminescence is read in Relative Light Units (RLU).

1.2 Extraction Buffer

To determine the optimal extraction buffer, different formulations were tested and compared to the Remel Xpect kit extraction buffer. This was evaluated on the Theranos system with some of the best pairs from the MTP Screen. The best pair selected was #4 and #6; where #4 serves as the capture antibody and #6 serves as detection antibody respectively.

Table [SEQ Table * ARABIC]: Extracted Buffer

Cab ID#	Dab ID#	Preparation	Sample	Mean RLU	CV%	Modulation
8	6	Theranos Flu Extraction Buffer	Positive Flu A	6708	34.0	**10.4**
		Theranos Flu Extraction Buffer	Negative Flu A	643	14.5	
		Theranos Flu Extraction Buffer	Flu B	848	25.3	1.3
		Remel Buffer	Positive Flu A	1896	17.5	**4.0**
		Remel Buffer	Negative Flu A	473	26.3	
4	6	Theranos Flu Extraction Buffer	Positive Flu A	21121	9.4	**25.0**
		Theranos Flu Extraction Buffer	Negative Flu A	844	25.3	
		Theranos Flu Extraction Buffer	Flu B	2986	11.7	3.5
		Remel Buffer	Positive Flu A	5970	16.5	**8.6**
		Remel Buffer	Negative Flu A	696	22.7	
13	4	Theranos Flu Extraction Buffer	Positive Flu A	6231	16.7	**8.6**
		Theranos Flu Extraction Buffer	Negative Flu A	727	16.9	
		Theranos Flu Extraction Buffer	Flu B	946	22.2	1.3
		Remel Buffer	Positive Flu A	1579	20.6	**4.5**
		Remel Buffer	Negative Flu A	352	22.1	
13	6	Theranos Flu Extraction Buffer	Positive Flu A	17715	10.8	**18.6**
		Theranos Flu Extraction Buffer	Negative Flu A	951	20.4	
		Theranos Flu Extraction Buffer	Flu B	3103	39.3	3.3
		Remel Buffer	Positive Flu A	1826	21.9	**4.5**
		Remel Buffer	Negative Flu A	404	35.1	

1.3 Specificity tests on the Theranos System

Specificity and cross reactivity studies were performed on the Theranos system. Microbix controls for the potential cross reactants were tested. The cross reactants tested were Respiratory Syncytial Virus, Mycoplasma pneumonia, Adenovirus, Parainfluenza A-III, Parainfluenza A-II and Parainfluenza A –I. All these samples did not show to cross react. Different strains of Flu A and Flu B were also tested to demonstrate specificity for the Flu A assay. Both Zeptometrix and Microbix controls were used for this test. Positive Flu A control swab from the Remel Xpect Flu kit was also used. The Zeptometrix controls and Microbix controls are prediluted controls. A sample volume of 200ul was mixed with 200ul of extraction buffer and tested for these prediluted samples. Swabs are processed using 400ul of extraction buffer for this experiment.

Table [SEQ Table * ARABIC]: Specificity Tests

Sample Type	Sample	Mean RLU	CV%
Microbix POS CTL	Flu A	127832	25.7
Zeptometrix -POS CTL	Flu A	24235	10.3
Swab-Remel (FDA)	Flu A	269726	11.2
Zeptometrix-Flu A Strain	Brisbane/59/07	202118	10.8
Zeptometrix-Flu A Strain	Brisbane/10/07	60655	14.2
Zeptometrix-Flu A Strain	Perth/16/2009	36571	14.0
Zeptometrix-Flu A Strain	Solomon Islands/03/2006	91428	11.8
Virusys	250ng/ml of Flu A	439907	16.3
	Mean Positive	**156559**	
Microbix	Respiratory Syncytial Virus	1744	21.3
Microbix	Mycoplasma pneumoniae	1798	23.2
Microbix	Adenovirus	1954	24.7
Microbix	Parainfluenza A-III	2162	22.0
Microbix	Parainfluenza A-II	2110	25.2
Microbix	Parainfluenza A -I	2108	20.3
Microbix NEG CTL	Flu A/B Negative	2072	28.0
Zeptometrix-Flu B Strain	Lee/40	2042	16.6
Zeptometrix-Flu B Strain	Florida/02/2006	2806	16.4
Zeptometrix-Flu B Strain	Brisbane/33/2008	2849	15.7
Zeptometrix-Flu B Strain	Panama/45/90	2536	26.9

1.4 Clinical evaluation of the Flu A assay on the Theranos system

An evaluation of the Flu A assay's performance on the Theranos system compared to the Remel FDA kit was performed. The assay works excellent. For the NIBSC strains, 50ul is added to the swab and treated like a sample swab. For the Zeptometrix panel controls (prediluted samples), 200ul of sample is mixed with 200ul of extraction buffer. For any sample swabs, swab is subjected to 500ul of extraction buffer and incubated for 3-5 minutes. This extracted sample is

then used on the Theranos system. Swabs and samples were processed on the Remel kit as directed on the FDA kit. The cutoff was determined to be Means of Normals + 5 * Stdev(Normals).

Table [SEQ Table * ARABIC]: Clinical Evaluation

Type	ID#	Ab Index Theranos System	Remel FDA
Normal Clinicals	1	0.02	
	6	0.02	
	7	0.02	
	8	0.02	
	10	0.02	
	11	0.01	
	12	0.02	
	13	0.01	
	15	0.02	
	16	0.04	
	17	0.02	
	18	0.02	
	2	0.32	
	3	0.23	
	4	0.20	
	9	0.05	
	14	0.29	
	19	0.95	
REMEL	FDA Swab	2.66	
Zeptometrix CTLS	Flu A POS	1.27	
Zeptometrix Flu A	Brisbane/10/07	2.58	
Zeptometrix Flu A	Solomon Islands/03/2006	3.25	
Zeptometrix Flu A	New Caledonia/20/99	2.57	
Zeptometrix Flu A	Brisbane/59/07	5.16	
NIBSC STANDARDS	Panama 45/90	0.06	
FLU B Strains	Influenza Antigen B-Johannesburg	0.06	
	Influenza Antigen B-Guangdong	0.08	
	Influenza Antigen B/Yamanashi/166/98.	0.11	
	Influenza Antigen B/Malaysia/2506/2004	0.02	
	Influenza Antigen B/Harbin/7/94	0.06	
	B:/Florida 4/2006	0.04	
NIBSC STANDARDS	Influenza Antigen A/California /7/2009-H1N1	6.88	
FLU A Strains	Influenza Antigen A/HongKong/1073/99 (H9N2)	8.56	
	Influenza Antigen A/Cambodia/RO405050/2007 (H5N1)	6.02	
	Influenza Antigen A/mallard/England/727/2006 (H2N3)	5.70	
	Influenza Antigen A/New York/107/2003 (H7N2) (NIBRG-109)	7.26	
	Influenza Antigen A/New York/55/2004 (H3N2) (NYMC X-157)	6.54	

1.5 Stability Studies

Stability monitoring is ongoing for the the assay reagents.