510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k112177

B. Purpose for Submission:
New device clearance

C. Measurand:
Influenza A and B virus nucleoprotein antigens in nasal swab, nasopharyngeal swab and nasopharyngeal aspirate/wash specimens.

D. Type of Test:
Qualitative immunofluorescence assay

E. Applicant:
Quidel Corporation

F. Proprietary and Established Names:

Sofia™ Analyzer and Influenza A+B FIA
Influenza A+B immunological test system

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.3330

2. Classification:
   Class I

3. Product code:
   GNX, KHO

4. Panel:
   Microbiology (83)

H. Intended Use:

1. Intended use(s):
   The Sofia Influenza A+B FIA employs immunofluorescence to detect influenza A and influenza B viral nucleoprotein antigens in nasal swab, nasopharyngeal swab, and nasopharyngeal aspirate/wash specimens taken directly from symptomatic patients. This

qualitative test is intended for use as an aid in the rapid differential diagnosis of acute influenza A and influenza B viral infections. The test is not intended to detect influenza C antigens. A negative test is presumptive and it is recommended these results be confirmed by virus culture or an FDA-cleared influenza A and B molecular assay. Negative results do not preclude influenza virus infections and should not be used as the sole basis for treatment or other management decisions. The test is intended for professional and laboratory use.

Performance characteristics for influenza A and B were established during February through March 2011 when influenza viruses A/California/7/2009 (2009 H1N1), A/Perth/16/2009 (H3N2), and B/Brisbane/60/2008 (Victoria-Like) were the predominant influenza viruses in circulation according to the Morbidity and Mortality Weekly Report from the CDC entitled "Update: Influenza Activity--United States, 2010-2011 Season, and Composition of the 2011-2012 Influenza Vaccine". Performance characteristics may vary against other emerging influenza viruses.

If infection with a novel influenza virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Virus culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

2. Indication(s) for use:
   Same as intended use

3. Special conditions for use statement(s):
   Prescription use only

4. Special instrument requirements:
   To be used only with the Sofia Analyzer

I. Device Description:

The Sofia Influenza A+B FIA employs immunofluorescence technology that is used with the Sofia Analyzer to detect influenza virus nucleoproteins.

The Sofia Influenza A+B FIA is a lateral-flow immunoassay that uses monoclonal antibodies that are specific for influenza antigens and have no known cross-reactivity to normal flora or other known respiratory pathogens.

Nasal swab, nasopharyngeal swab, and nasopharyngeal aspirate/wash specimens are used for this test. The patient specimen is placed in the Reagent Tube, during which time the virus particles in the specimen are disrupted, exposing internal viral nucleoproteins. After disruption, the specimen is dispensed into the cassette sample well. From the sample well, the specimen migrates through a test strip containing various unique chemical environments. If influenza viral antigen is present, they will be trapped in a specific location.

The Sofia Analyzer will scan the test strip and measure the fluorescent signal by processing the results using method-specific algorithms. The Sofia Analyzer will display the test results (Positive, Negative, or Invalid) on the screen.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   3M Rapid Detection Flu A+B Reader and Test (Response Biomedical Corporation)
   QuickVue Influenza A+B Test (Quidel Corporation)

2. Predicate 510(k) number(s):
   K071591 and K093116
   K031899, K053146 and K092698

3. Comparison with predicate:

Item	Proposed Device	Predicate Devices	
Features	**Sofia Analyzer and Influenza A+B FIA**	**3M Rapid Detectection Flu A+B Reader and Test (**K093116)	**QuickVue Influenza A+B Test (**K092698)
Intended Use	The Sofia Influenza A+B FIA employs immunofluorescence to detect influenza A and influenza B viral nucleoprotein antigens in nasal swab, nasopharyngeal swab, and nasopharyngeal aspirate/wash specimens taken directly from symptomatic patients. This qualitative test is intended for use as an aid in the rapid differential diagnosis of acute influenza A and influenza B viral infections. The test is not intended to detect influenza C antigens. A negative test is presumptive and it is recommended these results be confirmed by virus culture or an FDA-cleared influenza A and B molecular assay. Negative results do not preclude influenza virus infections and should not be used as the sole basis for treatment or other management decisions. The test is intended for professional and laboratory use. Performance characteristics for influenza A and B were established during February through March 2011 when influenza viruses A/California/7/2009 (2009 H1N1), A/Perth/16/2009 (H3N2), and B/Brisbane/60/2008 (Victoria-Like) were the predominant influenza viruses in circulation according to the Morbidity and Mortality Weekly Report from the CDC entitled	The 3M™ Rapid Detection Flu A+B Test is a qualitative immunochromatographic assay used to identify the presence of Influenza A and Influenza B nucleoprotein antigens in nasal wash, nasal aspirate, nasopharyngeal aspirate, and nasopharyngeal swab specimens from symptomatic patients. It is an *in vitro* diagnostic assay that aids in the rapid differential diagnosis of influenza viral infections in symptomatic patients. A negative test is presumptive and it is recommended these results be confirmed by cell culture. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other management decision.	The QuickVue® Influenza A+B test allows for the rapid, qualitative detection of influenza type A and type B antigens directly from nasal swab, nasopharyngeal swab, nasal aspirate, and nasal wash specimens. The test is intended for use as an aid in the rapid differential diagnosis of acute influenza type A and type B viral infections. The test is not intended to detect influenza C antigens. Negative results should be confirmed by cell culture; they do not preclude influenza virus infection and should not be used as the sole basis for treatment or other management decisions. The test is intended for professional and laboratory use.

	"Update: Influenza Activity--United States, 2010-2011 Season, and Composition of the 2011-2012 Influenza Vaccine". Performance characteristics may vary against other emerging influenza viruses. If infection with a novel influenza virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Virus culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.		
Qualitative	Yes	Yes	Yes
Read Results	Read results on instrument screen or print with optional printer	Read results on instrument screen or print with optional printer	Visual read for presence or absence of control and test lines
Instrument	Sofia Analyzer	3M Rapid Detection Reader	None
Automated Analysis	Yes	Yes	No, operator must be read results
Calibrator	Yes – Calibration Cassette and QC Card provided	Yes – Lot Card contains calibration and expiration information	Not Applicable
Test Principle	Immunofluorescence Device	Immunochromatographic Fluorescence Device	Immunoassay
Format	Lateral-flow Test Cassette	Lateral-flow Test Cassette	Lateral-flow dipstick
Antibodies Used	Monoclonal antibodies to influenza A nucleoprotein and monoclonal antibodies to influenza B nucleoprotein	Monoclonal antibodies to influenza A nucleoprotein and monoclonal antibodies to influenza B nucleoprotein	Monoclonal antibodies to influenza A nucleoprotein and monoclonal antibodies to influenza B nucleoprotein
Storage	Room Temperature	Room Temperature	Room Temperature
Specimen Types	Nasal swab, nasopharyngeal swab, and nasopharyngeal aspirate/wash	Nasopharyngeal swab, nasal wash, nasal aspirate, and nasopharyngeal aspirate	Nasal swab, nasopharyngeal swab, nasal aspirate, and nasal wash
Reagent	Lyophilized buffer containing detergents	Contains phosphate buffer, animal protein, surfactant, and preservatives	Lyophilized buffer containing detergents
Transfer Device	Fixed volume pipette used to transfer sample mixed with sample buffer into Test Cassette	Fixed volume device used to transfer sample mixed with sample buffer into Test Cassette	Dropper used to transfer liquid patient samples into test tube
Read Result Time	15 Minutes	15 Minutes	10 Minutes
Sample Transport	Various Viral Transport Media	Various Viral Transport Media	Various Viral Transport Media and dry container
External Controls	Test kit contains Positive and Negative Control swabs	Test kit contains Positive and Negative Control swabs	Test kit contains Positive and Negative Control swabs
Quality Control Features	Built-in features include: • Scanning of the procedural control zone to determine whether adequate flow occurred • Scanning of the negative control line to measure degree of non-specific binding	Built-in features include: • Detection system ensures sufficient mixed sample was applied, unbound fluorescent label washed away • Reader prevents used or expired cassette from being read by the reader • Cassette properly inserted	Built-in procedural control line and clearing of background

	• Analyzer prevents used or expired cartridge from being read by the reader • Cassette properly inserted		

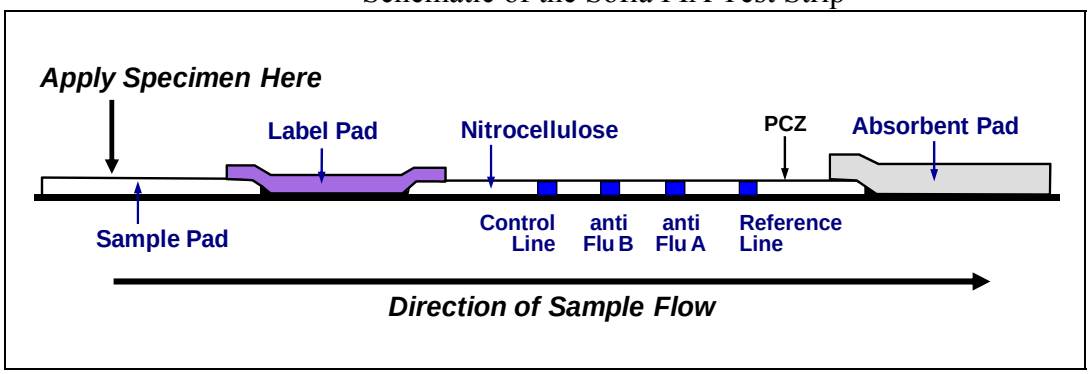
K. Standard/Guidance Document Referenced (if applicable):

Not applicable

L. Test Principle:

The Sofia Influenza A+B FIA is an immunoassay that uses a sandwich design to detect and differentiate Influenza A and Influenza B in patient specimens. This test uses a lateral flow design with location-dependent lines and zones. The Sofia Analyzer scans the test strip and displays the results (Positive, Negative or Invalid) after utilizing the method-specific algorithms. Basically Sofia Influenza A+B FIA involves the disruption of Influenza A and B viral antigens and exposure of internal nucleoproteins by placing the patient specimen in the reagent tube. After disruption the specimen is dispended into the Cassette sample well after which the sample is drawn by capillary action into and through the label pad, through the nitrocellulose strip and into the adsorbent pad. Within the label pad, the specimen comes into contact with antibodies that have been coupled to the europium chelate-impregnated microbeads. During this interaction, the independent subpopulations of beads containing bound anti-influenza A or anti-influenza B monoclonal antibodies bind corresponding influenza A or B nucleoprotein antigens that are present in the specimen. The bead-coupled antigen-antibody complexes then begin to flow through the test strip. Some of these beads bind non-specifically to the NC line. Most migrate on and, if influenza A or B antigen are present in the sample, they will be subsequently captured on the surface of the nitrocellulose by the respective location-fixed, analyte-specific anti-influenza A or anti-influenza B capture antibodies. The flow and capture of the fluorescent microbeads coated with influenza A and/or B nucleoprotein antigens allows the accumulation of a fluorescent signal at specific analyte line locations on the test strip. The conjugates that do not bind to the Negative Control line and Test Lines continue to flow with the remaining specimen and soon encounter the Reference line that is comprised of goat anti-mouse Ig. The fluorescent signal generated at this line serves as the location marker to direct the Sofia Analyzer to the other precise locations on the Nitrocellulose that are to be scanned. The remaining sample then flows into the Procedural Control Zone that is also scanned to confirm that adequate flow of the sample has occurred. The Sample with any remaining conjugate then flows on into the Adsorbent Pad. This process takes approximately fifteen minutes, at which time the Sofia Analyzer scans the strip, measuring the fluorescent signals across the strip's length and performs calculations and reports the test results. The assay's sensitivity is derived from the use of a unique polystyrene microbead that has been dyed with a chelate of europium that is temperature stable, resistant to bleaching in room light and yields a very efficient conversion of the UV energy from 365 nm to a wavelength of 618 nm.

Schematic of the Sofia FIA Test Strip



PCZ = Procedural Control Zone

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   Precision:

   The precision study spanned a period of at least 12 days, using two operators per day. It included the use of three different lots of the Sofia Influenza A+B FIA test cassettes and one Sofia Analyzer. One strain each of Influenza A and Influenza B virus was used. Viruses were spiked into the negative clinical matrix pool derived from negative clinical nasal swab samples in VTM to prepare High negative, Low positive (at or near the LoD) and Moderate positive (3.5 times the LoD) members of the precision study panel. Each operator tested two aliquots of each precision study panel member on each of the three lots of the test cassettes. A total of 1,008 observations (3 lots of cassettes x 2 operators x 2 aliquots/test runs x 12 days x 7 precision panel members = 1008) were included in the analysis. The three different cassette lots agreed with one another 100% of the time for the true negative (no spiked virus; clinical negative matrix only), type A moderate positive, type A low positive, type B moderate positive and type B low positive precision panel members. The high negative samples (C_5) for type A and type B gave 90% and 92% agreement, respectively. The overall agreement between the three different lots of test cassettes was 97% (981/1008).

Inter-Cassette-Lot Test Agreement

Cassette	Operator	Neg.	Type A High Neg	Type A Moderate Pos	Type A Low Pos	Type B High Neg	Type B Moderate Pos	Type B Low Pos	Overall Agreement
1	1	24/24	24/24	24/24	24/24	24/24	24/24	24/24	168/168
	2	24/24	24/24	24/24	24/24	24/24	24/24	24/24	168/168
2	1	24/24	21/24	24/24	24/24	21/24	24/24	24/24	162/168

Cassette	Operator	Neg.	Type A High Neg	Type A Moderate Pos	Type A Low Pos	Type B High Neg	Type B Moderate Pos	Type B Low Pos	Overall Agreement
	2	24/24	20/24	24/24	24/24	20/24	24/24	24/24	160/168
3	1	24/24	20/24	24/24	24/24	22/24	24/24	24/24	162/168
	2	24/24	20/24	24/24	24/24	21/24	24/24	24/24	161/168
Total All:		**144/144**	**129/144**	**144/144**	**144/144**	**132/144**	**144/144**	**144/144**	**981/1008**
% Correct Call:		**100% Neg**	**90% Neg**	**100% Pos**	**100% Pos**	**92% Neg**	**100% Pos**	**100% Pos**	**97% Agreement**
(95% CI):		(97-100%)	(83-94%)	(97-100%)	(97-100%)	(86-95%)	(97-100%)	(97-100%)	(96-98%)

Another inter-operator comparison was made to show the overall agreement between two distinct operators when testing the same samples using three different lots of cassettes. As shown in the table below, the overall agreement was 96.5% (834/864) between the operators.

Inter-Operator (Test) Agreement*

Operator	Neg.	Type A High Neg	Type A Moderate Pos	Type A Low Pos	Type B High Neg	Type B Moderate Pos	Type B Low Pos	Overall Agreement
1	72/72	65/72	72/72	72/72	67/72	72/72	72/72	420/432
2	72/72	64/72	72/72	72/72	65/72	72/72	72/72	414/432
Total All:	**144/144**	**129/144**	**144/144**	**144/144**	**132/144**	**144/144**	**144/144**	**834/864**
% Correct Call:	**100% Neg**	**90% Neg**	**100% Pos**	**100% Pos**	**92% Neg**	**100% Pos**	**100% Pos**	**96. 5% Agreement**
(95% CI):	(97-100%)	(83-94%)	(97-100%)	(97-100%)	(86-95%)	(97-100%)	(97-100%)	(96-98%)

*All data from all 3 cassettes pooled for each operator.

Reproducibility:

The reproducibility of the Sofia Influenza A+B FIA was evaluated at three different laboratories. Two different operators at each site tested a series of coded, contrived samples, prepared in negative nasal swab matrix, ranging from high negative to moderate positive influenza A and influenza B using formalin inactivated virus. The influenza strains used were Influenza A/California/07/2009 (2009 H1N1) and Influenza B/Allen/45. Testing occurred on five different days spanning over approximately a two-week period using six Sofia Analyzers. A total of 210 results (105 samples x 2 operators = 210 samples) per site were obtained. A total of 630 samples were tested by the combined three sites. The inter-laboratory agreement for negative samples (negative and high negative samples) was 94-100% and 98-100%

for positive samples (low positive and moderate positive samples). The intra-laboratory agreement for all samples ranged from 98-99%.

Sofia Influenza A+B Reproducibility Study Inter-Laboratory Agreement

Laboratory Site	Neg (no virus)	Flu A High Neg (C_5)	Flu A Low Pos (C_{95})	Flu A Mod Pos (C_{3X})	Flu B High Neg (C_5)	Flu B Low Pos (C_{95})	Flu B Mod Pos (C_{3X})
1	30/30	29/30	30/30	30/30	28/30	29/30	30/30
2	30/30	29/30	30/30	30/30	30/30	29/30	30/30
3	30/30	30/30	30/30	30/30	27/30	30/30	30/30
Total	90/90	88/90	90/90	90/90	85/90	88/90	90/90
% Overall Agreement with Expected Result (95% CI)	100% (95-100%)	98% (92-100%)	100% (95-100%)	100% (95-100%)	94% (87-98%)	98% (92-100%)	100% (95-100%)

Sofia Influenza A+B Reproducibility Study Intra-Laboratory Agreement

Lab. Site	Neg (no virus)	Flu A High Neg (C_5)	Flu A Low Pos (C_{95})	Flu A Mod Pos (C_{3X})	Flu B High Neg (C_5)	Flu B Low Pos (C_{95})	Flu B Mod Pos (C_{3X})	% Overall Agreement with Expected Result (95% CI)
1	30/30	29/30	30/30	30/30	28/30	29/30	30/30	98% (206/210) (95-100%)
2	30/30	29/30	30/30	30/30	30/30	29/30	30/30	99% (208/210) (96-100%)
3	30/30	30/30	30/30	30/30	27/30	30/30	30/30	99% (207/210) (96-100%)

b. Linearity/assay reportable range:
N/A

c. Traceability, Stability, Expected values (controls, calibrators, or methods):
N/A

d. Detection limit:
The limit of detection (LoD) for the Sofia Influenza A+B FIA was determined using a total of four strains of human influenza viruses, two influenza A and two influenza B viruses using the approach outlined in CLSI EP-17. All the virus serial dilutions were made in the influenza-negative clinical matrix prepared from negative nasal swab samples in M5 viral transport medium.

Limit of Detection with Human Isolates of Influenza A and B

Viral Strain	Viral Type	Sub-Type	Minimum Detectable Level (TCID50/mL)
A/California/07/2009	A	2009 H1N1	202
A/Hong Kong/8/68	A	H1N1	105
B/Allen/45	B		40
B/Malaysia/2506/04	B		24

$TCID_{50}$ levels were determined by either the Reed-Muench method or Rowe ELISA.

e. Analytical reactivity:

Analytical reactivity of Sofia Influenza A+B FIA was evaluated using a total of 29 strains of human influenza viruses comprised of 20 Influenza A and nine influenza B viruses. Serial ten-fold dilutions for each virus with starting $TCID_{50}$/mL concentrations ranging from $2x10^4$ down to $2x10^1$ were prepared in viral transport medium M5. Five replicates of each dilution were tested. The highest dilution where 100% of the replicates were positive was chosen to make a series of 2-fold dilutions in M5. Five replicates at each dilution were tested. The highest dilution that gave five out five positive results was reported.

Analytical Reactivity with Human Isolates of Influenza A and B

Viral Strain	Viral Type	Sub-Type	Minimum Detectable Level ($TCID_{50}$/mL)
A/Fort Monmouth/1/47	A	H1N1	50
A/New Caledonia/20/1999	A	H1N1	200
A/New Jersey/8/76	A	H1N1	500
A/NWS/33	A	H1N1	0.63
A/Puerto Rico/8/34	A	H1N1	100
A/Solomon Islands/3/06	A	H1N1	0.31
A/Taiwan/42/06	A	H1N1	200
A/WI/629-9/2008	A	H1N1	200
A1/Denver/1/57	A	H1N1	20
Influenza/Mexico/4108/2009	A	2009 H1N1	200
A/WI/629-S5 (D02312)/2009	A	2009 H1N1	50
A/WI/629-S7(D02473)/2009	A	2009 H1N1	25
A/Port Chalmers/1/73	A	H3N2	500
A/Victoria/3/75	A	H3N2	200
A/WI/629-2/2008	A	H3N2	20
A/Wisconsin/67/05	A	H3N2	20
A2/Aichi/2/68	A	H3N2	1.25
A/Anhui/01/2005	A	H5N1	5
A/GWT/LA/169GW/88	A	H10N7	20
A/Shearwater/Australia25 76/79	A	H15N9	10
B/Brisbane/60/2008	B		10
B/Florida/04/2006	B		250
B/Florida/07/2004	B		500
B/GL/1739/54	B		1000
B/Hong Kong/5/72	B		20
B/Lee/40	B		5
B/Maryland/1/59	B		50
B/Ohio/1/2005	B		50
B/Taiwan/2/62	B		50

$TCID_{50}$/mL = 50% tissue culture infectious dose. $TCID_{50}$ levels were determined by the Reed-Muench method.

Analytical reactivity was further evaluated using a total of 12 influenza A viruses isolated from birds. The Sofia Influenza A+B FIA detected all of the strains

examined.

Analytical Reactivity with Different Isolates of Avian Influenza A

Viral Strain*	Viral Type	Sub-Type	Minimum Detectable Level ($TCID_{50}$/mL)
A/Mallard/NY6750/78	A	H2N2	100
A/Mallard/OH/338/86	A	H4N8	50
A/Mallard/WI/34/75	A	H5N2	100
A/Chicken/CA/431/00	A	H6N2	50
A/Chicken/NJ/15086-3/94	A	H7N3	5
A/Blue Winged Teal/LA/B174/86	A	H8N4	10
A/Chicken/NJ/122210/97	A	H9N2	10
A/Chicken/NJ/15906-9/96	A	H11N9	50
A/Duck/LA/188D/87	A	H12N5	50
A/Gull/MD/704/77	A	H13N6	0.625
A/Mallard/GurjevRussia/262/82	A	H14N5	20
A/Shorebird/DE/172/2006	A	H16N3	2

*The performance characteristics for influenza A virus subtypes emerging as human pathogens have not been established.

f. Analytical specificity:

Cross Reactivity

The Sofia Influenza A+B FIA was evaluated with a total of 18 bacterial and fungal microorganisms and 16 non-influenza viral isolates. Bacterial and fungal isolates were evaluated at a concentration of $2x10^6$ cfu/mL. Viral isolates were evaluated at a concentration of $2x10^5$ $TCID_{50}$/mL. None of the organisms or non-influenza viruses listed in the table below showed any sign of cross reactivity in the assay. Flow of the sample and appearance of the Control Line were also not affected.

Analytical Specificity and Cross Reactivity

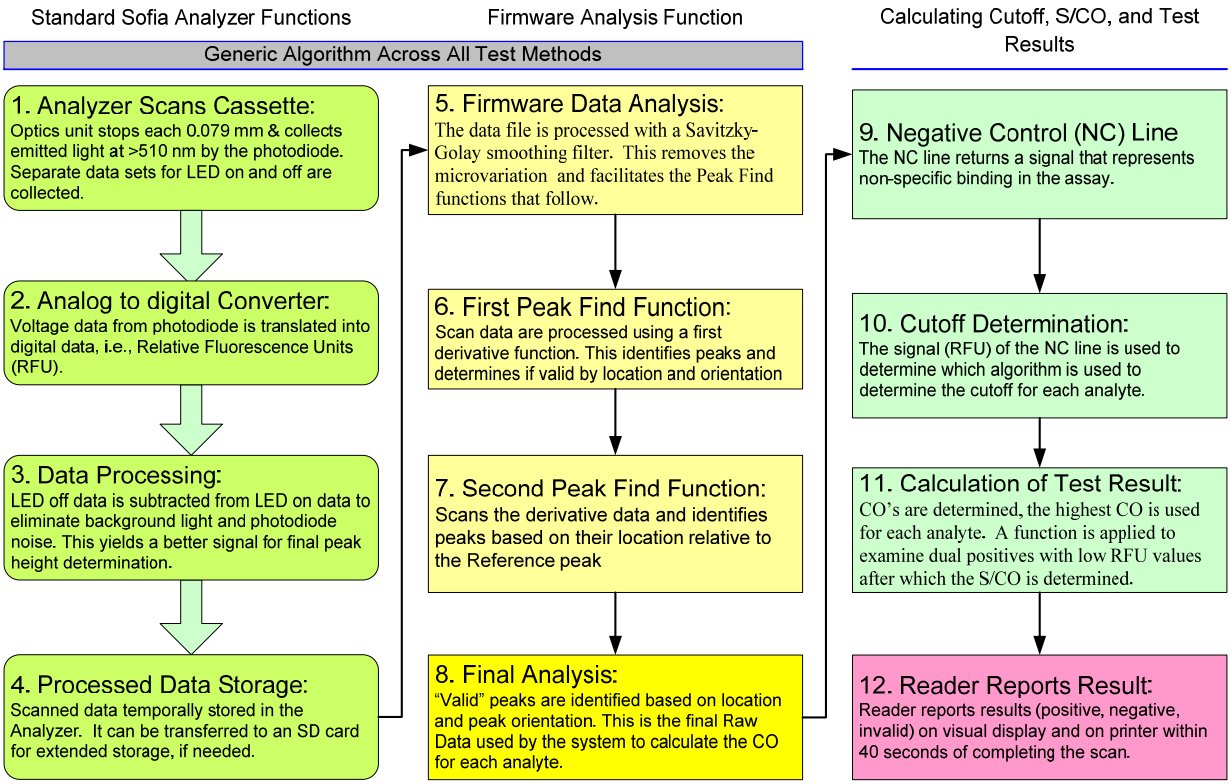
Organism/Non-Influenza Virus	Concentration*	Flu A Result	Flu B Result
Bordetella pertussis	$2x10^6$ cfu/mL	Negative	Negative
Canidida albicans	$2x10^6$ cfu/mL	Negative	Negative
Chlamydia trachomatis	$2x10^6$ cfu/mL	Negative	Negative
Corynebacterium diphtheriae	$2x10^6$ cfu/mL	Negative	Negative
Escherichia coli	$2x10^6$ cfu/mL	Negative	Negative
Haemophilus influenzae	$2x10^6$ cfu/mL	Negative	Negative
Lactobacillus plantarum	$2x10^6$ cfu/mL	Negative	Negative
Legionella pneumophila	$2x10^6$ cfu/mL	Negative	Negative
Moraxella catarrhalis	$2x10^6$ cfu/mL	Negative	Negative
Mycobacterium tuberculosis (avirulent)	$2x10^6$ cfu/mL	Negative	Negative
Mycoplasma pneumoniae	$2x10^6$ cfu/mL	Negative	Negative
Neisseria meningitidis	$2x10^6$ cfu/mL	Negative	Negative

Organism/Non-Influenza Virus	Concentration*	Flu A Result	Flu B Result
Neisseria subflava	$2x10^6$ cfu/mL	Negative	Negative
Pseudomonas aeruginosa	$2x10^6$ cfu/mL	Negative	Negative
Staphylococcus epidermidis	$2x10^6$ cfu/mL	Negative	Negative
Streptococcus pneumoniae	$2x10^6$ cfu/mL	Negative	Negative
Streptococcus pyogenes	$2x10^6$ cfu/mL	Negative	Negative
Streptococcus salivarius	$2x10^6$ cfu/mL	Negative	Negative
Adenovirus type 1	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Adenovirus type 7	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Human coronavirus (OC43)	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Human coronavirus (229E)	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Human coxsackievirus	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Cytomegalovirus	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Epstein Barr Virus	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Human parainfluenza type 1	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Human parainfluenza type 2	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Human parainfluenza type 3	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Measles	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Human metapneumovirus	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Mumps virus	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Respiratory syncytial virus type A	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Respiratory syncytial virus type B	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Rhinovirus type 1B	$2x10^5$ $TCID_{50}$/mL	Negative	Negative

*The levels of bacteria were determined by limiting dilution, bacterial culture, and colony counting to give cfu/mL. Virus concentrations were determined by standard virology methods, Reed-Muench.

g. Assay cut-off:

The Sofia Analyzer uses a constant set of calculations to generate processed peak heights. The peak heights are used by the test method file to calculate a final test result.



Sofia Analyzer Data Processing Work Flow

The last column of the Sofia Analyzer data processing workflow describes a series of step undertaken by the software to calculate the cutoffs (COs) and the signal over CO ratio (S/CO) immediately prior to reporting the result. Values from reference lots during development were used to establish the reference values used in these algorithms. Three cutoffs are generated for each analyte in each test and the algorithm then selects automatically the cutoff with the highest value for each analyte. This particular cutoff is the one ultimately used to calculate the test results for each analyte.

Cutoff Determination Type 1

Adjusts for the impact of the most strongly fluorescing Test Line on the adjacent Test Line.

The analysis of known positive and negative specimens during development led to a preliminary cutoff for both analytes. Additional analyses, similar to those described in CLSI EP17-A for determining the Limit of Blank (LoB), further established that the cutoff at 675 RFUs for influenza A and B was appropriate. These studies further demonstrated that the most strongly fluorescing signal on one Test Line would influence the signal on the adjacent Test Line. To adjust for this effect, the signal of the Test Line giving the highest signal is multiplied by 0.2 and the resulting product added to 675. This larger value becomes the cutoff for the analyte with the lower signal. This process compensates for the impact of the signal from the Test Line with the highest signal on the adjacent Test Line.

Cutoff Determination Type 2

Calculating cutoff when the NC value is less than the NC Threshold value.

For each test, if the NC signal is less than the NC Threshold value, then the fixed cutoff of 675 will be used.

Cutoff Determination Type 3

Calculating cutoff when the NC value is greater that the NC Threshold value.

When the NC value of any test is greater than the NC Threshold value, then the test's NC value multiplied by the analyte-specific correction factor (CF) yields the cutoff for each analyte in that test.

The method algorithm requires the calculation of the preliminary cutoffs described above. It then selects the cutoff with the highest RFU signal to complete the calculations of test results. These calculations and the ultimate cutoff employed are not visible to the user of the FIA test and Sofia Analyzer.

h. Interfering Substances:

Whole blood, mucin, heparin and 16 substances, many of which are common cold and flu medications were examined in this study. Two strains of Influenza A and B each were diluted in viral transport medium M5 to $TCID_{50}$/mL titers approximately three times the LoD for each virus and tested five replicates each.

None of the substances tested at the concentrations indicated interfere with the test results of negative or positive Influenza A and Influenza B samples in the Sofia Influenza A+B FIA. Whole blood at concentration of > 4% v/v or mucin at concentration of > 0.5% v/v interfered in the interpretation of the test.

Non-interfering Substances

Substance	Concentration
Whole Blood	4%
Mucin	0.5%
Ricola (Menthol)	1.5 mg/mL
Sucrets (Dyclonin/Menthol)	1.5 mg/mL
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL
Naso GEL (NeilMed)	5% v/v
CVS Nasal Drops (Phenylephrine)	15% v/v
Afrin (Oxymetazoline)	15% v/v
CVS Nasal Spray (Cromolyn)	15% v/v
Nasal Gel (Oxymetazoline)	10% v/v
Zicam	5% v/v
Homeopathic (Alkalol)	1:10 dilution
Fisherman's Friend	1.5 mg/mL

Sore Throat Phenol Spray	15% v/v
Tobramycin	4 µg/mL
Mupirocin	10 mg/mL
Fluticasone Propionate	5% v/v
Tamiflu (Oseltamivir Phosphate)	5 mg/mL

i. Virus Stability in Transport Media:

Saline and eight different commercially available viral transport media (VTM) were evaluated in this study. One influenza A and one influenza B isolate were used. Five different Sofia Analyzers were used. Two different temperature conditions were examined, including ambient room temperature (RT) and 2-8°C. The stability of viruses (2-3 times of LoD) that were stored in VTM or saline for 0 hours, 2 hours, 4 hours, 6 hours, 24 hours, 48 hours and 72 hours of incubation at 2-8°C and RT was examined in the Sofia Influenza A+B FIA. The study demonstrated that viruses stored in M4, UTM, M5, M6, M4-RT and Starplex Multitrans for up to 72 hours at either temperature still gave 100% correct results. Saline was not useable beyond 4 hours at RT, however, it could be used for up to 24 hours, if the samples were stored at 2°C to 8°C. Hank's and Stuart's were inferior and not recommended for storing specimens at RT. However, Hank's can be used for storing specimens up 24 hours and Stuart's up to 6 hours at 2°C to 8°C. All the media were shown to be compatible with the Sofia Influenza A+B FIA; none gave false positive or invalid results under the conditions examined.

Recommended Viral Transport Media

Viral Transport Media	Recommended Storage Condition	
	2-8°C	**25°C**
Copan Universal Transport Media	72 hours	72 hours
Hank's Balanced Salt Solution	24 hours	Not recommended
M4	72 hours	72 hours
M4-RT	72 hours	72 hours
M5	72 hours	72 hours
M6	72 hours	72 hours
Modified Liquid Stuarts	6 hours	Not recommended
Saline	24 hours	4 hours
Starplex Multitrans	72 hours	72 hours

j. Rehydrated Extraction Reagent Stability:

Rehydrated extraction solution was stored at room temperature and aliquots were tested at various time points spanning from zero to 24 hours after rehydrating the lyophilized extractant. Five Sofia Analyzers and one influenza A and one influenza B virus were used in this study. Influenza A and B viruses were diluted in saline to a

$TCID_{50}$/mL concentration that was approximately two to three times higher than the LoD. Five replicates were run for each condition.

All samples—influenza A and influenza B—tested 100% in agreement with the expected results, regardless of duration of storage of the rehydrated extractant. There were no false positive or negative results obtained at any condition with the virus-spiked mock specimens.

Performance of Sofia Influenza A+B FIA Using Stored Rehydrated Extraction Solution

Sample	Number of Positive Results Versus Duration (hrs.) of Storage of Rehydrated Extraction Reagent					
	0	**0.5**	**2**	**4**	**6**	**24**
Blank	0/5	0/5	0/5	0/5	0/5	0/5
A	5/5	5/5	5/5	5/5	5/5	5/5
B	5/5	5/5	5/5	5/5	5/5	5/5

k. Extracted Specimen Stability:

One of each influenza A and B virus was diluted to a $TCID_{50}$/mL concentration that was approximately two to three times higher than the LoD. For each virus, five dilutions were prepared using the following diluents: M5, M4, UTM and saline. The viruses were diluted in saline, M5, UTM or M4 to the desired concentration, thus mimicking a swab, nasopharyngeal aspirate, wash, or sample suspended in VTM. The rehydrated extraction solution containing the blank buffer or viruses were stored at room temperature and tested in replicates of five at the following time points: 1 min, 5 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 5 hr, and 24 hr. The results showed that extracted samples containing influenza A or influenza B viruses can be stored in the rehydrated extraction reagent for up to 24 hours at room temperature without significantly affecting the performance of the Sofia Influenza A+B FIA. Negative specimens, containing neither virus, tested negative throughout the time course, as well.

l. Comparison of Read Now/Walk Away modes:

The comparative performance of 19 Sofia Analyzers was examined when using the Read Now and Walk Away procedure modes. The exact same kit lot of Sofia Influenza A+B FIA was used for this study. One influenza A and one influenza B virus were used. The viruses were diluted in M5 to a $TCID_{50}$/mL concentration that was approximately two times their respective LoDs. The study results showed that all the negative (blank) and positive samples performed well, giving 100% agreement with the expected results when using either the Read Now or Walk Away modes. There were no false positive or false negative results obtained.

Impact of Incubating on the Bench Top or within the Analyzer

Incubation Condition	Virus	Media	Flu A and B Result
Read Now	NA	M5	38/38 A- B-
Walk Away	NA	M5	38/38 A- B-
Read Now	A/Hong Kong/8/68	M5	38/38 A+ B-
Walk Away	A/Hong Kong/8/68	M5	38/38 A+ B-
Read Now	B/Allen/45	M5	38/38 A- B+
Walk Away	B/Allen/45	M5	38/38 A- B+

m. Shelf Life/Stability studies:

Three lots of Sofia Influenza A+B FIA were selected for real-time and accelerated stability testing. Prior to placing the lots under the stress temperature conditions, replicates of the positive (at 0.75 of LoD and at LoD for Influenza A and 0.75 of LoD and at 1.75 of LoD for Influenza B) and negative samples, positive and negative controls, and various media were tested (Day 0). The test kits were then placed under the controlled stress temperature conditions. At each time point, units from each lot were removed from the stress temperature conditions and tested per the Package Insert according to the test schedule.

Summary of Stability Test Schedule

Sample Level	Number of Replicates	
	Day 0	Day 7 – Month 33
Sofia Influenza AB Pos. Control Swabs	20	10
Sofia Influenza Neg. Control Swabs	20	10
Sofia Influenza A Low-level Pos. Standard (0.75 of LoD)	20	10
Sofia Influenza B Low-level Pos. Standard (0.75 of LoD)	20	10
Sofia Influenza A High-level Pos. Standard (At LoD)	20	10
Sofia Influenza B High-level Pos. Standard (At 1.75 of LoD)	20	10
Media (M4, M5, UTM)	20/media	10/media

Real-time and accelerated data have been generated out to 3 months and 100 days, respectively. Stability studies are on-going and will continue for up to 33 months.

2. Comparison studies:

a. Method comparison with predicate device:
Not applicable

b. Matrix comparison:
Not applicable

3. Clinical studies:

The performance of the Sofia Influenza A+B FIA was compared to viral cell culture methods followed by DFA in a multi-center clinical field study during February through March 2011 in the United States. This study was conducted by health care personnel at seventeen (17) distinct sites in various geographical regions within the United States. In this multi-center, point-of-care (POC) field trial, two (2) nasal or two (2) nasopharyngeal swabs or nasopharyngeal aspirate/wash specimens were collected from each of two thousand forty-seven (2047) patients. Six hundred sixty-five (665) provided a nasal swab specimen, seven hundred thirty-three (733) provided a nasopharyngeal swab specimen and six hundred forty-nine (649) provided a nasopharyngeal aspirate/wash specimen. All clinical samples were collected from symptomatic patients. Seventy-one percent (71%) of the population tested were <6 years of age, 22% 6-21 years of age, 6% 22-59 years of age, and 1% ≥60 years of age. Fifty-three percent (53%) were male and forty-seven percent (47%) were female.

On-site testing of one nasal swab or nasopharyngeal swab or a portion of nasopharyngeal aspirate/wash specimen in the Sofia Influenza A+B FIA test was performed on the fresh specimen by medical personnel in the physician's office or hospital facility. The remaining sample was placed in viral transport media. The paired swab samples were randomized with respect to the order of testing in the Sofia Influenza A+B FIA versus culture. Cell culture was performed either at a local virus laboratory of the test site or transported cold on ice packs, not frozen, overnight to a central laboratory for culture within 48 hours.

Sofia Influenza A+B FIA Nasal Swab Results Versus Culture (All Age Groups)

TYPE A

	Culture	
	Pos	Neg
Sofia Pos	124	27
Sofia Neg	14	500

Sens = 124/138 = 90% (95% C.I. 84-94%)

Spec = 500/527 = 95% (95% C.I. 93-96%)

TYPE B

	Culture	
	Pos	Neg
Sofia Pos	100	23
Sofia Neg	12	530

Sens = 100/112 = 89% (95% C.I. 82-94%)

Spec = 530/553 = 96% (95% C.I. 94-97%)

Sofia Influenza A+B FIA Nasopharyngeal Swab Results Versus Culture (All Age Groups)

TYPE A

	Culture	
	Pos	Neg
Sofia Pos	100	34
Sofia Neg	3	596

Sens = 100/103 = 97% (95% C.I. 91-99%)

Spec = 596/630 = 95% (95% C.I. 93-96%)

TYPE B

	Culture	
	Pos	Neg
Sofia Pos	101	19
Sofia Neg	11	602

Sens = 101/112 = 90% (95% C.I. 83-95%)

Spec = 602/621 = 97% (95% C.I. 95-98%)

Sofia Influenza A+B FIA Nasopharyngeal Aspirate/Wash Results Versus Culture (All Age Groups)

TYPE A

	Culture	
	Pos	Neg
Sofia Pos	68	26
Sofia Neg	1	554

Sens = 68/69 = 99% (95% C.I. 91-100%)

Spec = 554/580 = 96% (95% C.I. 93-97%)

TYPE B

	Culture	
	Pos	Neg
Sofia Pos	46	22
Sofia Neg	6	575

Sens = 46/52 = 88% (95% C.I. 77-95%)

Spec = 575/597 = 96% (95% C.I. 94-98%)

Performance Compared to Culture for Each Specimen Type by Age Group for Influenza A

	Nasal Swabs		Nasopharyngeal Swabs		Nasopharyngeal Aspirate/Wash	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
All Ages	90% (124/138) (95%CI=84-94%)	95% (500/527) (95%CI=93-96%)	97% (100/103) (95%CI=91-99%)	95% (596/630) (95%CI=93-96%)	99% (68/69) (95%CI=91-100%)	96% (554/580) (95%CI=93-97%)
<6 years	95% (62/65) (95%CI=87-99%)	95% (210/221) (95%CI=91-97%)	97% (61/63) (95%CI=86-100%)	94% (444/470) (95%CI=92-96%)	99% (68/69) (95%CI=91-100%)	95% (544/570) (95%CI=93-97%)
6 to 21 years	87% (46/53) (95%CI=75-94%)	95% (193/204) (95%CI=91-97%)	97% (35/36) (95%CI=85-100%)	94% (136/144) (95%CI=89-97%)	N/A (0/0)	100% (10/10) (95%CI=68-100%)
22 to 59 years	78% (14/18) (95%CI=54-92%)	96% (82/85) (95%CI=90-99%)	100% (4/4) (95%CI=45-100%)	100% (15/15) (95%CI=76-100%)	N/A (0/0)	N/A (0/0)
60 Years and up	100% (2/2) (95%CI=29/100%)	88% (15/17) (95%CI=64-98%)	N/A (0/0)	100% (1/1) (95%CI=17/100%)	N/A (0/0)	N/A (0/0)

Performance Compared to Culture for Each Specimen Type by Age Group for Influenza B

	Nasal Swabs		Nasopharyngeal Swabs		Nasopharyngeal Aspirate/Wash	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
All Ages	89% (100/112) (95%CI=82-94%)	96% (530/553) (95%CI=94-97%)	90% (101/112) (95%CI=83-95%)	97% (602/621) (95%CI=95-98%)	88% (46/52) (95%CI=77-95%)	96% (575/597) (95%CI=94-98%)
<6 years	90% (35/39) (95%CI=76-97%)	96% (238/247) (95%CI=93-98%)	87% (54/62) (95%CI=76-94%)	97% (455/471) (95%CI=95-98%)	87% (39/45) (95%CI=73-94%)	96% (572/594) (95%CI=94-98%)
6 to 21 years	92% (56/61) (95%CI=82-97%)	95% (187/196) (95%CI=91-98%)	94% (45/48) (95%CI=83-98%)	98% (130/132) (95%CI=94-100%)	100% (7/7) (95%CI=60-100%)	100% (3/3) (95%CI=38-100%)
22 to 59 years	73% (8/11) (95%CI=43-91%)	97% (89/92) (95%CI=90-99%)	100% (2/2) (95%CI=29-100%)	94% (16/17) (95%CI=71-100%)	N/A (0/0)	N/A (0/0)

60 Years and up	100% (1/1) (95%CI=17-100%)	89% (16/18) (95%CI=66-98%)	N/A (0/0)	100% (1/1) (95%CI=17-100%)	N/A (0/0)	N/A (0/0)

A total of 2047 prospective clinical specimens were tested and gave valid results during this clinical study. These results were included in the performance tables above. There were nineteen (19) additional specimens (less than 1% of the total collected) that gave invalid results. The invalid results were excluded from the performance tables above because new patient specimens were not collected for re-testing. Of these 2047, five were dual positives by Sofia Influenza A+B FIA. Only one out of these five was positive for both Influenza A and B by virus culture.

4. Clinical cut-off**:**
   Not applicable

5. Expected values/Reference range:
   The prevalence observed with the reference test (virus culture) during the 2011 clinical study for Sofia Influenza A+B FIA was 15% for influenza A and 13% for influenza B.

N. Instrument Name:

Sofia Analyzer

O. System Descriptions:

1. Modes of Operation:

   The Sofia Analyzer is a bench top instrument intended to be used with cassette based immunofluorescent *in vitro* diagnostic assays manufactured by Quidel Corporation. After the extracted patient sample has been added to the test cassette, the test is developed at room temperature for a pre-specified period of time. The cassette is then placed into the Analyzer where it is scanned, and the fluorescent signal of the test is processed using a test-specific algorithm. The algorithm calculates the Signal to Cut-off (S/CO) value for each test line. This is the ratio of the signal on the Test Line expressed in RFUs divided by the calculated cutoff for each analyte expressed in RFUs. When the S/CO ratio is equal to or greater than 1, the test result for that analyte is positive. When the S/CO is less than 1, the test result for that analyte is negative.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___X___ or No _____

3. Specimen Identification:
   The user ID, patient ID, and order # can be entered via a handheld barcode scanner or by

manually entering the information onto the keypad of the Sofia Analyzer.

4. Specimen Sampling and Handling:
   Not applicable

5. Calibration:
   The Calibration cassette is shipped with the Sofia Analyzer and the calibration check procedure details are provided in the Sofia Analyzer user manual. The Calibration check is the required function that checks the Sofia Analyzer optics and calculation systems using the specific Calibration Cassette. The procedure is automatic and hence requires no user input and should be performed every 30 days.

6. Quality Control:
   There are three types of Quality Control for the Sofia Analyzer and Cassette: Sofia Analyzer calibration procedure, built-in procedural control features, and External Controls.

   Built-in Procedural Controls:

   The Sofia Influenza A+B FIA contains a built-in procedural control feature. Each time a test is run in the Sofia Analyzer, the procedural control zone is scanned by the Sofia Analyzer and the result is displayed on the Analyzer screen.

   The manufacturer's recommendation for daily control is to document the results of these built-in procedural controls for the first sample tested each day. This documentation is automatically logged in the Analyzer with each test result.

   A valid result obtained from the procedural control demonstrates that the test flowed correctly and the functional integrity of the Cassette was maintained. The procedural control is interpreted by the Sofia Analyzer after the Cassette has developed for fifteen (15) minutes. If the test does not flow correctly, the Sofia Analyzer will indicate that the result **is invalid.** Should this occur, review the procedure and repeat the test with a new patient sample and a new test Cassette.

   External Quality Control

   External Controls may also be used to demonstrate that the reagents and assay procedure perform properly.

   Quidel recommends that Positive and Negative External Controls be run once for each untrained operator, once for each new shipment of kits – provided that each different lot received in the shipment is tested – and as deemed additionally necessary by your internal quality control procedures, and in accordance with local, state and federal regulations or accreditation requirements.

   The user must first select Run QC on the main Menu of the Sofia Analyzer and then, when prompted, scan the QC Card (located on kit box). This card provides information specific to the kit lot, including lot number and expiration date.

The Analyzer will then prompt the user to run the External Control swabs.

External Positive and Negative Control swabs are supplied in the kit and should be tested using the Swab Test Procedure provided in this Package Insert or in the Quick Reference Instructions. Note: the Influenza Positive Control Swab should give a positive result for both influenza A and influenza B.

Do not perform patient tests or report patient test results if the control tests do not produce the expected results. Repeat the test or contact Quidel Technical Support before testing patient specimens.

Additional External Control swabs may be obtained separately by contacting Quidel's Customer Support Services.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In the "Performance Characteristics" Section above:

Not applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.