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Solana®
Influenza A+B ASSAY

FOR USE WITH SOLANA
For the detection and differentiation of influenza A and influenza B viral RNA in nasal and nasopharyngeal swabs from patients with signs and symptoms of respiratory infection.

For *in vitro* diagnostic use.

℞ ONLY

A symbols glossary can be found at quidel.com/glossary.

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INTENDED USE

The Solana Influenza A+B Assay is a qualitative *in vitro* diagnostic test for the detection and differentiation of influenza A and influenza B viral RNA in nasal and nasopharyngeal swabs from patients with signs and symptoms of respiratory infection. This test is intended for use as an aid in the differential diagnosis of influenza A and influenza B viral infections in humans in conjunction with clinical and epidemiological risk factors. The assay does not detect the presence of influenza C virus.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions.

Performance characteristics for influenza A were established during the spring of 2016 when influenza A/H3 and 2009 H1N1 influenza were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel influenza A 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. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

SUMMARY AND EXPLANATION

Influenza viruses (family Orthomyxoviridae) contain a single-stranded RNA genome which is present in eight separate segments of ribonucleoprotein. This segmentation of the genome is rare among viruses and probably contributes to the rapid development of new influenza strains through interchange of gene segments if two different viruses infect the same cell. There are three types of influenza – A, B and C. Type A has counterparts in birds and pigs as well as humans, while types B and C are known only in humans.[1] Due to the possibility of another pandemic caused by influenza A, as occurred in 1918 when 30 to 50 million people worldwide died,[2] the Centers for Disease Control (CDC) and the World Health Organization (WHO) maintain surveillance of influenza strains and make predictions of suitable strains for vaccine production.

CDC estimates that from the 1976-1977 season to the 2006-2007 flu season, flu-associated deaths ranged from a low of about 3,000 to a high of about 49,000 people.[3] Worldwide, annual epidemics of influenza result in about three to five million cases of severe illness, and about 250,000-500,000 deaths.[4] Pandemics of influenza A occur about every 10 to 30 years and epidemics of influenza A or B occur annually. Infections are seasonal, typically extending from November to April in the northern hemisphere. Complications tend to occur in the young, elderly and persons with chronic cardio-pulmonary diseases.

Incubation time is 1 to 3 days with rapid spread by inhalation via aerial droplets and fomites. It is characterized by fever, cough, sore throat, runny or stuffy nose, muscle or body aches, headache, fatigue, and in some cases vomiting and diarrhea (though this is more common in children than adults).[5]

Solana influenza A+B Assay allows for the rapid, accurate detection of influenza A and influenza B viral RNA. The assay is performed in the Solana instrument, where influenza RNA is amplified by isothermal Reverse Transcriptase Helicase Dependent Amplification (RT-HDA) reaction, which amplifies an influenza A and/or influenza B specific sequence in the presence of a process control sequence.[6,7] The amplicons are simultaneously detected by fluorescence probes.

PRINCIPLE OF THE TEST

The Solana Influenza A+B Assay amplifies and detects viral RNA present in viral transport media containing nasopharyngeal or nasal swab specimens obtained from symptomatic patients.

The assay consists of two major steps: (1) specimen preparation, and (2) amplification and detection of target sequences specific to influenza A and/or influenza B using isothermal Reverse Transcriptase – Helicase-Dependent Amplification (RT-HDA) in the presence of target-specific fluorescence probes.

A patient nasal or nasopharyngeal swab specimen in viral transport media is transferred to a Process Buffer Tube, subjected to heat treatment at 95°C for 5 minutes and mixed. The processed sample is transferred to a Reaction Tube. The Reaction Tube contains lyophilized RT-HDA reagents, dNTPs, primers and probes. Once rehydrated with the processed sample, the Reaction Tube is placed in Solana for amplification and detection of influenza A and influenza B-specific target sequences. In Solana, the target sequences are amplified by influenza A and influenza B specific primers and detected by influenza A and influenza B specific fluorescence probes, respectively. A competitive process control (PRC) is included in the Reaction Tube to monitor sample processing, inhibitory substances in clinical samples, reagent failure or device failure. The PRC target is amplified by influenza B specific primers and detected by a PRC specific fluorescence probe.

The two target probes and PRC probe are labeled with a quencher on one end and a fluorophore on the other end. In addition, the two target probes and PRC probe have one or more bases that are comprised of ribonucleic acid. Upon annealing to influenza A, influenza B or PRC amplicons, the fluorescence probes are cleaved by RNaseH2 and the fluorescence signal increases due to physical separation of fluorophore from quencher. Solana measures and interprets the fluorescent signal, using on-board method-specific algorithms. Solana then reports the test results to the user on its display screen, and it can print out the results via an integrated printer.

MATERIALS PROVIDED

Cat. #M300
48 Tests per Kit

Component	Quantity	Storage
Process Buffer	48 tubes/kit 1.55 mL	2°C to 8°C
Reaction Tubes	48 tubes/kit	2°C to 8°C

MATERIALS REQUIRED BUT NOT PROVIDED

- External controls for Influenza A and Influenza B (e.g. Solana Influenza A+B Control Set (Cat. #M122), which contains positive and negative controls, serves as an external processing control)
- Sterile DNAse-free filter-blocked positive displacement micropipettor tips
- Micropipettor
- Stopwatch or timer
- Vortex Mixer
- Scissors or a blade
- Workflow tray
- Transfer Rack
- Heat block capable of 95°C $\pm$ 2°C temperature
- Thermometer
- Solana instrument
- Transport Media (BD/Copan UTM, Remel M4, Remel M4RT, Remel M5, Remel M6, or Copan eSwab)

WARNINGS AND PRECAUTIONS

- All reagents are for *in vitro* diagnostic use only.
- Refer to the Solana Operator's Manual for further information regarding instrument installation and operation.
- Only use the protocol described in this package insert. Deviations from the protocol may give erroneous results.

- Treat all specimen/samples as potentially infectious. Follow universal precautions when handling samples, this kit and its contents.
- Influenza A and influenza B are stable in Copan eSwab™ transport media at 2°C to 8°C only for up to 48 hours.
- All tubes should be capped tightly prior to vortexing.
- Proper sample collection, storage and transport are essential for correct results.
- Store assay reagents as indicated on their individual labels.
- Reagents are not interchangeable between lots.
- Never pool reagents from different tubes even if they are from the same lot.
- Do not use the reagents after their expiration date.
- Do not interchange caps among reagents as contamination may occur and compromise test results.
- Only open the tubes when adding aliquots into tubes or removing aliquots from tubes. Keep the tubes closed at any other time to avoid contamination.
- For accurate results, pipette carefully using only calibrated equipment. Use of inaccurate volumes may give erroneous results.
- To avoid contamination of the environment with influenza amplicons, do not open the reaction tubes post-amplification.
- Avoid microbial and ribonuclease (RNAse) contamination of reagents when removing aliquots from tubes.
- Performing the assay outside of the recommended time ranges can produce invalid results. Assays not completed within specified time ranges should be repeated.
- Additional controls may be tested according to guidelines or requirements of local, state, provincial and/or federal regulations or accrediting organizations.
- Do not pipette by mouth.
- Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
- Maintenance and decontamination of workspace and equipment should follow and be performed according to established laboratory protocols and schedules. Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE AND HANDLING OF KIT REAGENTS

Store the Assay Kit at 2°C to 8°C until the expiration date listed on the outer kit box.

SPECIMEN COLLECTION, STORAGE AND HANDLING[8]

Nasal and nasopharyngeal specimens should be collected, transported, stored, and processed according to CLSI M41-A. Specimens should be stored at 2°C to 8°C until tested. Specimens collected in BD UTM™ (1- and 3- mL), Thermo Fisher Scientific™ Remel™ MicroTest™ M4® (3-mL), Remel™ MicroTest™ M4RT® (3-mL), Remel™ MicroTest™ M5® (3-mL), and Remel™ MicroTest™ M6® (3-mL) are stable at 2°C to 8°C for up to 9 days.

NOTE: Specimens collected in Copan eSwab™ transport media are stable at 2°C to 8°C for up to 48 hours.

TEST PROCEDURE

1. Turn on Solana by pressing the power button and wait until it completes self-testing.
   NOTE: Do not open the lid during the self-testing.
2. Place the required number of Process Buffer Tubes in the Workflow tray. Mark the Process Buffer Tubes on the cap and/or side of the tube.
   NOTE: One (1) Process Buffer Tube is required for each specimen or control to be tested.
   NOTE: A maximum of 12 tests can be performed per test run in a single Solana instrument.
3. Remove the required number of Reaction Tubes from the protective pouch and place in the Workflow tray. Mark the Reaction Tubes on the cap. Remove the excess air and reseal the pouch.
4. Mix the specimen received in viral transport media by vortexing the tubes for 5 seconds.
5. Remove 50 µL of the mixed specimen or External control and add to labeled Process Buffer Tubes and then vortex the Tubes for 5 seconds.

NOTE: Samples are stable in process buffer up to 48 hours at 2°C to 8°C, 25°C and –20°C after being added and prior to the heat step.

6. Heat the Process Buffer Tubes at 95 ±2°C for 5 minutes and then vortex the Tubes for 5 seconds.
   NOTE: Begin 5-minute lysis procedure after placing tubes in block and waiting until block returns to 95°C.
   NOTE: Samples are stable in process buffer up to 48 hours at 2°C to 8°C, 25°C and –20°C after the heat step.
7. Rehydrate the marked Reaction Tubes with 50 µL of each Process Buffer by vigorously pipetting up and down 5 times. The solution should be clear, free of solid material.
8. Using the Solana Transfer Rack to hold Reaction Tubes at eye-level, visually inspect each Reaction Tube to ensure pellet rehydration
9. Open the lid and place the Reaction Tubes in Solana via the Transfer Rack. Close the lid**.**
   NOTE: Be sure that all tubes are in tight contact with heat block.
10. Enter User ID, press ↵ (ENTER) and enter Password and press ↵ (ENTER).
11. Select "NEW TEST." If Solana displays a different screen, go to the home screen.
12. Select the tube positions to use.
13. Scan the assay barcode or manually enter Lot ID/Exp Date, then select "FLU Assay" from the Select Test drop-down menu and press "▶."
14. Select sample type (patient or QC) from the drop-down menu and enter Sample IDs (optional; see 2nd Note in next step).
15. Press "Start" to initiate the Solana Influenza A+B Assay. Solana will display the progress and the count-down to assay completion. Test results will be displayed on the screen in approximately 40 minutes.
    NOTE: To avoid laboratory contamination, once the tube has been closed and the amplification reaction started, **DO NOT** open the Reaction Tube.
    NOTE: While the test is running, sample ID can be entered or edited by pressing the pencil icon.
16. After the run is completed the results can be printed by selecting the print button.
    NOTE: Do not navigate away from this screen before printing results. Once the screen is gone, it cannot be revisited. If this occurs, the results can be viewed individually by going to Home and then selecting Review Results.
18. To determine if sample is positive for influenza A and/or B press the tube sample number. Separate results for the influenza A and influenza B channels will be displayed.

INTERPRETATION OF RESULTS

The Solana software automatically determines the specimen results for influenza A virus and influenza B virus. A positive result indicates that the viral RNA for the respective influenza virus was detected. A negative result indicates that influenza A virus and influenza B virus RNA were not detected and the process control was detected. Solana reports a specimen result as invalid when both influenza A virus and influenza B virus were not detected and the process control was undetected. The process control (PRC) is used to monitor sample processing, to detect HDA inhibitory specimens, to confirm the integrity of assay reagents and the operation of the Solana instrument.

Single Sample Results Screen	
Assay Result	**Interpretation**
INFLUENZA B NEGATIVE INFLUENZA A POSITIVE	Influenza A RNA detected
INFLUENZA B POSITIVE INFLUENZA A NEGATIVE	Influenza B RNA detected
INFLUENZA B POSITIVE INFLUENZA A POSITIVE	Influenza B RNA detected and Influenza A RNA detected*
INFLUENZA B NEGATIVE INFLUENZA A NEGATIVE	No Influenza B RNA detected/PRC detected and No Influenza A RNA detected/PRC detected
INFLUENZA B INVALID/ INFLUENZA A INVALID	No Influenza A or B RNA and No PRC detected; for invalid test results, re-process another aliquot of the same sample or obtain a new sample and re-test.

* Dual infections are rare. Re-process another aliquot of the same sample and re-test. If the retest confirms this result, collect and test a new specimen. Contact Quidel if multiple samples provide this result.

QUALITY CONTROL

The Solana Influenza A+B Assay incorporates several controls to monitor assay performance.

- The process control (PRC) is used to monitor sample processing, to detect HDA inhibitory specimens, to confirm the integrity of assay reagents and the operation of the Solana instrument. The process control is included in the Reaction Tube.
- The external positive control may be treated as a patient specimen. The control should be sampled and tested as if it were a patient specimen and processed as described above in the Assay Procedure. The external positive control is intended to monitor substantial reagent and instrument failure.
- The external negative control may be treated as a patient specimen. The control should be sampled and tested as if it were a patient specimen and processed as described above in the Assay Procedure. The external negative control is used to detect reagent or environmental contamination (or carry-over) by influenza A or B RNA or amplicon.

LIMITATIONS

- This test is not intended to differentiate influenza A subtypes. Additional testing is required if subtype differentiation is required.
- Negative results do not preclude infection with influenza virus and should not be the sole basis of a patient treatment decision. Improper collection, storage or transport of specimens may lead to false negative results.
- Errors in following the assay procedure may lead to false negative results.
- Recent patient exposure to LAIV (FluMist) may cause inaccurate dual positive results.
- A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- Analyte targets (viral sequences) may persist in vivo, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(s) are infectious, nor are the causative agents for clinical symptoms.
- There is a risk of false negative values due to the presence of sequence variants in the viral targets of the assay.
- The performance of the assay has not been established in individuals who received nasally administered Influenza A vaccine.
- The assay performance was not established in immunocompromised patients.
- Positive and negative predictive values are highly dependent on prevalence. The assay performance was established during the spring 2016 season. The performance may vary depending on the prevalence and population tested.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

EXPECTED VALUES

The expected values of the Solana Influenza A+B Assay were established during a prospective study conducted between February and April 2016. One thousand four hundred seventy-three (1473) specimens (fresh (742) and frozen (731)) have been included in this study at five (5) sites across the United States. A single specimen was collected per patient. The specimens were processed and tested with Solana Influenza A+B Assay on the Solana instrument at the sites.

The expected value of influenza A and influenza B with the Solana Influenza A+B Assay has been calculated for the combined sites based on the age of the patient.

Fifty-three (53) of the one thousand four hundred seventy-three (1473) specimens were removed from analysis: (three (3) specimens did not have the age provided; fifty (50) specimens were invalid). The table below provides the percentage of influenza A and influenza B positive cases per specified age group, as determined by the Solana Influenza A+B Assay, for the remaining one thousand four hundred twenty (1420) specimens.

Expected Values (N=1420)						
Age Group	**Influenza A**			**Influenza B**		
	Number of Patients	Number of Positives	Prevalence	Number of Patients	Number of Positives	Prevalence
≤ 5 years	377	91	24.1%	377	26	6.9%
6 to 21 years	297	89	30.0%	297	48	16.2%
22 to 59 years	504	191	37.9%	504	17	3.4%
≥ 60 years	242	37	15.3%	242	3	1.2%

The prospective clinical study had a dual infection rate for Influenza A and Influenza B of 0.2% (3/1420) using the Solana Influenza A+B Assay. All three (3) of these dual detections were only positive for influenza A by culture and DSFA and also by an alternate molecular comparator.

CLINICAL PERFORMANCE

Performance characteristics of the Solana Influenza A+B Assay were established during a prospective study with specimens collected between February and April 2016. One thousand four hundred seventy-three (1473) prospectively collected specimens have been included in this study at five (5) sites across the United States. A single nasal or nasopharyngeal swab specimen (302 and 1171, respectively) was collected per patient in viral transport media (BD™/Copan UTM™, Remel™ M5™, Remel™ M6™). All specimens were transported to a central location at 2°C to 8°C for testing by the comparator methods (culture for influenza A and B using the R-Mix Too mixed cells and direct specimen DFA (DSFA), and extraction with the NucliSENS® easyMAG® and testing with a FDA-cleared Influenza A+B molecular assay). The specimens were processed and tested with Solana Influenza A+B Assay on the Solana instrument at the sites.

The gender and age demographics of the patients enrolled in the study are shown below.

Combined Study – Age and Gender Distribution		
Gender*	Female	Male
Total	798	672
Age		
≤ 5 years	195	197
6 to 21 years	139	167
22 to 59 years	328	197
≥ 60 years	136	111

***** Three (3) specimens did not have gender or age provided.

Comparison Versus Culture with DFA and DSFA

One thousand four hundred seventy-three (1473) fresh specimens were included in this study. Each specimen was cultured for influenza A and B using the R-Mix Too mixed cells and stained with an FDA-cleared device and processed for direct specimen DFA (DSFA). All comparator testing was performed on fresh specimens within 72 hours of their collection. A specimen was recorded as positive for influenza A or B if either comparator test was positive. Seven hundred and forty-two (742) of these specimens were tested using the Solana Influenza A+B Assay for the presence influenza A or B unfrozen.

Seven hundred and forty-two (742) of these specimens were tested fresh using the Solana Influenza A+B Assay for the presence influenza A or B. Seven hundred and thirty-one (731) specimens were frozen and stored at –70°C prior to testing with the Solana Influenza A+B Assay. Fifteen (15) specimens were contaminated or toxic in the cell culture (1.0%). Fifty (50) specimens were invalid in the Solana Assay (3.4%). These sixty-five (65) specimens have been excluded from further analysis. The tables below detail the performance of the Solana Assay for influenza A and influenza B respectively for the remaining one thousand four hundred eight (1408) specimens, across all testing sites combined, as compared to viral culture with DSFA results.

Performance Characteristics of the Solana Influenza A+B Assay for Influenza A Compared to Culture and DSFA (Across all Sites Combined)							
Source Category	N	TP	FP	TN	FN	Sensitivity% (95% CI)	Specificity% (95% CI)
Fresh	709	180	24	503	2	98.9 (96.1 to 99.7)	95.4 (93.3 to 96.9)
Frozen	699	176	27	493	3	98.3 (95.2 to 99.4)	94.8 (92.6 to 96.4)
All	1408	356	51*	996	5**	98.6 (96.8 to 99.4)	95.1 (93.7 to 96.3)

*Of the fifty-one (51) discordant specimens (Solana Positive/Culture and DSFA Negative), twenty-eight (28) of these specimens were positive by an FDA-cleared alternate molecular assay.

**Of the five (5) discordant specimens (Solana Negative/Culture and DSFA Positive), two (2) of these specimens were positive by an alternate FDA cleared molecular assay.

Performance Characteristics of the Solana Influenza A+B Assay for Influenza B Compared to Culture and DSFA (Across all Sites Combined)							
Source Category	N	TP	FP	TN	FN	Sensitivity% (95% CI)	Specificity% (95% CI)
Fresh	709	62	1	646	0	100 (94.2 to 100)	99.8 (99.1 to 100)
Frozen	699	23	8	668	0	100 (85.7 to 100)	98.8 (97.7 to 99.4)
All	1408	85	9*	1314	0	100 (95.7 to 100)	99.3 (98.7 to 99.6)

*Of the nine (9) discordant specimens (Solana Positive/Culture and DSFA Negative), two (2) of these specimens were positive by an alternate FDA cleared molecular assay.

Comparison with a FDA-cleared Influenza A+B Molecular Assay

One thousand four hundred seventy-three (1473) specimens were processed using the NucliSENS easyMAG and tested with a FDA-cleared Influenza A+B molecular assay according to the assay's package insert. The comparator testing was performed on fresh specimens within 72-hours of their collection.

Seven hundred and thirty-one (731) of the original specimens were frozen and stored at –70°C prior to testing with the Solana Influenza A+B Assay. Seven hundred and forty-two (742) of the original specimens were tested fresh using the Solana Influenza A+B Assay for the presence of influenza A or B. Thirty-one (31) specimens were invalid in the comparator assay (2.1%). Fifty (50) specimens were invalid in the Solana Assay (3.4%) (one specimen was invalid in both assays). These eighty (80) specimens have been excluded from further analysis. The table below details the positive percent agreement (PPA) and the negative percent agreement (NPA) of the Solana Influenza A+B Assay results for influenza A, as compared with an FDA cleared molecular comparator, for the remaining one thousand three hundred ninety-three (1393) specimens.

Percent Agreement of the Solana Influenza A+B Assay for Influenza A Compared to an FDA cleared Influenza A+B Molecular Assay (Across all Sites Combined)							
Source Category	N	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
Fresh	710	195	9	499	7	96.5 (93.0 to 98.3)	98.2 (98.7 to 99.1)
Frozen	683	180	24	475	4	97.8 (94.5 to 99.2)	95.2 (92.9 to 96.7)
All	1393	375	33	974	11	97.2 (95.0 to 98.4)	96.7 (95.4 to 97.7)

There were a total of forty-four (44) discordant specimens among the one thousand three hundred ninety-three (1393) specimens evaluated. Of the thirty-three (33) discordant specimens (Solana Positive/Comparator Negative), nine (9) of these specimens were positive by culture/DSFA. Of the eleven (11) discordant specimens (Solana Negative/ Comparator Positive), two (2) of these specimens were positive by culture/DSFA.

Percent Agreement of the Solana Influenza A+B Assay for Influenza B Compared to an FDA-cleared Influenza A+B Molecular Assay (Across all Sites Combined)							
Source Category	N	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
Fresh	710	57	6	647	0	100 (93.7 to 100)	99.1 (98.0 to 99.6)
Frozen	683	23	8	652	0	100 (85.7 to 100)	98.8 (97.6 to 99.4)
All	1393	80	14	1299	0	100 (95.4 to 100)	98.9 (98.2 to 99.4)

There were a total of fourteen (14) discordant specimens among the one thousand three hundred ninety-three (1393) specimens evaluated. Of the fourteen (14) discordant specimens (Solana Positive/ Comparator Negative), seven (7) of these specimens were positive by culture/DSFA.

ANALYTICAL PERFORMANCE

Analytical Sensitivity (Limit of Detection)

The analytical sensitivity (limit of detection or LOD) of the Solana Influenza A+B Assay was determined using quantified ($TCID_{50}$/mL) cultures of three (3) influenza A strains and two (2) influenza B strains, serially diluted in negative

nasopharyngeal matrix. Each dilution was run as 20 replicates in the Solana Influenza A+B assay. Analytical sensitivity (LOD) is defined as the lowest concentration at which at least 95% of all replicates tested positive. The demonstrated LOD for each strain tested is shown below:

LOD Values		
Influenza A Virus	Subtype	$TCID_{50}$/mL
A/Taiwan/42/06	H1N1	$7.5x10^2$
A/California/07/2009	H1N1p	$4.7x10^2$
A/Texas/50/2012	H3N2	$6.3x10^0$
Influenza B Virus	Lineage	
B/Brisbane/60/08	Victoria	$8.5x10^1$
B/Massachusetts/2/2012	Yamagata	$3.3x10^1$

ANALYTICAL REACTIVITY (INCLUSIVITY)

The reactivity of the Solana Influenza A+B Assay was evaluated against multiple strains of influenza A and influenza B viruses. The influenza panel consisted of fourteen (14) influenza A strains, and eight (8) Influenza B strains at concentrations near the level of detection (LOD) of the assay.

Inclusivity Strains			
Strain	**Subtype/Lineage**	**$TCID_{50}$/mL**	**Inclusive (Yes or No)**
Influenza A			
A/Mexico/4108/2009	H1N1p	$2.3x10^3$	Yes
A/Denver/1/57	H1N1	$2.3x10^3$	Yes
A/New Jersey/8/76	H1N1	$2.3x10^3$	Yes
A/PR/8/34	H1N1	$2.3x10^3$	Yes
A/FM/1/47	H1N1	$2.3x10^3$	Yes
A/Solomon Islands/3/06	H1N1	$2.3x10^3$	Yes
A/New Caledonia/20/1999	H1N1	$2.3x10^3$	Yes
A/Victoria/361/11	H3N2	$2.3x10^3$	Yes
A/Port Chalmers/1/73	H3N2	$1.4x10^4$	Yes
A/Aichi/2/68	H3N2	$2.3x10^3$	Yes
A/Victoria/3/75	H3N2	$2.3x10^3$	Yes
A/Hong Kong/8/68	H3N2	$2.3x10^3$	Yes
A/Wisconsin/67/2005	H3N2	$2.3x10^3$	Yes
A/WS/33	H1N1	$2.3x10^3$	Yes
Influenza B			
B/Malaysia/2506/04	Victoria	$2.6x10^2$	Yes
B/Florida/07/2004	Victoria	$7.7x10^2$	Yes
B/Maryland/1/59	Yamagata	$2.6x10^2$	Yes
B/Allen/45	Yamagata	$2.6x10^2$	Yes
B/Lee/40	Yamagata	$2.6x10^2$	Yes
B/Florida/04/2006	Yamagata	$7.7x10^2$	Yes
B/Panama/45/90	Yamagata	$2.6x10^2$	Yes
B/Hong Kong/5/72	Victoria	$2.6x10^2$	Yes
B/Malaysia/25/06/04	Victoria	$2.6x10^2$	Yes

Due to restrictions and availability of a number of influenza A strains, *in silico* analysis was performed for three additional strain designations:

- A total of four (4) H3N2v (1 human strain and 3 swine) sequences were analyzed *in silico*. All four sequences demonstrated 100% homology.
- A total of three hundred forty (340) H5N1 strains were analyzed *in silico*. Three hundred thirty-nine (339) strains in the database demonstrated ≥95% overall homology and ≥88% homology to any individual primer or probe sequence. One H5N1 strain demonstrated an overall homology of 88% and ≥82% homology to any individual primer or probe sequence.

- A total of one hundred sixty-four (164) H7N9 sequences were analyzed *in silico*. All 164 sequences demonstrated 100% homology.
- Fourteen (14) non-clinical avian restricted influenza A viruses (table below) were analyzed *in silico*.

Non-clinical Avian Restricted Influenza A Viruses	
Subtype	**Strain**
H2N2	A/Mallard/NY/6750/78 (H2N2)
H7N3	A/Chicken/NJ/15086-3/94 (H7N3)
H9N2	A/Chicken/NJ/12220/97 (H9N2)
H4N8	A/Mallard/OH/338/86 (H4N8)
H6N2	A/Chicken/CA/431/00 (H6N2)
H8N4	A/Blue Winged Teal/LA/B174/86 (H8N4)
H5N1	A/Anhui/01/2005(H5N1)-PR8-IBCDC-RG5
H10N7	A/GWT/LA/169GW/88 (H10N7)
H11N9	A/Chicken/NJ/15906-9/96 (H11N9)
H12N5	A/Duck/LA/188D/87 (H12N5)
H13N6	A/Gull/MD/704/77 (H13N6)
H14N5	A/Mallard/GurjevRussia/262/82 (H14N5)
H15N9	A/Shearwater/Australia/2576/79 (H15N9)
H16N3	A/Shorebird/DE/172/2006(H16N3)

A total of twenty-seven (27) sequences were available for analysis. The Solana FluA primers and probe are 90% to 100% conserved to the specified avian strains and to representative avian strains.

REPRODUCIBILITY STUDY

The reproducibility of the Solana Influenza A+B Assay was evaluated at three laboratory sites. A four-sample panel consisting of three levels of a combined influenza A and influenza B contrived samples and a negative contrived sample were tested in this study. Influenza A and influenza B viruses (Influenza A/California/07/2009 and Influenza B/Brisbane/60/08, respectively) were diluted in negative nasal matrix to 2 x LOD for moderate positive, 1 x LOD for low positive and diluted to C20 to C80 for high negative / low positive. Negative nasal matrix without spiked virus was used for the negative sample. The Solana Influenza A+B assay was used according to the instructions for use.

Panels and controls were tested at each site by two operators per instrument for five (5) days, each sample tested in three (3) replicates, for a total of 90 results per level for each virus for each instrument (2 operators x 5 days x 3 sites x 3 replicates).

Reproducibility Summary									
Source	SITE						Overall Percent Agreement		95% Confidence Interval
	Site #1		Site #2		Site #3				
	#Detected positive/# tested	*% Agreement with Expected Result*	*#Detected positive/# tested*	*% Agreement with Expected Result*	*#Detected positive/# tested*	*% Agreement with Expected Result*			
Influenza A/California/07/2009 High Negative (1.4×10^2 $TCID_{50}$/mL)	10/30	33.3	25/30	83.3	23/30	76.7	58/90	64.4	54.1 to 73.6
Influenza A/California/07/2009 Low Positive (4.7×10^2 $TCID_{50}$/mL)	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
Influenza A/California/07/2009 Moderate Positive	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100

Reproducibility Summary									
Source	SITE						Overall Percent Agreement		95% Confidence Interval
	Site #1		Site #2		Site #3				
	#Detected positive/# tested	*% Agreement with Expected Result*	*#Detected positive/# tested*	*% Agreement with Expected Result*	*#Detected positive/# tested*	*% Agreement with Expected Result*			
($9.4x10^2$ $TCID_{50}$/mL)									
Negative	0/30	100	0/30	100	0/30	100	0/90	100	96.5 to 100
Influenza A Positive Control	15/15	100	15/15	100	15/15	100	45/45	100	94.2 to 100
Influenza A Negative Control	0/15	100	0/15	100	0/15	100	0/45	100	94.2 to 100
Influenza B/Brisbane/60/08 High Negative ($2.6 x10^1$ $TCID_{50}$/mL)	9/30	30	5/30	16.7	10/30	33.3	24/90	26.7	18.6 to 36.6
Influenza B/Brisbane/60/08 Low Positive ($8.5x10^1$$TCID_{50}$/mL)	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
Influenza B/Brisbane/60/08 Moderate Positive ($1.7x10^2$ $TCID_{50}$/mL)	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
Negative	0/30	100	0/30	100	0/30	100	0/90	100	96.5 to 100
Influenza B Positive Control	15/15	100	15/15	100	15/15	100	45/45	100	94.2 to 100
Influenza B Negative Control	0/15	100	0/15	100	0/15	100	0/45	100	94.2 to 100

ANALYTICAL SPECIFICITY – MICROBIAL INTERFERENCE

A study was performed to evaluate the performance of the Solana Influenza A+B Assay in the presence of forty-four (44) microorganisms (24 bacteria, 1 yeast, 19 viruses) potentially found in specimens that are collected from nasal passages of patients symptomatic for influenza. Each microorganism was diluted in negative nasal matrix to the desired concentration (10^6 or higher CFU/mL for bacteria and yeast, and 10^5 or higher pfu/mL or $TCID_{50}$/mL for viruses). Each organism was tested with the Solana Influenza A+B assay in triplicate in the presence of influenza A and influenza B viruses (Influenza A/California/07/2009 and Influenza B/Brisbane/60/08, respectively) at 2x LOD. No microbial interference was observed. The organisms and their concentrations included in the interference study are shown in the table below.

Potential Interfering Organisms	
Organism	**Concentration Tested**
Adenovirus 1	$1.0x10^5$ $TCID_{50}$/mL
Adenovirus 11	$1.0x10^5$ $TCID_{50}$/mL
Bordetella bronchiseptica	$1.0x10^6$ CFU/mL
Bordetella pertussis	$1.0x10^6$ CFU/mL
Candida albicans	$1.0x10^6$ CFU/mL
Chlamydophila pneumoniae	$5.0x10^4$ $TCID_{50}$/mL*
Coronavirus 229E	$1.0x10^5$ $TCID_{50}$/mL
Corynebacterium diptheriae	$1.0x10^6$ CFU/mL
Coxsackievirus B5/10/2006	$1.0x10^5$ $TCID_{50}$/mL
Echovirus 11	$1.0x10^5$ $TCID_{50}$/mL

Potential Interfering Organisms	
Organism	**Concentration Tested**
Echovirus 6	$1.0x10^5$ $TCID_{50}$/mL
Enterovirus 70	$1.0x10^5$ $TCID_{50}$/mL
Enterovirus 71	$2.0x10^4$ $TCID_{50}$/mL*
Epstein Barr virus	$1.0x10^5$ $TCID_{50}$/mL
Escherichia coli	$1.0x10^6$ CFU/mL
Haemophilus influenzae	$1.0x10^6$ CFU/mL
HSV 1 MacIntyre Strain	$1.0x10^5$ $TCID_{50}$/mL
HSV 2 G strain	$1.0x10^5$ $TCID_{50}$/mL
Human Rhinovirus	$1.0x10^5$ $TCID_{50}$/mL
Klebsiella pneumoniae	$1.0x10^6$ CFU/mL
Lactobacillus plantarum	$1.0x10^6$ CFU/mL
Legionella pneumophila	$1.0x10^6$ CFU/mL
Measles	$1.0x10^5$ $TCID_{50}$/mL
Metapneumovirus A1	$1.0x10^5$ $TCID_{50}$/mL
Moraxella catarrhalis	$1.0x10^6$ CFU/mL
Mumps	$1.0x10^5$ $TCID_{50}$/mL
Mycobacterium avium	$1.0x10^6$ CFU/mL
Mycobacterium tuberculosis	$1.0x10^6$ CFU/mL
Mycoplasma pneumoniae	$1.0x10^6$ CFU/mL
Neisseria gonorrhoeae	$1.0x10^6$ CFU/mL
Neisseria meningitides	$1.0x10^6$ CFU/mL
Parainfluenza Type 1	$1.0x10^5$ $TCID_{50}$/mL
Parainfluenza Type 2	$1.0x10^5$ $TCID_{50}$/mL
Parainfluenza Type 3	$1.0x10^5$ $TCID_{50}$/mL
Proteus mirabilis	$1.0x10^6$ CFU/mL
Proteus vulgaris	$1.0x10^6$ CFU/mL
Pseudomonas aeruginosa	$1.0x10^6$ CFU/mL
Respiratory syncytial virus	$1.0x10^5$ $TCID_{50}$/mL
Staphylococcus aureus	$1.0x10^6$ CFU/mL
Staphylococcus epidermidis	$1.0x10^6$ CFU/mL
Streptococcus mutans	$1.0x10^6$ CFU/mL
Streptococcus pneumoniae	$1.0x10^5$ CFU/mL*
Streptococcus pyogenes	$1.0x10^6$ CFU/mL
Streptococcus salivarius	$1.0x10^6$ CFU/mL

*Due to low concentration of the stock organism, the concentration tested was below the target. The actual concentration tested is listed in the table.

No interference was observed with the forty-four (44) microorganisms tested with the Solana Influenza A+B Assay.

ANALYTICAL SPECIFICITY – CROSS-REACTIVITY

A study was performed to evaluate the cross-reactivity of the Solana Influenza A+B Assay with forty-four (44) microorganisms (24 bacteria, 1 yeast, 19 viruses) potentially found in specimens that are collected from patients symptomatic for influenza. Each microorganism was diluted in negative nasal matrix to the desired concentration (10^6 or higher CFU/mL for bacteria, yeast and 10^5 or higher pfu/mL or $TCID_{50}$/mL for viruses) and tested with the Solana Influenza A+B assay. No cross reactivity was observed with the organisms at concentrations shown in the table below.

Potential Cross-reacting Organisms	
Organism	**Concentration Tested**
Adenovirus 1	$1.0x10^5$ $TCID_{50}$/mL
Adenovirus 11	$1.0x10^5$ $TCID_{50}$/mL
Bordetella bronchiseptica	$1.0x10^6$ CFU/mL
Bordetella pertussis	$1.0x10^6$ CFU/mL
Candida albicans	$1.0x10^6$ CFU/mL
Chlamydophila pneumoniae	$5.0x10^4$ $TCID_{50}$/mL*
Coronavirus 229E	$1.0x10^5$ $TCID_{50}$/mL
Corynebacterium diptheriae	$1.0x10^6$ CFU/mL
Coxsackievirus B5/10/2006	$1.0x10^5$ $TCID_{50}$/mL
Enterovirus 70	$1.0x10^5$ $TCID_{50}$/mL
Enterovirus 71	$2.0x10^4$ $TCID_{50}$/mL*
Echovirus 11	$1.0x10^5$ $TCID_{50}$/mL
Echovirus 6	$1.0x10^5$ $TCID_{50}$/mL
Epstein Barr virus	$1.0x10^5$ $TCID_{50}$/mL
Escherichia coli	$1.0x10^6$ CFU/mL
Haemophilus influenzae	$1.0x10^6$ CFU/mL
HSV 1 MacIntyre Strain	$1.0x10^5$ $TCID_{50}$/mL
HSV 2 G strain	$1.0x10^5$ $TCID_{50}$/mL
Human Rhinovirus	$1.0x10^5$ $TCID_{50}$/mL
Klebsiella pneumoniae	$1.0x10^6$ CFU/mL
Lactobacillus plantarum	$1.0x10^6$ CFU/mL
Legionella pneumophila	$1.0x10^6$ CFU/mL
Measles	$1.0x10^5$ $TCID_{50}$/mL
Metapneumovirus A1	$1.0x10^5$ $TCID_{50}$/mL
Moraxella catarrhalis	$1.0x10^6$ CFU/mL
Mumps	$1.0x10^5$ $TCID_{50}$/mL
Mycobacterium avium	$1.0x10^6$ CFU/mL
Mycobacterium tuberculosis	$1.0x10^6$ CFU/mL
Mycoplasma pneumoniae	$1.0x10^6$ CFU/mL
Neisseria gonorrhoeae	$1.0x10^6$ CFU/mL
Neisseria meningitides	$1.0x10^6$ CFU/mL
Parainfluenza Type 1	$1.0x10^5$ $TCID_{50}$/mL
Parainfluenza Type 2	$1.0x10^5$ $TCID_{50}$/mL
Parainfluenza Type 3	$1.0x10^5$ $TCID_{50}$/mL
Proteus mirabilis	$1.0x10^6$ CFU/mL
Proteus vulgaris	$1.0x10^6$ CFU/mL
Pseudomonas aeruginosa	$1.0x10^6$ CFU/mL
Respiratory syncytial virus	$1.0x10^5$ $TCID_{50}$/mL
Staphylococcus aureus	$1.0x10^6$ CFU/mL
Staphylococcus epidermidis	$1.0x10^6$ CFU/mL
Streptococcus mutans	$1.0x10^6$ CFU/mL
Streptococcus pneumoniae	$1.0x10^5$ CFU/mL*
Streptococcus pyogenes	$1.0x10^6$ CFU/mL
Streptococcus salivarius	$1.0x10^6$ CFU/mL

* Due to low concentration of the stock organism, the concentration tested was below the target. The actual concentration tested is listed in the table.

ANALYTICAL SPECIFICITY – INTERFERING SUBSTANCES

The performance of Solana Influenza A+B Assay was evaluated with potentially interfering substances that may be present in nasal and nasopharyngeal specimens. The potentially interfering substances were evaluated with influenza A

(A/Mexico/ 4108/2009) and influenza B (Influenza B/Brisbane/60/08) at concentrations of 2x LOD. There was no evidence of interference caused by the substances tested at the concentrations shown below.

Potential Interfering Substances		
Substances	**Active Ingredient**	**Concentration Tested**
Purified mucin protein	Mucin protein	2.5 mg/mL
Blood (human)	Blood	5.0%
Afrin – nasal spray	Oxymetazoline	5.0%
Saline nasal spray	Saline	15.0%
Phenylephrine hydrochloride	Phenylephrine hydrochloride	15.0%
Flonase	Fluticasone	5.0%
Zicam Gentle Allergy Relief NasalGel	*Galphimia glauca*, *Histaminum hydrochloricum*, *Luffa operculata*, Sulfur	5.0%
Mupirocin	Mupirocin	12.0 mg/mL
Oseltamivir	Oseltamivir	2.2 µg/mL
Zanamivir	Zanamivir	282.0 ng/mL
Tobramycin	Tobramycin	2.5 mg/mL
Chloraseptic	Benzocaine, Menthol	0.68 g/mL
Amantadine hydrochloride	Amantadine hydrochloride	282.0 ng/mL
Nasocort Allergy 24 hour	Triamcinolone	5.0%
Sinus Buster Nasal Spray	*Capsicum annuum* (Capsaicin)	5.0%
NasalCrom Nasal Allergy Spray	Cromolyn Sodium	5.0%
Rhinocort	Budesonide (Glucocorticoid)	5.0%
Air-Vita Allergy Multi-Symptom Relief	Allium cepa, Ambrosia artemisiaefolia, Apis mellifica, Chamomilla, Eucalyptol, Eucalyptus globulus, Euphrasia officinalis, Galphimia glauca, Histaminum hydrochloricum, Natrum muriaticum, Nux vomica, Quercus robur, Silicea, Wyethia helenioides	5.0%
Ipratropium bromide	Ipratropium bromide	10.0 mg/mL
Olopatadine hydrochloride	Olopatadine hydrochloride	10.0 mg/mL
Amantadine hydrochloride	Amantadine hydrochloride	282.0 ng/mL

CARRYOVER AND CROSS-CONTAMINATION STUDIES

Positive samples consisting of an influenza A strain and an influenza B strain formulated in pooled negative nasal matrix at concentrations greater or equal to $1x10^5$ $TCID_{50}$/mL each. The negative samples consisted of pooled negative nasal matrix. In each of 5 rounds of testing, 6 positive samples and 6 negative samples were tested in alternating order to assess the risk of cross contamination.

Consecutive testing of alternating high positive samples and negative samples resulted in no observed carry over or cross contamination as 30/30 positive samples tested positive and 30/30 negative samples tested negative.

CUSTOMER AND TECHNICAL SUPPORT

If you have any questions regarding the use of this product, please contact Quidel Technical Support at 1.800.874.1517 (in the U.S.) or technicalsupport@quidel.com. If outside the U.S., further information can be obtained from your distributor, or directly from Quidel at one of the numbers listed below. Reference **quidel.com** to see more options for Support.

Country	Phone	E-Mail Address
Europe, Middle East and Africa	+353 (91) 412 474 (main) 0 1800 200441 (toll free)	emeatechnicalsupport@quidel.com
Austria	+43 316 231239	
France	0 (805) 371674	
Germany	+49 (0) 7154 1593912	
Netherlands	0 800 0224198	
Switzerland	0 800 554864	
United Kingdom	0 800 3688248	
Italy	+39 (800) 620 549	
North America, Asia-Pacific, Latin America	858.552.1100	technicalsupport@quidel.com
Canada	437.266.1704 (main) 888.415.8764 (toll free)	technicalsupport@quidel.com
China	0400 920 9366 or +86 021 3217 8300	chinatechnicalservice@quidel.com

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Dye compounds in this product are sold under license from Biosearch Technologies, Inc., and protected by U.S. and worldwide patents either issued or under application.

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REF M300 – Solana Influenza A+B Assay – 48-Test Kit

IVD

CE

EC REP

MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

Quidel Corporation
2005 East State Street, Suite 100
Athens, OH 45701 USA
quidel.com

PIM300010EN01 (10/20)

Revision Changes:

- Add Intellectual Property section.

GLOSSARY

REF Catalogue number	CE mark of conformity
EC REP Authorized Representative in the European Community	LOT Batch code
Use by	Manufacturer
Temperature limitation	Intended use
℞ ONLY Prescription use only	Consult e-labeling instructions for use
IVD For *In Vitro* diagnostic use	Σ 48 Contains sufficient for 48 determinations
CONT Contents/Contains	