This Procedural Bulletin is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete Package Insert. It is the obligation of every manufacturer of medical devices labeled FOR *IN VITRO* DIAGNOSTIC USE to provide a complete Package Insert in accordance with FDA labeling regulation (21 CFR 809.10).

Quidel Corporation provides CLSI procedures for your use. The procedures are required to include the same information as listed in the Package Insert. Any modifications to this document are the sole responsibility of the Laboratory.

Solana RSV + hMPV Assay

CLIA Complexity: Moderate

INTENDED USE

The Solana RSV + hMPV Assay is a qualitative *in vitro* diagnostic test for the detection and differentiation of RSV and hMPV 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 RSV and hMPV viral infections in humans in conjunction with clinical and epidemiological risk factors. This test is not intended to differentiate the two subtypes of RSV or the four genetic sub-lineages of hMPV.

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

Conversely, positive results do not rule-out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. The use of additional laboratory testing and clinical presentation must be considered in order to obtain the final diagnosis of respiratory viral infection.

SUMMARY AND EXPLANATION

RSV: Human respiratory syncytial virus (RSV) is a negative single-stranded RNA virus of the family Paramyxoviridae. RSV is the major cause of lower respiratory tract infection and hospital visits during infancy and childhood.

In the United States, 60% of infants are infected during their first RSV season, and nearly all children will have been infected with the virus by 2–3 years of age.[1] Of those infected with RSV, 2–3% will develop bronchiolitis, necessitating hospitalization.[2] Natural infection with RSV induces protective immunity that wanes over time—possibly more so than other respiratory viral infections--and thus people can be infected multiple times. Sometimes an infant can become symptomatically infected more than once, even within a single RSV season. Severe RSV infections have increasingly been found among elderly patients.

RSV subtypes A and B are present either simultaneously or alternately during yearly epidemics and there are several supporting studies indicating that RSV-A induced bronchiolitis is more severe than an RSV-B induced one.[3]

hMPV: Human metapneumovirus (hMPV) is a negative single-stranded RNA virus of the family Paramyxoviridae first isolated in 2001 in the Netherlands.[4] hMPV may be the second most common cause (after RSV) of lower respiratory infection in young children. Compared with RSV, infection with hMPV tends to occur in slightly older children and produce disease that is less severe. Co-infection with both viruses can occur and is generally associated with more severe disease.

hMPV accounts for approximately 7.1% of respiratory tract infections.[5] The virus appears to be distributed worldwide and has a seasonal distribution with its incidence comparable to that for the influenza viruses during winter. Serologic studies have shown that by the age of five, virtually all children have been exposed to the virus.[6] hMPV generally causes mild respiratory tract infection. However, small children, the elderly, and immuno-compromised individuals are at risk for severe disease and hospitalization.

Sequence analyses of the hMPV genome have shown that hMPV strains circulating around the world can be divided into two main genetic lineages (A and B) representing two serotypes, each comprising two sublineages (A1, A2, B1, and B2).[7]

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

PRINCIPLE OF THE TEST

The Solana RSV + hMPV 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 RSV and/or hMPV using isothermal Reverse Transcriptase – Helicase-Dependent Amplification (RT-HDA) in the presence of target-specific fluorescence probes.

A patient nasal or nasopharyngeal swab specimen collected 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 RSV and hMPV-specific target sequences. In Solana, the target sequences are amplified by RSV and hMPV specific primers and detected by RSV and hMPV 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 test failure. The PRC target is amplified by RSV and hMPV 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 incorporate one or more RNA bases. Upon annealing to RSV, hMPV 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 attached printer.

MATERIALS PROVIDED

Cat. # M306
48 Tests per Kit

Components	**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 RSV and hMPV (e.g. Solana RSV + hMPV Control Set, CAT. #M123 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° ± 2°C temperature
- Thermometer
- Solana instrument

- Transport Media (BD™ Universal Viral Transport Medium (UVT) / COPAN Universal Transport Medium (UTM™) 3.0 mL, Thermo Fisher Scientific™ Remel™ MicroTest™ M4® 3.0 mL, Remel™ MicroTest™ M4RT® 3.0 mL, Remel™ MicroTest™ M5® 3.0 mL, Remel™ MicroTest™ M6® 3.0 mL, or COPAN eSwab™ 1.0 mL)

WARNINGS AND PRECAUTIONS

- All reagents are for *in vitro* diagnostic use only.
- Refer to the Solana User 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.
- 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.
- To avoid contamination of the environment with 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 incorrect results. Assays not completed within specified time ranges should be repeated.
- Additional controls may be tested per guidelines or requirements of local, state, provincial and/or federal regulations or accrediting organizations.
- For accurate results, pipette carefully using only calibrated equipment. Use of inaccurate volumes may give erroneous results.
- Gloves must always be worn and must be changed before going from one area to another. Gloves must be changed before manipulating the reagents.
- Wash hands thoroughly after performing the test.
- Do not pipette by mouth.
- Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
- Testing should be performed in an area with adequate ventilation.
- Dispose of unused reagents and waste in accordance with county, federal, provincial, state and local regulations.
- Wear suitable protective clothing, gloves, eyes and face protection when using this kit.
- Thoroughly clean and disinfect all surfaces with a 1% bleach solution followed by water.
- 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

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™ UVT (1 and 3 mL), Thermo Fisher Scientific™ Remel™ MicroTest™ M4® (3 mL), Remel™ MicroTest™ M4RT® (3 mL), Remel™ MicroTest™ M5® (3 mL), Remel™ MicroTest™ M6® (3 mL), and COPAN eSwab™ are stable at 2°C to 8°C for up to 8 days and at –70°C for up to 10 weeks.

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 theTubes 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 when the heat block measures 95° ± 2°C. The timer must be stopped if the temperature falls out of range at any time during the 5-minute period and cannot be restarted until the heat block returns to 95° ± 2°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 "RSV + hMPV Assay" from the Select Test drop-down menu and press "▶".
14. Enter Sample ID.
15. Press "Start" to initiate the Solana RSV + hMPV Assay. Solana will display the progress, and the 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.
18. To determine if sample is positive for RSV and/or hMPV press the tube sample number. Separate results for the RSV and hMPV channels will be displayed.

INTERPRETATION OF RESULTS

The Solana software automatically determines the specimen results for RSV and hMPV. A positive result indicates that the viral RNA for RSV and/or hMPV was detected. A negative result indicates that RSV and hMPV were not detected and the process control was detected. Solana reports a specimen result as invalid when both RSV and hMPV 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.

Assay Result	Interpretation
RSV POSITIVE hMPV NEGATIVE	RSV RNA detected
RSV NEGATIVE hMPV POSITIVE	hMPV RNA detected
RSV POSITIVE hMPV POSITIVE	RSV RNA detected and hMPV RNA detected
RSV NEGATIVE hMPV NEGATIVE	No RSV RNA detected/PRC detected and No hMPV RNA detected/PRC detected
RSV INVALID hMPV INVALID	No RSV or hMPV 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.

QUALITY CONTROL

The Solana RSV + hMPV 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 RSV or hMPV RNA or amplicon.

It is recommended that the reactivity of each new lot and each new shipment of the Solana RSV + hMPV Assay be verified on receipt and before use. External control tests should be performed thereafter in accordance with appropriate federal, state and local guidelines. The Solana RSV + hMPV Assay should not be used in patient testing if the external controls do not produce the correct results.

LIMITATIONS

- This test is not intended to differentiate RSV or hMPV subtypes. Additional testing is required if subtype differentiation is required.
- Negative results do not preclude infection with RSV or hMPV 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.
- 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 assay performance was not established in immunocompromised patients.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

EXPECTED VALUES

The expected values of the Solana RSV + hMPV Assay were established during a prospective study conducted between January and May 2017. Two thousand sixty-four (2064) prospectively collected specimens (from all-comers) have been included in this study at six (6) sites across the United States. Four (4) of the fresh specimens were removed from the study due to protocol deviations (inappropriate specimen type). A single specimen was collected per patient. The specimens were processed and tested with Solana RSV + hMPV Assay on the Solana instrument at six (6) sites.

The expected value of RSV and hMPV with the Solana RSV + hMPV Assay has been calculated for the combined sites based on the age of the patient.

Eighteen (18) of the two thousand sixty-four (2064) specimens were removed from analysis: (four (4) specimens were removed from the study due to protocol deviations (inappropriate specimen type); fourteen (14) specimens were invalid). The table below provides the percentage of RSV and hMPV positive cases per specified age group, as determined by the Solana RSV + hMPV Assay, for the remaining two thousand forty-six (2046) specimens.

Age Group	**RSV**			**hMPV**		
	Number of Patients	Number of Positives	Positive Detection rate	Number of Patients	Number of Positives	Positive Detection rate
<1 year	235	46	19.6%	235	15	6.4%
1 to 5 years	390	41	10.5%	390	30	7.7%
6 to 10 years	186	6	3.2%	186	11	5.9%

Age Group	RSV			hMPV		
	Number of Patients	Number of Positives	Positive Detection rate	Number of Patients	Number of Positives	Positive Detection rate
11 to 15 years	125	5	4.0%	125	4	3.2%
16 to 21 years	109	2	1.8%	109	0	0.0%
22 to 50 years	358	11	3.1%	358	11	3.1%
51 to 65 years	259	15	5.8%	259	7	2.7%
> 65 years	384	23	6.0%	384	11	2.9%
Combined Age Groups	2046	149	7.3%	2046	89	4.3%

CLINICAL PERFORMANCE

Performance characteristics of the Solana RSV + hMPV Assay were established during a prospective study with specimens collected between January and May 2017. Two thousand sixty-four (2064) specimens prospectively collected specimens have been included in this study at six (6) sites across the United States. Specimens were tested fresh (773) or after freezing (1291) at –70°C. A single nasal or nasopharyngeal swab specimen (300 and 1760, respectively) was collected per patient in viral transport media (BD/Copan UTM, Remel M5, Remel M6). Four (4) of the fresh specimens were removed from the study due to protocol deviations (inappropriate specimen type). All specimens were transported to a central location for extraction with the NucliSENS® easyMAG® and testing with a FDA-cleared RSV + hMPV molecular assay. The specimens were processed and tested with Solana RSV + hMPV Assay on the Solana instrument at one of the six (6) sites.

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

Gender	Female	Male
Total	1053	1007
Age		
<1 year	99	139
1 to 5 years	172	218
6 to 10 years	95	91
11 to 15 years	63	62
16 to 21 years	60	51
22 to 50 years	197	165
51 to 65 years	146	115
> 65 years	221	166

Comparison with an FDA-cleared RSV + hMPV Molecular Assay

Two thousand sixty (2060) specimens were processed using the NucliSENS® easyMAG® and tested with a FDA-cleared RSV + hMPV molecular assay per the assay's package insert.

Of 2060 specimens evaluated in the study, 769 specimens were tested fresh (nonfrozen) using the Solana RSV + hMPV Assay and the Lyra RSV + hMPV Assay for the presence of RSV and hMPV. One thousand two hundred ninety-one (1291) specimens were frozen after collection and stored at –70°C prior to testing with the Solana RSV + hMPV Assay and the Lyra RSV + hMPV Assay for the presence of RSV and hMPV. Fourteen (14) specimens (9 fresh and 5 frozen) were invalid in the Solana® Assay when initially tested and upon repeat testing (invalid rate of 0.7%, with 95% CI 0.4% to 1.1%). These fourteen (14) specimens have been excluded from further analysis.

The tables below detail the positive percent agreement (PPA) and the negative percent agreement (NPA) of the Solana RSV + hMPV Assay results for RSV and hMPV, respectively, as compared with an FDA cleared molecular comparator, for the 2046 specimens with valid results.

Percent Agreement of the Solana RSV + hMPV Assay for RSV Compared to an FDA cleared RSV + hMPV Molecular Assay (Across all Sites Combined)							
Source / Category	N	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
Fresh	760	12	0	747	1	92.3 (66.7 to 98.6)	100 (99.5 to 100)
Frozen	1286	136	1	1143	6	95.8 (91.1 to 98.0)	99.9 (99.5 to 100)
All	2046	148	1	1890	7	95.5 (91.0 to 97.8)	99.9 (91.0 to 97.8)

There was a total of eight (8) discordant specimens among the two thousand forty-six (2046) specimens evaluated for RSV. The one (1) discordant specimen (Solana Positive/Comparator Negative) was positive for RSV by an alternative FDA-cleared molecular test. Of the seven (7) discordant specimens (Solana Negative/ Comparator Positive) six (6) were positive for RSV by an alternative FDA-cleared molecular test.

Percent Agreement of the Solana RSV + hMPV Assay for hMPV Compared to an FDA-cleared RSV + hMPV Molecular Assay (Across all Sites Combined)							
Source / Category	N	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
Fresh	760	24	2	733	1	96.0 (80.5 to 99.3)	99.7 (99.0 to 99.9)
Frozen	1286	62	1	1220	3	95.4 (87.3 to 98.4)	99.9 (99.5 to 100)
All	2046	86	3	1953	4	95.6 (89.1 to 98.3)	99.8 (99.6 to 99.9)

There was a total of seven (7) discordant specimens among the two thousand forty-six (2046) specimens evaluated for hMPV. Of the three (3) discordant specimens (Solana Positive/ Comparator Negative) reported, all of these specimens were negative for hMPV by an alternative FDA-cleared molecular test. Of the four (4) discordant specimens (Solana Negative/ Comparator Positive), all were positive for hMPV by an alternative FDA-cleared molecular test.

ANALYTICAL PERFORMANCE

Analytical Sensitivity (Limit of Detection)

The analytical sensitivity (limit of detection or LOD) of the Solana RSV + hMPV Assay was determined using quantified ($TCID_{50}$/mL) cultures of one RSV A, one RSV B, one hMPV A1, one hMPV A2, one hMPV B1 and one hMPV B2 strain, serially diluted in negative nasopharyngeal matrix. Each dilution was run as 20 replicates in the Solana RSV + hMPV 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	
Virus	$TCID_{50}$/mL
RSV	
RSV A, A2 (VR-1540)	$7.9x10^3$
RSV B, Wash/18537/62 (VR-1580)	$3.9x10^2$
hMPV	
hMPV 16 Type A1, IA10-2003	$3.7x10^2$
hMPV 20 Type A2 IA14-2003 G gene	$1.2x10^4$
hMPV 5 Type B1, Peru3-2003	$3.8x10^3$
hMPV 4 Type B2, Peru1-2002	$2.3x10^3$

Analytical Reactivity (Inclusivity)

The reactivity of the Solana® RSV + hMPV Assay was evaluated against four (4) additional strains of RSV, which include two (2) RSV A and two (2) RSV B strains and four (4) additional strains of hMPV, which include one (1) each of hMPV A1, hMPV A2, hMPV B1 and hMPV B2 at concentrations near the level of detection (LOD) of the assay.

Inclusivity Strains		
Strain	$TCID_{50}$/mL	Inclusive (Yes or No)
RSV		
RSV A, strain Long (VR-26)	$1.6x10^4$	Yes
RSV A, strain 4/2015 Isolate #1	$1.6x10^4$	Yes
RSV B, strain 9320 (VR-955)	$7.9x10^2$	Yes
RSV B, strain WV/14617/85 (VR-1400)	$7.9x10^2$	Yes
hMPV		
hMPV 9 Type A1, strain IA3-2002	$7.4x10^2$	Yes
hMPV 27 Type A2, strain IA27-2004	$2.4x10^4$	Yes
hMPV 3 Type B1, strain Peru2-2002	$7.6x10^3$	Yes
hMPV 18 Type B2, strain IA18-2003	$4.5x10^3$	Yes

Reproducibility Study

A four-sample panel consisting of three levels of a combined RSV and hMPV (two (2) strains of each virus) contrived samples and a negative contrived sample were tested in this study. RSV A strain A2 (VR-1540) and hMPV 20 Type A2 (IA14-2003 G gene) (Set 1), or RSV B strain Wash/18537/62 (VR-1580) and hMPV 4 Type B2 (Peru1-2002, B2) (Set 2) were diluted in negative nasal matrix to 2x LOD for moderate positive, 1x 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. Positive and negative controls were run in triplicate along with the panels. The panels were run by two operators at each testing site for five (5) non-consecutive days. The Solana RSV + hMPV assay was used per the instructions for use.

Panels and controls were tested at each site by two (2) operators per instrument for five (5) days, each sample tested in three (3) replicates, for a total of 45 results per level for each virus strain (2 operators x 5 days x 3 sites x 3 replicates).

Reproducibility Summary for RSV									
Samples	**SITE**						**Overall Percent Agreement**		**95% Confidence Interval**
	Site #1		**Site #2**		**Site #3**				
	# Expected Result/# tested	*% Agreement with Expected Result*	*# Expected Result/# tested*	*% Agreement with Expected Result*	*# Expected Result/# tested*	*% Agreement with Expected Result*			
RSV A strain A2 (VR-1540) High Negative (0.2x LOD) ($1.6x10^3$ TCID50/mL)*	8/15	53.3	14/15	93.3	10/15	66.7	32/45	71.1	56.6 to 82.3
RSV A strain A2 (VR-1540) Low Positive (1x LOD) ($7.9x10^3$ TCID50/mL)	15/15	100	15/15	100	15/15	100	45/45	100	92.1 to 100
RSV A strain A2 (VR-1540) Moderate Positive (2x LOD) ($1.6x10^4$ TCID50/mL)	15/15	100	15/15	100	15/15	100	45/45	100	92.1 to 100
RSV B strain Wash/18537/62 (VR-1580) High Negative (0.2x LOD)	6/15	40.0	10/15	66.7	6/15	40.0	22/45	48.9	35.0 to 63.0

Reproducibility Summary for RSV									
Samples	SITE						Overall Percent Agreement		95% Confidence Interval
	Site #1		Site #2		Site #3				
	# Expected Result/# tested	*% Agreement with Expected Result*	*# Expected Result/# tested*	*% Agreement with Expected Result*	*# Expected Result/# tested*	*% Agreement with Expected Result*			
(1.4 x10^2 TCID50/mL)*									
RSV B strain Wash/18537/62 (VR-1580) Low Positive (1x LOD) (4.7x10^2 TCID50/mL)	15/15	100	15/15	100	15/15	100	45/45	100	92.1 to 100
RSV B strain Wash/18537/62 (VR-1580) Moderate Positive (2x LOD) (9.4x10^2 TCID50/mL)	15/15	100	15/15	100	15/15	100	45/45	100	92.1 to 100
Negative	0/30	100	0/30	100	0/30	100	0/90	100	96.0 to 100
RSV Positive Control	30/30	100	30/30	100	30/30	100	90/90	100	96.0 to 100
RSV Negative Control	0/30	100	0/30	100	0/30	100	0/90	100	96.0 to 100

* An expected result for the high negative sample is a negative result.

Reproducibility Summary for hMPV									
Samples	SITE						Overall Percent Agreement With Expected Results		95% Confidence Interval
	Site #1		Site #2		Site #3				
	# Expected Result/# tested	*% Agreement with Expected Result*	*# Expected Result/# tested*	*% Agreement with Expected Result*	*# Expected Result/# tested*	*% Agreement with Expected Result*			
hMPV 20 Type A2 (IA14-2003 G gene) High Negative (0.2x LOD) (2.4x10^2 $TCID_{50}$/mL)*	11/15	73.3	14/15	93.3	10/15	66.7	35/45	77.8	63.7 to 87.5
hMPV 20 Type A2 (IA14-2003 G gene) Low Positive (1x LOD) (1.2x$10^4$$TCID_{50}$/mL)	15/15	100	15/15	100	15/15	100	45/45	100	92.1 to 100
hMPV 20 Type A2 (IA14-2003 G gene) Moderate Positive (2x LOD) (2.4x10^4 $TCID_{50}$/mL)	15/15	100	15/15	100	15/15	100	45/45	100	92.1 to 100
hMPV 4 Type B2 (Peru1-2002, B2) High Negative (0.2x LOD) (4.6x10^2 $TCID_{50}$/mL)*	9/15	60	9/15	60	9/15	60	27/45	60.0	45.5 to 73.0
hMPV 4 Type B2 (Peru1-2002, B2) Low Positive (1x LOD) (2.3x10^3 $TCID_{50}$/mL)	15/15	100	15/15	100	15/15	100	45/45	100	92.1 to 100
hMPV 4 Type B2 (Peru1-2002, B2) Moderate Positive	15/15	100	15/15	100	15/15	100	45/45	100	92.1 to 100

Reproducibility Summary for hMPV									
Samples	SITE						Overall Percent Agreement With Expected Results		95% Confidence Interval
	Site #1		Site #2		Site #3				
	# Expected Result/# tested	*% Agreement with Expected Result*	*# Expected Result/# tested*	*% Agreement with Expected Result*	*# Expected Result/# tested*	*% Agreement with Expected Result*			
(2x LOD) ($4.6x10^3 TCID_{50}$/mL)									
Negative	0/30	100	0/30	100	0/30	100	0/90	100	96.0 to 100
hMPV Positive Control	30/30	100	30/30	100	30/30	100	90/90	100	96.0 to 100
hMPV Negative Control	0/30	100	0/30	100	0/30	100	0/90	100	96.0 to 100

* An expected result for the high negative sample is a negative result.

Analytical Specificity – Microbial Interference

A study was performed to evaluate the performance of the Solana RSV + hMPV Assay in the presence of forty-six (46) microorganisms (25 bacteria, 1 yeast, 20 viruses) potentially found in specimens that are collected from nasal passages of patients symptomatic for RSV and/or hMPV. 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 in triplicate in the presence of RSV (RSV A, A2 (VR-1540) and RSV B, Wash/18537/62 (VR-1580)) and hMPV (hMPV 16 Type A1, IA10-2003 and hMPV 5 Type B1, Peru3-2003) at 3x LOD.

No microbial interference was observed.

There was no competitive interference between the detection of RSV and hMPV.

The organisms and their concentrations included in the interference study are shown in the table below.

Potential Interfering Organisms		
Organism	**Concentration Tested**	**Units**
Adenovirus 1	1.00E+05	$TCID_{50}$/mL
Adenovirus 11	1.00E+05	$TCID_{50}$/mL
Bordetella bronchiseptica	1.00E+06	CFU/mL
Bordetella pertussis	1.00E+06	CFU/mL
Candida albicans	1.00E+06	CFU/mL
Chlamydophila pneumoniae	1.00E+06	IFU/mL
Chlamydia trachomatis	1.00E+06	IFU/mL
Coronavirus 229E	1.00E+05	$TCID_{50}$/mL
Corynebacterium diptheriae	1.00E+06	CFU/mL
Coxsackievirus B5/10/2006	1.00E+05	$TCID_{50}$/mL
Cytomegalovirus (VR-977)	1.00E+05	$TCID_{50}$/mL
Echovirus 11	1.00E+05	$TCID_{50}$/mL
Echovirus 6	1.00E+05	$TCID_{50}$/mL
Enterovirus, Type 71	1.00E+05	$TCID_{50}$/mL
Epstein Barr virus	1.00E+05	$TCID_{50}$/mL
Escherichia coli	1.00E+06	CFU/mL
Haemophilus influenzae	1.00E+06	CFU/mL
HSV 2 G strain	1.00E+05	$TCID_{50}$/mL
hMPV Peru1-2002, B2[1]	1.00E+05	$TCID_{50}$/mL
Influenza A/Texas/50/2012	1.00E+05	$TCID_{50}$/mL
Influenza B/Panama/45/90	1.00E+05	$TCID_{50}$/mL
Klebsiella pneumoniae	1.00E+06	CFU/mL

Potential Interfering Organisms		
Organism	**Concentration Tested**	**Units**
Lactobacillus plantarum	1.00E+06	CFU/mL
Legionella pneumophila	1.00E+06	CFU/mL
Measles	1.00E+05	$TCID_{50}$/mL
Moraxella catarrhalis	1.00E+06	CFU/mL
Mumps	1.00E+05	$TCID_{50}$/mL
Mycobacterium avium	1.00E+06	CFU/mL
Mycobacterium tuberculosis	1.00E+06	CFU/mL
Mycoplasma pneumoniae	1.00E+06	CFU/mL
Neisseria gonorrhoeae	1.00E+06	CFU/mL
Neisseria meningitidis	1.00E+06	CFU/mL
Parainfluenza Type 1	1.00E+05	$TCID_{50}$/mL
Parainfluenza Type 2	1.00E+05	$TCID_{50}$/mL
Parainfluenza Type 3	1.00E+05	$TCID_{50}$/mL
Proteus mirabilis	1.00E+06	CFU/mL
Proteus vulgaris	1.00E+06	CFU/mL
Pseudomonas aeruginosa	1.00E+06	CFU/mL
Rhinovirus Type 7	1.00E+05	$TCID_{50}$/mL
RSV A2 (VR-1540)[2]	1.00E+05	$TCID_{50}$/mL
Staphylococcus aureus	1.00E+06	CFU/mL
Staphylococcus epidermidis	1.00E+06	CFU/mL
Streptococcus mutans	1.00E+06	CFU/mL
Streptococcus pneumoniae	1.00E+06	CFU/mL
Streptococcus pyogenes	1.00E+06	CFU/mL
Streptococcus salivarius	1.00E+06	CFU/mL

[1]All three replicates tested positive for hMPV and negative for RSV in the Solana RSV + hMPV assay.

[2]All three replicates tested negative for hMPV and positive for RSV in the Solana RSV + hMPV assay

Analytical Specificity – Cross-reactivity

A study was performed to evaluate the cross-reactivity of the Solana RSV + hMPV Assay with forty-six (46) microorganisms (25 bacteria, 1 yeast, 20 viruses) potentially found in specimens that are collected from patients symptomatic for RSV and/or hMPV. 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 RSV + hMPV Assay. No cross reactivity was observed with the organisms at concentrations shown in the table below.

Potential Cross-reactive Organisms		
Organism	**Concentration Tested**	**Units**
Adenovirus 1	1.00E+05	$TCID_{50}$/mL
Adenovirus 11	1.00E+05	$TCID_{50}$/mL
Bordetella bronchiseptica	1.00E+06	CFU/mL
Bordetella pertussis	1.00E+06	CFU/mL
Candida albicans	1.00E+06	CFU/mL
Chlamydophila pneumoniae	1.00E+06	IFU/mL
Chlamydia trachomatis	1.00E+06	IFU/mL
Coronavirus 229E	1.00E+05	$TCID_{50}$/mL
Corynebacterium diptheriae	1.00E+06	CFU/mL
Coxsackievirus B5/10/2006	1.00E+05	$TCID_{50}$/mL
Cytomegalovirus (VR-977)	1.00E+05	$TCID_{50}$/mL
Echovirus 11	1.00E+05	$TCID_{50}$/mL
Echovirus 6	1.00E+05	$TCID_{50}$/mL
Enterovirus, Type 71	1.00E+05	$TCID_{50}$/mL
Epstein Barr virus	1.00E+05	$TCID_{50}$/mL

Potential Cross-reactive Organisms		
Organism	**Concentration Tested**	**Units**
Escherichia coli	1.00E+06	CFU/mL
Haemophilus influenzae	1.00E+06	CFU/mL
HSV 2 G strain	1.00E+05	$TCID_{50}$/mL
hMPV Peru1-2002, B2	1.00E+05	$TCID_{50}$/mL
Influenza A/Texas/50/2012	1.00E+05	$TCID_{50}$/mL
Influenza B/Panama/45/90	1.00E+05	$TCID_{50}$/mL
Klebsiella pneumoniae	1.00E+06	CFU/mL
Lactobacillus plantarum	1.00E+06	CFU/mL
Legionella pneumophila	1.00E+06	CFU/mL
Measles	1.00E+05	$TCID_{50}$/mL
Moraxella catarrhalis	1.00E+06	CFU/mL
Mumps	1.00E+05	$TCID_{50}$/mL
Mycobacterium avium	1.00E+06	CFU/mL
Mycobacterium tuberculosis	1.00E+06	CFU/mL
Mycoplasma pneumoniae	1.00E+06	CFU/mL
Neisseria gonorrhoeae	1.00E+06	CFU/mL
Neisseria meningitidis	1.00E+06	CFU/mL
Parainfluenza Type 1	1.00E+05	$TCID_{50}$/mL
Parainfluenza Type 2	1.00E+05	$TCID_{50}$/mL
Parainfluenza Type 3	1.00E+05	$TCID_{50}$/mL
Proteus mirabilis	1.00E+06	CFU/mL
Proteus vulgaris	1.00E+06	CFU/mL
Pseudomonas aeruginosa	1.00E+06	CFU/mL
Rhinovirus Type 7	1.00E+05	$TCID_{50}$/mL
RSV A2 (VR-1540)	1.00E+05	$TCID_{50}$/mL
Staphylococcus aureus	1.00E+06	CFU/mL
Staphylococcus epidermidis	1.00E+06	CFU/mL
Streptococcus mutans	1.00E+06	CFU/mL
Streptococcus pneumoniae	1.00E+06	CFU/mL
Streptococcus pyogenes	1.00E+06	CFU/mL
Streptococcus salivarius	1.00E+06	CFU/mL

Analytical Specificity – Interfering Substances

The performance of Solana RSV + hMPV Assay was evaluated with twenty (20) potentially interfering substances that may be present in nasal and nasopharyngeal specimens. The potentially interfering substances were evaluated with RSV (RSV A, A2 (VR-1540) and RSV B, Wash/18537/62 (VR-1580)) and hMPV (hMPV 16 Type A1, IA10-2003 and hMPV 5 Type B1, Peru3-2003) at concentrations of 3x 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%

Potential Interfering Substances		
Substances	**Active Ingredient**	**Concentration Tested**
Neo-Synephrine	Phenylephrine hydrochloride	15.0%
Flonase	Fluticasone	5.0%
Zicam Gentle Allergy Relief NasalGel	*Galphimia glauca*, *Histaminum hydrochloricum*, *Luffa operculata*, Sulfur	5.0%
Bactroban	Mupirocin	12.0 mg/mL
TamiFlu	Oseltamivir	2.2 µg/mL
Relenza	Zanamivir	282.0 ng/mL
Tobramycin	Tobramycin sulfate	2.5 mg/mL
Chloraseptic spray	Benzocaine, Menthol	0.68 g/mL
SYMMETREL®	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%
Atrovent® Nasal Spray	Ipratropium bromide	10.0 mg/mL
Patanase Nasal Spray	Olopatadine hydrochloride	10.0 mg/mL

Carryover and Cross-contamination Studies

Positive samples consisting of an RSV and hMPV were 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.

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