REF 300901 NeuMoDx™ FluA/FluB/RSV/SARS-CoV-2 Test Strip

℞ only

CAUTION: For US Export Only

IVD For *in vitro* diagnostic use with the NeuMoDx 288 and NeuMoDx 96 Molecular Systems

Electronic version is available at www.qiagen.com/neumodx-ifu

For detailed instructions, refer to the NeuMoDx 288 Molecular System Operator's Manual; P/N 40600108

For detailed instructions, refer to the NeuMoDx 96 Molecular System Operator's Manual; P/N 40600317

INTENDED USE

The NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay is a multiplexed, *in vitro* real-time RT-PCR diagnostic test intended for the simultaneous qualitative detection and differentiation of Influenza A virus (Flu A), Influenza B virus (Flu B), Respiratory Syncytial Virus (RSV), and SARS-CoV-2 RNA from nasopharyngeal (NP) swab specimens collected in transport medium from individuals with signs and symptoms of Influenza like illness (ILI).

The NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay as performed on the NeuMoDx 288 Molecular System and NeuMoDx 96 Molecular System incorporates automated RNA extraction to isolate target nucleic acids from the specimen and real-time RT-PCR targeting a single conserved region for Flu A and RSV, and two conserved regions for SARS-CoV-2 and Flu B.

The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results are indicative of the presence of SARS-CoV-2 and/or Flu A and/or Flu B and/or RSV RNA, but do not rule out bacterial infection or co-infection with other viruses. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status.

Negative results do not preclude Flu A, Flu B, RSV, or SARS-CoV-2 infection and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results must be combined with clinical observations, patient history, and/or epidemiological information.

The NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and *in vitro* diagnostic procedures and/or NeuMoDx Molecular Systems. The NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay is not intended for self-testing or point-of-care use.

SUMMARY AND EXPLANATION

NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay is a qualitative assay for the use on the NeuMoDx 96 and the NeuMoDx 288 instrument systems for the detection of SARS-CoV-2, Influenza A, Influenza B, and/or RSV RNA in nasopharyngeal swab samples. The assay does not differentiate between RSV A and RSV B RNA. Nasopharyngeal swab specimens are collected in Copan Universal Transport Medium (UTM-RT®) (Copan UTM-RT, Copan, CA, USA) or BD™ Universal Viral Transport System (UVT) (BD™ UVT, BD, NJ, USA). The test uses an RNA Internal Sample Process Control (SPC2) that is incorporated during sample preparation and serves to monitor the entire sample preparation, reverse transcription, and PCR amplification process. The NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay allows up to two specimen processing workflows based on laboratory need: a direct workflow and a pretreated workflow. The NeuMoDx Molecular System automatically performs all the steps required to extract the target nucleic acids; prepares the isolated RNA for real-time reverse transcriptase polymerase chain reaction (RT-PCR); and, if present, reverse transcribes, amplifies, and detects the products of amplification. The NeuMoDx FluA/FluB/RSV/SARS-CoV-2 assay targets the conserved regions of the SARS-CoV-2 Nsp2 and O-ribose methyltransferase genes, regions in the matrix protein of Influenza A Virus and Respiratory Syncytial Virus and the matrix protein and nonstructural protein NS1 genes of Influenza B Virus.

PRINCIPLES OF THE PROCEDURE

The current state of the art for detection of acute FluA/FluB/RSV/SARS-CoV-2 infection is nucleic acid amplification of conserved regions within the target's genome, which is in alignment with the real-time reverse transcription PCR employed by NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay, as performed on the NeuMoDx 288 Molecular System and NeuMoDx 96 Molecular System.

The NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay combines automated RNA extraction and amplification/detection of SARS-CoV-2, Flu A, Flu B, and/or RSV RNA by real-time RT-PCR. Nasopharyngeal swab samples are collected in the Copan UTM-RT System or BD™ UVT System. The Direct Workflow allows the primary swab collection tube or an aliquot of the transport medium in a secondary tube to be barcoded and loaded onto the NeuMoDx System for processing. Alternatively, the swab specimen in transport medium can be first treated with an equal volume of NeuMoDx Vantage Viral Lysis Buffer (VVLB) before loading onto the System without further user intervention. The NeuMoDx System automatically aspirates either an aliquot of specimen to mix with NeuMoDx Lysis Buffer 3 for the Direct Workflow or an aliquot of pretreated specimen to mix with Lysis Buffer 2 and the reagents contained in the NeuMoDx Extraction Plate to begin processing. Specifically, using the Direct Workflow, the primary collection tube (with swab and cap removed) or an aliquot of the sample medium in a secondary tube is barcoded and loaded onto the NeuMoDx System using a designated specimen tube carrier. For the Pretreated Workflow, the specimen in transport medium is first treated with an equal volume of NeuMoDx VVLB before it is loaded onto the System. For the Direct Workflow, a 400 µL aliquot of the sample is aspirated by the NeuMoDx System and mixed with an equal volume of NeuMoDx Lysis Buffer 3 while for the Pretreated Workflow 550µL of the pretreated sample is combined with an equal volume of Lysis Buffer 2. The NeuMoDx System automates and integrates RNA extraction and concentration, reagent preparation, and nucleic acid amplification/detection of the target sequences using real-time RT-PCR. The Sample Process Control (SPC2) helps monitor for the presence of inhibitory substances and for system, process, or reagent failures. No operator intervention is necessary once the specimen is loaded onto the NeuMoDx System.

The NeuMoDx System uses a combination of heat, lytic enzyme, and extraction reagents to automatically perform lysis, RNA extraction, and removal of inhibitors. The released nucleic acids are captured by paramagnetic particles. The particles with bound nucleic acid are loaded into the NeuMoDx Cartridge where the unbound elements are washed away with NeuMoDx Wash Reagent. The bound RNA is then eluted using NeuMoDx Release Reagent. The NeuMoDx System uses the eluted RNA to rehydrate proprietary NeuDry™ amplification reagents containing all the elements necessary for amplification of the Flu A, Flu B, RSV, SARS-CoV-2 and SPC2 targets. This enables simultaneous amplification and detection of all targets and sample process control RNA sequences. Upon reconstitution of the dried RT-PCR reagents, the NeuMoDx System dispenses the

prepared RT-PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Reverse transcription, amplification, and detection of the control and target sequences (if present) occur in the PCR chamber. The NeuMoDx Cartridge is designed to contain the generated amplicon following RT-PCR, virtually eliminating the risk of post-amplification contamination.

The amplified targets are detected in real time using hydrolysis probe chemistry (commonly referred to as TaqMan® chemistry) using fluorogenic oligonucleotide probe molecules specific to the amplicons of their respective targets. TaqMan probes consist of a fluorophore covalently attached to the 5'-end of the oligonucleotide probe and a quencher at the 3'-end. While the probe is intact, the fluorophore and the quencher are in proximity, allowing the quencher molecule to suppress the fluorescence emitted by the fluorophore via Förster Resonance Energy Transfer (FRET).

TaqMan probes are designed such that they anneal within a cDNA region amplified by a specific set of primers. As the Taq DNA polymerase extends the primer and synthesizes the new strand, the 5' to 3' exonuclease activity of the Taq DNA polymerase degrades the probe that has annealed to the template. Degradation of the probe releases the fluorophore and breaks its proximity to the quencher, thereby overcoming the quenching effect due to FRET and allowing detection of the fluorophore. The resulting fluorescent signal detected in the NeuMoDx System RT-PCR thermal cycler is directly proportional to the fluorophore released and can be correlated to the amount of target present.

The fluorescent detection channel for each NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay target is presented in the table below. The NeuMoDx System software monitors the fluorescent signal emitted by the TaqMan probes at the end of each amplification cycle. When thermal cycling is complete, the NeuMoDx System software analyzes the data and reports a result (POSITIVE/NEGATIVE/INDETERMINATE/NO RESULT/UNRESOLVED).

Table 1. Detection Channels

Target	Target Region	Probe Fluorophore	Excitation/Emission	Detection Channel
Influenza A	Matrix protein	FAM	530/555 nm	Green
Influenza B	Matrix protein	HEX	470/510 nm	Yellow
	Nonstructural protein NS1			
SARS-CoV-2	Nsp2 gene	Texas Red	585/610 nm	Orange
	O-ribose methyltransferase			
Respiratory Syncytial Virus	Matrix protein	Q705	680/715 nm	Far Red
SPC2	Assembly Protein (MS2)	Q670	625/660 nm	Red

REAGENTS / CONSUMABLES

Material Provided

REF	Contents	Units per package	Tests per unit	Tests per package
300901	**NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strip** *Dried RT-PCR reagents containing FluA/FluB/RSV/SARS-CoV-2 specific TaqMan probes and primers, and SPC2-specific TaqMan probe and primers.* *Contains 21.1% Tris-HCl, 8.4% dNTP and other inactive ingredients*	6	16	96

Materials Required but Not Provided (Available separately from NeuMoDx)

REF	Contents
901200	**NeuMoDx FluA/FluB/RSV/SARS-CoV-2 External Controls** *Single use sets of FluA/FluB/RSV/SARS-CoV-2 Positive and Negative Controls to establish daily validity of NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay (1 vial of each control = 1 set)*
100200	**NeuMoDx Extraction Plate** *Dried paramagnetic particles, lytic enzyme, and sample process controls*
400500**	**NeuMoDx Lysis Buffer 2**
400600*	**NeuMoDx Lysis Buffer 3**
401500**	**NeuMoDx Vantage Viral Lysis Buffer**
400100	**NeuMoDx Wash Reagent**
400200	**NeuMoDx Release Reagent**
100100	**NeuMoDx Cartridge**
235903	**Hamilton® CO-RE / CO-RE II Tips (300 µL) with Filters**
235905	**Hamilton CO-RE / CO-RE II Tips (1000 µL) with Filters**

** Required only for processing of samples using the Direct Workflow, without a pretreatment step. See "Instructions for Use" section below.*
*** Required only if processing samples using the Pretreated Workflow, with a pretreatment step. See "Instructions for Use" section below.*

Swabs and Transport Media (Not Provided)

Sample Type	Recommended Collection Device	Recommended Swab
Nasopharyngeal Swab	3 mL Universal Transport Medium (Copan UTM-RT, Copan, CA, USA, 305C) **or** 3 mL Universal Viral Transport System (BD UVT, BD, NJ, USA, BD 220531)	Flexible Minitip Nylon® Flocked Swab (Copan, CA, USA) **or** Flexible Minitip Flocked Swab (BD, NJ, USA)

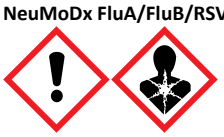
Instrumentation Required

NeuMoDx 288 Molecular System [REF 500100] or **NeuMoDx 96 Molecular System** [REF 500200]
NeuMoDx System Software version 1.9.2.6 or higher

WARNINGS AND PRECAUTIONS

- The NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay is for *in vitro* diagnostic use with NeuMoDx Systems only.
- Do not use the reagents or consumables after the listed expiration date.
- Do not use any reagents if the safety seal is broken or if the packaging is damaged upon arrival.
- Do not use consumables or reagents if the protective pouch is open or broken upon arrival.
- Minimum specimen volume of secondary aliquots is dependent on the tube size/specimen tube carrier as defined below. Specimen volume below the specified minimum may result in a "Quantity Not Sufficient" error.
- The use of specimens stored at improper temperatures or beyond the specified storage times may produce invalid or erroneous results.
- Avoid microbial and ribonuclease (RNase) contamination of all reagents and consumables. The use of sterile, RNase-free, disposable transfer pipettes is recommended when using secondary tubes. Use a new pipette for each specimen.
- To avoid contamination, do not handle or break apart any NeuMoDx Cartridge post-amplification. Do not retrieve NeuMoDx Cartridges from the Biohazard Waste Container (NeuMoDx 288 Molecular System) or Biohazard Waste Bin (NeuMoDx 96 Molecular System) under any circumstances. The NeuMoDx Cartridge is designed to prevent contamination.
- In cases where open-tube PCR tests are also conducted by the laboratory, care must be taken to ensure that the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strip, the additional consumables and reagents required for testing, personal protective equipment such as gloves and lab coats, and the NeuMoDx System are not contaminated.
- Clean, powder-free, nitrile gloves should be worn when handling NeuMoDx reagents and consumables. Care should be taken not to touch the top surface of the NeuMoDx Cartridge, the foil seal surface of the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strip and the NeuMoDx Extraction Plate, or the top surface of the NeuMoDx Lysis Buffer container; handling of the consumables and reagents should be done by touching side surfaces only.
- NeuMoDx FluA/FluB/RSV/SARS-CoV-2 External Controls [REF 901200] must be processed every 24 hours when testing with the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay.
- Safety Data Sheets (SDS) are provided for each reagent (as applicable) at www.qiagen.com/neumodx-ifu.
- Wash hands thoroughly after performing the test.
- Do not pipette by mouth. Do not smoke, drink, or eat in areas where specimens or reagents are being handled.
- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in *Biosafety in Microbiological and Biomedical Laboratories*[1] and in CLSI Document M29-A4.[2]
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDS).
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state, and local regulations.

NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strip



Contains: boric acid; Ethoxylated nonylphenol. Danger! Causes skin irritation. Causes serious eye irritation. May damage fertility or the unborn child. Harmful to aquatic life with long lasting effects. Obtain special instructions before use. Do not handle until all safety precautions have been read and understood. Wear protective gloves/ protective clothing/ eye protection/ face protection. IF exposed or concerned: Get medical advice/ attention. Store locked up. Dispose of contents/ container to an approved waste disposal plant.

Emergency information
CHEMTREC
Outside USA & Canada +1 703-527-3887

Disposal
The product contains ethoxylated nonylphenol, an endocrine disrupting substance that can have adverse effects on the environment.
Dispose of as hazardous waste in compliance with local and national regulations. This also applies to unused products.
Do not dispose of liquid waste into sewer.
Follow recommendations in the Safety Data Sheet (SDS).

PRODUCT STORAGE, HANDLING AND STABILITY

- NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strips are stable in the primary packaging through the stated expiration date on the immediate product label when stored at 15°C to 28°C.
- Do not reload any test product that has previously been loaded onto another NeuMoDx System.
- Once loaded, the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strip may remain onboard the NeuMoDx System for 14 days. Remaining shelf life of loaded test strips is tracked by the software and reported to the user in real time. Removal of a test strip that has been in use beyond its allowable period will be prompted by the NeuMoDx System.
-

SPECIMEN COLLECTION, TRANSPORT, AND STORAGE

Handle all specimens as if they are capable of transmitting infectious agents.

1. Specimens should be collected using the Copan UTM-RT System or BD™ UVT System using the validated nylon flocked swabs (see Swabs and Transport Media). In addition, flocked swabs, polyester, and nylon swabs are acceptable swab types. Follow manufacturer instructions for specimen collection, transport, and storage.
2. Specimens may be tested in compatible primary collection tubes or secondary specimen tubes.
3. Specimens may be stored on the NeuMoDx System for up to 8 hours prior to processing. If additional storage time is required, it is recommended that the specimens be either refrigerated or frozen as secondary aliquots.
4. Prepared specimens should be stored at 2 to 8 °C for no longer than 7 days prior to testing.
5. If specimens are shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations.
6. Proceed to *Test Preparation* section.

TEST PREPARATION

The NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay accommodates two different workflows, depending on user/laboratory preference:

Workflow 1: **DIRECT** – swab specimen in transport medium is loaded directly onto the NeuMoDx System in a primary collection tube or a secondary specimen tube

-or-

Workflow 2: **PRETREATED** – swab specimen in transport medium is pretreated with NeuMoDx Vantage Viral Lysis Buffer before it is loaded onto the NeuMoDx System in a primary collection tube or a secondary specimen tube

Test Preparation – DIRECT Workflow for Direct Swab Specimens

1. Apply a specimen barcode label to a specimen tube compatible with the NeuMoDx System as described in step 3below.
2. If testing the specimen in the primary collection tube, place the barcoded tube into a Specimen Tube Carrier and ensure the cap and swab are removed prior to loading onto the NeuMoDx System.
3. Alternatively, an aliquot of the transport medium may be transferred to a barcoded secondary tube and placed into a Specimen Tube Carrier. If using a secondary tube, transfer an aliquot of the transport medium to the barcoded specimen tube compatible with the NeuMoDx System according to the volumes defined below:
   - Specimen Tube Carrier (32-tube): 11 – 14 mm in diameter and 60 – 120 mm in height; minimum fill volume ≥ 600 µL
   - Specimen Tube Carrier (24-tube): 14.5 – 18 mm in diameter and 60 – 120 mm in height; minimum fill volume ≥ 1000 µL
   - Low Volume Specimen Tube Carrier (32-tube): 1.5 mL conical bottom microcentrifuge tube; minimum fill volume ≥ 500 µL

Test Preparation – PRETREATED Workflow for Pretreated Swab Specimens

Note: Bring Vantage Viral Lysis Buffer to room temperature (15 to 30 °C) before using.

WARNING: Pretreatment of swab samples with NeuMoDx Vantage Viral Lysis Buffer does not guarantee inactivation of any virus present. All samples should be handled as if they are capable of transmitting infectious agents.

1. Pretreat the specimen transport medium with a 1:1 volume of NeuMoDx Vantage Viral Lysis Buffer. This can be done in the primary swab collection tube if the volume of transport medium is known. Alternatively, pretreatment can be done in a secondary tube by combining an aliquot of the transport medium with an equal volume of NeuMoDx Vantage Viral Lysis Buffer. The resulting mixture should meet the minimum volume requirements specified in step 4 below.
2. Mix gently with a pipette to ensure uniform distribution of the NeuMoDx Vantage Viral Lysis Buffer.
3. If testing the pretreated specimen in the primary collection tube, place the barcoded tube into a Specimen Tube Carrier and ensure the cap and swab are removed prior to loading onto the NeuMoDx System.
4. If using a secondary tube, transfer an aliquot of the pretreated sample to a barcoded specimen tube compatible with the NeuMoDx System and place it into a Specimen Tube Carrier according to the volumes defined below:
   - Specimen Tube Carrier (32-tube): 11 – 14 mm in diameter and 60 – 120 mm in height; minimum fill volume ≥ 750 µL
   - Specimen Tube Carrier (24-tube): 14.5 – 18 mm in diameter and 60 – 120 mm in height; minimum fill volume ≥ 1100 µL
   - Low Volume Specimen Tube Carrier (32-tube): 1.5 mL conical bottom microcentrifuge tube; minimum fill volume ≥ 650 µL

NeuMoDx System Operation

For detailed instructions, refer to the NeuMoDx 288 and 96 Molecular Systems Operator's Manuals (P/N 40600108 & 40600317)

1. Load the test order onto the NeuMoDx System according to the workflow used for test preparation:
   - Untreated, neat swab specimens prepared using the DIRECT workflow are tested by defining the specimen as "**Transport Medium**"
   - Swab specimens pretreated with NeuMoDx Vantage Viral Lysis Buffer using the PRETREATED workflow are tested by defining the specimen as "**UserSpecified1**"

   If not defined in the test order, the Transport Medium specimen type (direct workflow), in a **Secondary Tube**, will be used as default.
2. Populate one or more NeuMoDx System Test Strip Carrier(s) with NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strip(s) and use the touchscreen to load the Test Strip Carrier(s) into the NeuMoDx System.
3. If prompted by the NeuMoDx System software, add the necessary required consumables to the NeuMoDx System consumable carriers and use the touchscreen to load carrier(s) into the NeuMoDx System.
4. If prompted by the NeuMoDx System software, replace NeuMoDx Wash Reagent and/or NeuMoDx Release Reagent and empty the Priming Waste, Biohazard Waste Container (NeuMoDx 288 Molecular System only), Tip Waste Bin (NeuMoDx 96 Molecular System only), and/or Biohazard Waste Bin (NeuMoDx 96 Molecular System only), as appropriate.
5. Load the specimen tube(s) into a Specimen Tube Carrier and ensure caps and any swabs are removed from all tubes.
6. Place the Specimen Tube Carrier(s) on the autoloader shelf and use the touchscreen to load the carrier(s) into the NeuMoDx System. This will initiate processing of the loaded specimens for the tests identified, given a valid test order is present in the system.

LIMITATIONS

1. The NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strip can only be used on NeuMoDx Systems.
2. The performance of the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strip has been established for clinician-collected nasopharyngeal swab specimens in transport medium. Use of the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strip with other specimen types and collection media has not been assessed and performance characteristics are unknown.
3. Because detection of viral targets is generally dependent on the number of viral particles present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
4. Erroneous results could occur from improper specimen collection, handling, storage, technical error, or specimen tube mix-up. In addition, false-negative results could occur because the number of viral particles in the sample is below the limit of detection of the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay.
5. Operation of the NeuMoDx System is limited to use by personnel trained on the use of the NeuMoDx System.
6. If Flu A, Flu B, RSV and SARS-CoV-2 targets and the SPC2 target do not amplify, an invalid result (Indeterminate or Unresolved) will be reported, and the test should be repeated.
7. If a system error occurs prior to completion of sample processing, "No Result" will be reported and the test should be repeated.
8. A positive result does not necessarily indicate the presence of viable influenza A, influenza B, SARS-CoV-2 and/or RSV. However, a positive result is presumptive for the presence of influenza A, influenza B, SARS-CoV-2 and/or RSV RNA.
9. Deletions or mutations in the conserved regions targeted by the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay may affect detection and could lead to an erroneous result.
10. Results from the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay should be used as an adjunct to clinical observations and other information available to the physician.
11. Good Laboratory Practices, including changing gloves between handling patient specimens, are recommended to avoid contamination.

RESULTS

Available results may be viewed or printed from the 'Results' tab in the Results window on the NeuMoDx System touchscreen. NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay results are automatically generated by the NeuMoDx System software using the decision algorithm and results processing parameters specified in the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay Definition File (FluA-B-RSV-CoV-2 ADF version 4.0.0 or higher). A NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay result may be reported as Negative, Positive, Indeterminate, No Result, or Unresolved based on the amplification status of the target(s) and SPC2. Results are reported based on the ADF results processing decision algorithm, summarized below in *Table 2*.

Table 2. NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay Results Interpretation

OVERALL RESULT	TARGET 1 (Flu A) FAM	TARGET 2 (Flu B) HEX	TARGET 3 (SARS-CoV-2) TX RED	TARGET 4 (RSV) Far Red	PROCESS CONTROL (SPC2) Red	INTERPRETATION
POSITIVE (Target RNA Detected)	**AMPLIFIED** [5 ≤ Ct < 25 AND EPR > 2.0 AND EP ≥ 750] **OR** (25 ≤ Ct ≤ 37 AND EP ≥ 750)	N/A	N/A	N/A	N/A	Flu A RNA Detected
	N/A	**AMPLIFIED** [5 ≤ Ct < 28 AND EPR > 1.5 AND EP ≥ 600] **OR** [28 ≤ Ct ≤ 37 AND EP ≥ 600]	N/A	N/A	N/A	Flu B RNA Detected
	N/A	N/A	**AMPLIFIED** [5 ≤ Ct < 25 AND EPR > 1.5 AND EP ≥ 1200] **OR** [25 ≤ Ct ≤ 37 AND EP ≥ 1200]	N/A	N/A	SARS-CoV-2 RNA Detected
	N/A	N/A	N/A	**AMPLIFIED** [5 ≤ Ct < 30 AND EPR > 1.15 AND EP ≥ 1200] **OR** [30 ≤ Ct ≤ 37 AND EP ≥ 1200]	N/A	RSV RNA Detected
NEGATIVE (Target RNA Not Detected)	**NOT AMPLIFIED** N/A **OR** (5 ≤ Ct < 25 AND EPR ≤ 2.0) **OR** (25 ≤ Ct ≤37 AND EP < 750) **OR** (Ct > 37)	**NOT AMPLIFIED** N/A **OR** (5 ≤ Ct < 28 AND EPR ≤ 1.5) **OR** (28 ≤ Ct ≤ 37 AND EP < 600) **OR** (Ct > 37)	**NOT AMPLIFIED** N/A **OR** (5 ≤ Ct < 25 AND EPR ≤ 1.5) **OR** (25 ≤ Ct ≤ 37 AND EP < 1200) **OR** (Ct > 37)	**NOT AMPLIFIED** N/A **OR** (5 ≤ Ct < 30 AND EPR ≤ 1.15) **OR** (30 ≤ Ct ≤ 37 AND EP < 1200) **OR** (Ct > 37)	**AMPLIFIED** (24≤ Ct ≤31 AND EP ≥1800)	Flu A, Flu B, RSV, and SARS-CoV-2 RNA not detected
NR*	Not Amplified, System Error Detected, Sample Processing Aborted					Sample processing was aborted; retest sample
IND*	Not Amplified, System Error Detected, Sample Processing Completed					All target results were invalid; retest sample
UNR*	Not Amplified, No System Error Detected					All target results were invalid; retest sample

* The System allows optional Rerun/Repeat capability to enable automatic reprocessing in the event of an invalid result to minimize delays in result reporting.

Invalid Results

If a NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay performed on the NeuMoDx System fails to produce a valid result, it will be reported as either Indeterminate, No Result, or Unresolved based on the type of error that occurred, and the test should be repeated to obtain a valid result.

An Indeterminate result will be reported if a NeuMoDx System error is detected during sample processing. In the event of an Indeterminate result, a retest is recommended.

A No Result will be reported if a NeuMoDx System error is detected and sample processing is aborted. In the event of a No Result, a retest is recommended.

An Unresolved result will be reported if no target is detected and there is no amplification of the Sample Process Control, which indicates possible reagent failure or the presence of inhibitors. In the event of an Unresolved result, a retest is recommended as a first step. If the retest fails, a diluted specimen may be used to mitigate the effect of possible inhibition.

See the NeuMoDx 288 Molecular System Operator's Manual (PN: 40600108) or the NeuMoDx 96 Molecular System Operator's User Manual (PN: 40600317) for a list of error codes that may be associated with any Invalid Results.

The NeuMoDx System is equipped with automatic Rerun/Repeat capability that the end user can choose to use to ensure that an INVALID result is automatically reprocessed to minimize delays in result reporting.

Quality Control

Local regulations typically specify that the laboratory is responsible for control procedures that monitor accuracy and precision of the complete analytical process and must establish the number, type, and frequency of testing control materials using verified performance specifications for an unmodified, approved test system.

External Controls

1) It is required that users process one set of NeuMoDx FluA/FluB/RSV/SARS-CoV-2 External Controls [REF 901200] every 24 hours and prior to processing patient samples. If a set of valid external control results does not exist, the NeuMoDx System software will prompt the user for controls to be processed before sample results can be reported.

2) If external controls are required, process the controls (1 positive control and 1 negative control):

NeuMoDx FluA/FluB/RSV/SARS-CoV-2 External Control	Label Color Scheme
NeuMoDx Flu A/FluB/RSV/SARS-CoV-2 Positive Control(s)	Red
NeuMoDx Flu A/FluB/RSV/SARS-CoV-2 Negative Control(s)	Black

3) When processing External Controls, place the controls in a specimen tube carrier and use the touchscreen to load the carrier into NeuMoDx System from the autoloader shelf. The NeuMoDx System will recognize the barcodes and start processing controls unless reagents or consumables required for testing are not available.

4) Validity of these external controls will be assessed by the NeuMoDx System based on the expected results.

NeuMoDx FluA/FluB/RSV/SARS-CoV-2 External Control	FluA/FluB/RSV/SARS-CoV-2 Result	SPC2 Result
NeuMoDx Flu A/FluB/RSV/SARS-CoV-2 Positive Control(s)	FluA, FluB, RSV, SARS-CoV-2 RNA Detected	N/A
NeuMoDx Flu A/FluB/RSV/SARS-CoV-2 Negative Control(s)	FluA, FluB, RSV, SARS-CoV-2 RNA Not Detected	SPC2 Positive

5) Discrepant result handling for external controls should be performed as follows:

   a) A Positive test result reported for a negative control sample may indicate contamination and the laboratories quality control procedures need to be examined to find a root cause. Ensure to use separate areas for sample preparation, control handling and RT-PCR set up. Please refer to *NeuMoDx 288 or 96 Molecular System Operator's Manual* for additional troubleshooting tips.
   b) A Negative test result reported for a positive control sample may indicate that there is a reagent or NeuMoDx System related problem. Please refer to *NeuMoDx 288 or 96 Molecular System Operator's Manual* for troubleshooting tip.
   c) In either of the above instances, or in the event of a No Result (NR), Unresolved (UNR), or Indeterminant (IND) result, repeat the failed control with freshly thawed vial(s) of the control(s) failing the validity test.
   d) If the Positive Control continues to report a Negative test result, contact QIAGEN Technical Support.
   e) If the Negative Control continues to report a Positive test result, attempt to eliminate all sources of potential contamination, including replacing all reagents and repeating the run before contacting QIAGEN Technical Support.
   f) If the External Controls do not provide the expected results, it is required to repeat a set of positive and negative controls. Patient results will not be reported if controls do not give expected results.

Sample Process (Internal) Controls

An exogenous Sample Process Control (SPC2) is incorporated in the NeuMoDx Extraction Plate and undergoes the entire process of nucleic acid extraction and real-time RT-PCR amplification with each sample. Primers and Probes specific to SPC2 are also included in each NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strip well, enabling detection of SPC2 with the target RNA (if present) via multiplex PCR. Detection of SPC2 amplification allows the NeuMoDx System software to monitor the efficacy of the RNA extraction and RT-PCR amplification processes.

Prior to RT-PCR, the NeuMoDx System automatically performs a 'FILL CHECK' to ensure that the PCR chamber is filled with solution and contains an adequate amount of fluorescent probe.

The NeuMoDx System software continuously monitors on-board sensors and actuators to ensure a safe and effective operation of the System.

Multiple fluidic error recovery modes are implemented by active monitoring of aspiration and dispensing operations to ensure that the System can either complete processing of all samples in a safe and effective manner or provide an appropriate error code.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The Analytical Sensitivity of the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay on the NeuMoDx Molecular Systems was characterized in two parts. The Limit of Detection (LoD) was characterized using pooled leftover deidentified clinical negative nasopharyngeal swab specimens collected in UVT matrix and model strains of each target. The model strains used for each target are presented in *Table 3*. First, a dilution series using model strains of each target in UVT were prepared with the Direct and Pretreated Workflows and then processed by the NeuMoDx System to determine a preliminary Limit of Detection (LoD) value. In the second part of testing, these preliminary LoD values were confirmed using a hit-rate study on both the NeuMoDx 288 and the NeuMoDx 96 Molecular Systems for both workflows. The preliminary LoD was accepted if the hit-rate testing achieved a 95% positivity rate for both workflows on both Systems. Detection rates for the preliminary LoD are depicted in *Table 4* while *Table 5* details the hit-rate confirmation for the N288 System and *Table 6* details the hit-rate confirmation for the N96 System. The final LoD claims in *Table 4* are indicated in **bold** font.

Table 3. Strain Used for Each Target

Target/Strain	Source	Cat #	Lot #	Material Type
Flu A, Idaho/07/2018 (H1N1) pdm09	IRR	FR-1688	70031602	Clarified supernatant from infected cells
Flu A, Wisconsin/505/2018 (H1N1) pdm09	IRR	FR-1690	70032253	Clarified supernatant from infected cells
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	Virapur	N/A	B1904J	Live Crude
Flu A, Hong Kong/2671/2019 (H3N2)	Virapur	N/A	C2030D	Live Crude
Flu B, Hong Kong/286/2017 (Victoria)	IRR	FR-1619	70015942	Clarified supernatant from infected cells
Flu B, Colorado/6/2017 (Victoria)	IRR	FR-1592	70013310	Clarified supernatant from infected cells
Flu B, Florida/78/2015 (Victoria)	ATCC	VR-1931	70020870	Clarified culture fluid and cell lysate
Flu B, Phuket/3073/2013 (Yamagata)	Virapur	N/A	B1904N	Live Crude
RSV A2	ATCC	VR-1540	60430286	Culture Fluid and cell lysate
RSV B (WV/14617/85)	ATCC	VR-1400	70013461	Culture Fluid and cell lysate
SARS-CoV-2, 1st WHO International Standard	NIBSC	20/146	N/A	Lyophilized acid and heat inactivated virus
SARS-CoV-2, Isolate USA-WA1/2020	BEI	NR-52285	70037779	Heat inactivated virus

Table 4. Positive Detection Rates for Preliminary LoD Determination of the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay – (a) Pretreated Workflow; (b) Direct Workflow

(a) Pretreated Workflow

Target/Strain	Level	Unit	# Valid Results (n/N)	# Pos	% Pos	Ct Avg	Ct SD
Flu A, Idaho/07/2018 (H1N1) pdm09	0.02	$TCID_{50}$/mL	10/10	7	70%	33.97	0.90
	0.06		10/10	10	100%	33.36	0.96
	0.17		10/10	10	100%	32.17	0.45
	0.5		10/10	10	100%	31.05	0.42
	1.5		10/10	10	100%	31.01	0.45
Flu A, Wisconsin/505/2018 (H1N1) pdm09	0.17	$TCID_{50}$/mL	10/10	8	80%	33.72	1.00
	0.5		10/10	10	100%	32.97	0.51
	1.5		10/10	10	100%	32.28	0.60
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	0.17	$TCID_{50}$/mL	10/10	8	80%	32.81	0.38
	0.5		10/10	10	100%	31.68	0.84
	1.5		10/10	10	100%	31.69	0.65
Flu A, Hong Kong/2671/2019 (H3N2)	0.17	$TCID_{50}$/mL	20/20	15	75%	32.15	1.70
	0.5		10/10	9	90%	32.37	0.50
	1.5		10/10	10	100%	32.63	1.35
Flu B, Hong Kong/286/2017 (Victoria)	0.01	$TCID_{50}$/mL	10/10	8	80%	32.90	1.27
	0.03		10/10	10	100%	32.26	0.48
	0.08		10/10	10	100%	31.48	0.78
	0.25		10/10	10	100%	30.59	0.40
Flu B, Colorado/6/2017 (Victoria)	0.003	$TCID_{50}$/mL	10/10	10	100%	33.97	0.58
	0.01		10/10	10	100%	33.90	0.39
	0.03		10/10	10	100%	33.85	0.56
Flu B, Florida/78/2015 (Victoria)	0.083	$TCID_{50}$/mL	20/20	18	90%	34.39	0.84
	0.25		10/10	10	100%	32.53	0.21
	0.75		10/10	10	100%	32.57	0.40
Flu B, Phuket/3073/2013 (Yamagata)	0.33	$TCID_{50}$/mL	20/20	15	75%	33.58	1.50
	1		10/10	10	100%	34.03	0.69
	3		10/10	10	100%	32.30	0.66
RSV A2	0.17	$TCID_{50}$/mL	10/10	5	50%	32.68	0.43
	0.5		10/10	10	100%	31.72	0.85
	1.5		10/10	10	100%	31.71	1.35
RSV B (WV/14617/85)	0.017	$TCID_{50}$/mL	10/10	5	50%	32.20	1.10
	0.05		10/10	10	100%	31.50	0.49
	0.15		10/10	10	100%	29.94	0.93
SARS-CoV-2, 1st WHO International Standard	50	IU/mL	10/10	6	60%	34.36	0.64
	150		10/10	10	100%	34.20	0.31
	450		10/10	10	100%	33.04	0.63
SARS-CoV-2, Isolate USA-WA1/2020	50	copies/mL	10/10	6	60%	34.20	1.19
	150		10/10	10	100%	33.46	0.58
	450		10/10	10	100%	32.62	1.06

(b) Direct Workflow

Target/Strain	Level	Unit	# Valid Results (n/N)	# Pos	% Pos	Ct Avg	Ct SD
Flu A, Idaho/07/2018 (H1N1) pdm09	0.02	$TCID_{50}$/mL	20/20	17	85%	33.11	1.30
	0.06		10/10	10	100%	33.18	0.86
	0.17		10/10	10	100%	32.63	1.14
	0.5		10/10	10	100%	31.33	0.74
	1.5		10/10	10	100%	30.79	0.31
Flu A, Wisconsin/505/2018 (H1N1) pdm09	0.17	$TCID_{50}$/mL	20/20	18	90%	33.41	1.10
	0.5		10/10	9	90%	32.54	1.03
	1.5		10/10	10	100%	32.05	0.26
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	0.17	$TCID_{50}$/mL	10/10	7	70%	33.39	0.16
	0.5		10/10	10	100%	32.70	1.01
	1.5		10/10	10	100%	31.12	1.07
Flu A, Hong Kong/2671/2019 (H3N2)	0.17	$TCID_{50}$/mL	10/10	8	80%	34.11	0.69
	0.5		10/10	10	100%	33.68	0.50
	1.5		10/10	10	100%	32.27	1.29
Flu B, Hong Kong/286/2017 (Victoria)	0.01	$TCID_{50}$/mL	20/20	18	90%	33.31	0.95
	0.03		10/10	10	100%	31.51	0.94
	0.08		10/10	10	100%	31.76	0.46
	0.25		10/10	10	100%	30.11	0.45
Flu B, Colorado/6/2017 (Victoria)	0.003	$TCID_{50}$/mL	10/10	9	90%	34.82	0.39
	0.01		10/10	10	100%	34.37	0.55
	0.03		10/10	10	100%	33.64	0.34
Flu B, Florida/78/2015 (Victoria)	0.083	$TCID_{50}$/mL	20/20	18	90%	33.78	1.11
	0.25		10/10	10	100%	33.89	0.69
	0.75		10/10	10	100%	32.38	0.47
Flu B, Phuket/3073/2013 (Yamagata)	0.25	$TCID_{50}$/mL	10/10	8	80%	33.23	1.17
	0.75		20/20	19	95%	32.63	1.22
	2.25		10/10	10	100%	31.24	1.58
RSV A2	0.42	$TCID_{50}$/mL	10/10	7	70%	32.61	0.70
	1.25		10/10	10	100%	30.99	1.55
	3.75		10/10	10	100%	31.49	1.04
RSV B (WV/14617/85)	0.017	$TCID_{50}$/mL	10/10	6	60%	33.63	1.49
	0.05		10/10	10	100%	32.42	1.12
	0.15		10/10	10	100%	31.81	0.81
SARS-CoV-2, 1st WHO International Standard	50	IU/mL	10/10	7	70%	34.80	0.56
	150		20/20	19	95%	32.88	1.22
	450		10/10	10	100%	33.38	0.46
SARS-CoV-2, Isolate USA-WA1/2020	66.7	copies/mL	10/10	7	70%	33.53	0.58
	200		10/10	10	100%	32.63	1.25
	600		10/10	10	100%	32.69	0.86

Table 5. Positive Detection Rates for Confirmatory LoD Determination for the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay – N288, (a) Pretreated Workflow; (b) Direct Workflow

(a) Pretreated Workflow

Target/Strain	Level	Unit	# Valid Results (n/N)	# Pos	% Detection	Ct Avg	Ct SD
Flu A, Idaho/07/2018 (H1N1) pdm09	**0.06**	$TCID_{50}$/mL	30/30	30	100%	33.89	0.57
Flu A, Wisconsin/505/2018 (H1N1) pdm09	**0.5**	$TCID_{50}$/mL	30/30	29	96.7%	33.81	0.44
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	**0.5**	$TCID_{50}$/mL	30/30	30	100%	33.17	0.47
Flu A, Hong Kong/2671/2019 (H3N2)	**0.5**	$TCID_{50}$/mL	30/30	29	96.7%	33.77	0.52
Flu B, Hong Kong/286/2017 (Victoria)	**0.03**	$TCID_{50}$/mL	29/30	29	100%	32.32	1.09
Flu B, Colorado/6/2017 (Victoria)	**0.01**	$TCID_{50}$/mL	30/30	29	96.7%	34.50	0.68
Flu B, Florida/78/2015 (Victoria)	**0.25**	$TCID_{50}$/mL	30/30	30	100%	33.83	0.44
Flu B, Phuket/3073/2013 (Yamagata)	**1**	$TCID_{50}$/mL	29/30	29	100%	33.04	0.69
RSV A2	**0.5**	$TCID_{50}$/mL	30/30	29	96.7%	32.17	1.23
RSV B (WV/14617/85)	**0.05**	$TCID_{50}$/mL	30/30	30	100%	32.39	0.41
SARS-CoV-2, 1st WHO International Standard	**150**	IU/mL	30/30	30	100%	33.63	0.61
SARS-CoV-2, Isolate USA-WA1/2020	**150**	copies/mL	29/30	28	96.6%	33.59	1.01

(b) Direct Workflow

Target/Strain	Level	Unit	# Valid Results (n/N)	# Pos	% Detection	Ct Avg	Ct SD
Flu A, Idaho/07/2018 (H1N1) pdm09	**0.06**	$TCID_{50}$/mL	30/30	30	100%	33.92	0.69
Flu A, Wisconsin/505/2018 (H1N1) pdm09	**0.5**	$TCID_{50}$/mL	30/30	30	100%	33.75	0.57
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	**0.5**	$TCID_{50}$/mL	30/30	30	100%	32.96	0.48
Flu A, Hong Kong/2671/2019 (H3N2)	**0.5**	$TCID_{50}$/mL	30/30	30	100%	33.67	0.48
Flu B, Hong Kong/286/2017 (Victoria)	**0.03**	$TCID_{50}$/mL	29/30	28	96.6%	31.74	1.19
Flu B, Colorado/6/2017 (Victoria)	0.0033	$TCID_{50}$/mL	10/10	8	80%	34.88	0.95
	0.01	$TCID_{50}$/mL	30/30	30	100%	34.22	0.51
Flu B, Florida/78/2015 (Victoria)	**0.25**	$TCID_{50}$/mL	30/30	30	100%	33.55	0.38
Flu B, Phuket/3073/2013 (Yamagata)	**0.75**	$TCID_{50}$/mL	30/30	30	100%	33.33	0.74
RSV A2	**1.25**	$TCID_{50}$/mL	30/30	29	96.7%	31.87	0.95
RSV B (WV/14617/85)	**0.05**	$TCID_{50}$/mL	30/30	29	96.7%	32.46	0.72
SARS-CoV-2, 1st WHO International Standard	**150**	IU/mL	30/30	29	96.7%	33.78	0.77
SARS-CoV-2, Isolate USA-WA1/2020	**200**	copies/mL	30/30	30	100%	34.18	0.83

Table 6. Positive Detection Rates for Hit-Rate Confirmation of LoD for the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay – N96, (a) Pretreated Workflow; (b) Direct Workflow

(a) Pretreated Workflow

Target/Strain	Level	Unit	# Valid Results (n/N)	# Pos	% Detection	Ct Avg	Ct SD
Flu A, Idaho/07/2018 (H1N1) pdm09	**0.06**	$TCID_{50}$/mL	30/30	30	100%	33.05	0.81
Flu A, Wisconsin/505/2018 (H1N1) pdm09	**0.5**	$TCID_{50}$/mL	30/30	29	96.7%	33.53	0.75
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	**0.5**	$TCID_{50}$/mL	30/30	29	96.7%	32.33	1.11
Flu A, Hong Kong/2671/2019 (H3N2)	**0.5**	$TCID_{50}$/mL	30/30	30	100%	32.98	0.96
Flu B, Hong Kong/286/2017 (Victoria)	**0.03**	$TCID_{50}$/mL	30/30	29	96.7%	32.75	0.69
Flu B, Colorado/6/2017 (Victoria)	0.0033	$TCID_{50}$/mL	10/10	4	40%	34.75	0.58
	0.01	$TCID_{50}$/mL	30/30	30	100%	33.91	0.75
Flu B, Florida/78/2015 (Victoria)	**0.25**	$TCID_{50}$/mL	30/30	29	96.7%	33.25	0.97
Flu B, Phuket/3073/2013 (Yamagata)	**1**	$TCID_{50}$/mL	30/30	29	96.7%	33.21	0.96
RSV A2	**0.5**	$TCID_{50}$/mL	29/30	28	96.6%	32.39	1.10
RSV B (WV/14617/85)	**0.05**	$TCID_{50}$/mL	30/30	30	100%	32.06	0.76
SARS-CoV-2, 1st WHO International Standard	**150**	IU/mL	30/30	29	96.7%	33.79	0.67
SARS-CoV-2, Isolate USA-WA1/2020	**150**	copies/mL	30/30	29	96.7%	33.59	1.05

(b) Direct Workflow

Target/Strain	Level	Unit	# Valid Results (n/N)	# Pos	% Detection	Ct Avg	Ct SD
Flu A, Idaho/07/2018 (H1N1) pdm09	**0.06**	$TCID_{50}$/mL	30/30	30	100%	33.42	0.54
Flu A, Wisconsin/505/2018 (H1N1) pdm09	**0.5**	$TCID_{50}$/mL	30/30	30	100%	33.35	1.10
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	**0.5**	$TCID_{50}$/mL	30/30	30	100%	32.17	1.24
Flu A, Hong Kong/2671/2019 (H3N2)	**0.5**	$TCID_{50}$/mL	30/30	30	100%	33.22	0.50
Flu B, Hong Kong/286/2017 (Victoria)	**0.03**	$TCID_{50}$/mL	30/30	30	100%	32.78	0.56
Flu B, Colorado/6/2017 (Victoria)	**0.01**	$TCID_{50}$/mL	30/30	30	100%	34.21	0.50
Flu B, Florida/78/2015 (Victoria)	**0.25**	$TCID_{50}$/mL	30/30	30	100%	33.41	0.65
Flu B, Phuket/3073/2013 (Yamagata)	**0.75**	$TCID_{50}$/mL	30/30	29	96.7%	33.36	1.04
RSV A2	**1.25**	$TCID_{50}$/mL	30/30	29	96.7%	32.29	0.99
RSV B (WV/14617/85)	**0.05**	$TCID_{50}$/mL	30/30	30	100%	32.17	0.75
SARS-CoV-2, 1st WHO International Standard	**150**	IU/mL	30/30	29	96.7%	33.50	0.78
SARS-CoV-2, Isolate USA-WA1/2020	**200**	copies/mL	29/30	29	100%	34.45	0.39

The levels accepted as the LoD values for the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay on the NeuMoDx Systems are summarized in *Table 7*.

Table 7. Summary of Limit of Detection Study

Target	Strain	Limit of Detection		
		Pretreated Workflow	Direct Workflow	Unit
Influenza A (Flu A) – H1N1	Idaho/07/2018 (H1N1) pdm09	0.06	0.06	$TCID_{50}$/mL
	Wisconsin/505/2018 (H1N1) pdm09	0.5	0.5	
Influenza A (Flu A) – H3N2	Singapore/INIFMIH-16-0019/2016 (H3N2)	0.5	0.5	
	Hong Kong/2671/2019 (H3N2)	0.5	0.5	
Influenza B (Flu B) – Victoria lineage	Hong Kong/286/2017	0.03	0.03	
	Colorado/6/2017	0.01	0.01	
	Florida/78/2015	0.25	0.25	
Influenza B (Flu B) – Yamagata lineage	Phuket/3073/2013	1	0.75	
RSV A	A2	0.5	1.25	
RSV B	(WV/14617/85)	0.05	0.05	
SARS-CoV-2	1st WHO International Standard	150	150	IU/mL
	Isolate USA-WA1/2020	150	200	copies/mL

Competitive Interference for Target Organisms: Flu A, Flu B, RSV, and SARS-CoV-2
Competitive interference of the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay was evaluated using panels of viral targets spiked in clinical negative nasopharyngeal swab specimens collected in UVT. Ten panels contained one or two targets near their Limit of Detection (3-10X LoD) and a single target at ≥1E5 copies/mL, representing the coinfected target. An eleventh panel contained one of each of the four targets at 2X LoD. The presence of two to three viruses at varying concentrations in a single specimen and their effects on the analytical sensitivity are shown in *Table 8*.

Influenza A- and RSV A-negative results should be considered presumptive in samples that have a positive SARS-CoV-2 result, and RSV-negative results should be considered presumptive in samples that have a positive influenza A result. Competitive Interference studies showed that SARS-CoV-2 virus, when present at concentrations at or above 1E5 copies/mL, can inhibit the detection and amplification of influenza A and RSV A RNA if present at or below 1.5 $TCID_{50}$/mL or 6.25 $TCID_{50}$/mL, respectively, and may lead to false-negative results. Additionally, influenza A virus, when present at concentrations at or above 1E5 cp/mL, can inhibit detection and amplification of RSV A virus RNA if present at or below 3.75 $TCID_{50}$/mL and may lead to false negative results for RSV. If co-infection with influenza A or RSV virus is suspected in samples with a positive SARS-CoV-2 result, or co-infection with RSV virus is suspected in samples with a positive influenza A result, the sample should be re-tested with another FDA cleared, approved, or authorized influenza or RSV test, if influenza or RSV virus detection would change clinical management.

Table 8. Summary of Competitive Interference Study

Panel	Target	Panel Level	Target Conc.	Valid Results	# Pos	% Detection
1	Flu A	3X	1.5 $TCID_{50}$/mL	24	24	100%
	RSV A	3X	3.75 $TCID_{50}$/mL	24	23	96%
	Flu B	High	1E5 cp/mL	24	24	100%
2 (Run 1)	Flu A	3X	1.5 $TCID_{50}$/mL	24	19	79%
	RSV A	3X	3.75 $TCID_{50}$/mL	24	8	33%
	SARS-CoV-2	High	1E5 cp/mL	24	24	100%
2 (Run 2)	Flu A	5X	2.5 $TCID_{50}$/mL	24	24	100%
	RSV A	5X	6.25 $TCID_{50}$/mL	24	16	67%
	SARS-CoV-2	High	1E5 cp/mL	24	24	100%
2 (Run 3)	Flu A	5X	2.5 $TCID_{50}$/mL	24	24	100%
	RSV A	10X	12.5 $TCID_{50}$/mL	24	24	100%
	SARS-CoV-2	High	1E5 cp/mL	24	24	100%
3	Flu A	3X	1.5 $TCID_{50}$/mL	24	24	100%
	SARS-CoV-2	3X	450 IU/mL	24	24	100%
	RSV B	High	1E5 cp/mL	24	24	100%
4	Flu B	3X	0.75 $TCID_{50}$/mL	24	24	100%
	RSV B	3X	0.15 $TCID_{50}$/mL	24	24	100%
	Flu A	High	1E5 cp/mL	24	24	100%
5	Flu B	3X	0.75 $TCID_{50}$/mL	24	24	100%
	RSV B	3X	0.15 $TCID_{50}$/mL	24	24	100%
	SARS-CoV-2	High	1E5 cp/mL	24	24	100%
6	Flu B	3X	0.75 $TCID_{50}$/mL	24	24	100%
	RSV B	High	1E5 cp/mL	24	24	100%
7	SARS-CoV-2	3X	450 IU/mL	24	24	100%
	Flu A	High	1E5 cp/mL	24	24	100%
8	SARS-CoV-2	3X	450 IU/mL	24	24	100%
	Flu B	High	1E5 cp/mL	24	24	100%
9 (Run 1)	RSV A	3X	3.75 $TCID_{50}$/mL	24	20	83%
	Flu A	High	1E5 cp/mL	24	24	100%
9 (Run 2)	RSV A	5X	6.25 $TCID_{50}$/mL	24	23	96%
	Flu A	High	1E5 cp/mL	24	24	100%
10	RSV B	3X	0.15 $TCID_{50}$/mL	24	23	96%
	Flu B	High	1E5 cp/mL	24	24	100%
11	Flu A	2X	1 $TCID_{50}$/mL	24	24	100%
	Flu B	2X	0.5 $TCID_{50}$/mL	24	24	100%
	RSV B	2X	0.1 $TCID_{50}$/mL	24	24	100%
	SARS-CoV-2	2X	300 IU/mL	24	24	100%

Analytical Reactivity and Inclusivity

The analytical reactivity of the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay was evaluated against multiple strains/isolates of Influenza A, Influenza B, RSV, and SARS-CoV-2. The reactivity of each strain/isolate was characterized in two parts. The initial assessment of reactivity levels for each target was performed with each individual target strain tested at 3 concentrations in simulated nasopharyngeal swab matrix (prepared with 3000 human epithelial cells per mL of UVT), *Table 9*. In the second part, the lowest level that obtained a 100% positive rate in phase 1 was confirmed as the reactivity level by testing a minimum of 20 replicates, *Table 10*. A total of 14 Flu A strains, 6 Flu B strains, 1 RSV A isolate, 1 RSV B isolate, and 6 isolates of SARS-CoV-2 were tested.

Table 9. Flu A, Flu B, RSV A, RSV B, and SARS-CoV-2 Strains – Preliminary Analysis of Reactivity Level

Preliminary Analysis					
Target	**Strain**		**Levels Tested**	**# Valid Results**	**% Pos**
Flu A	H1N1	Brisbane/02/2018	0.5 $TCID_{50}$/mL	8	75.0%
			1.5 $TCID_{50}$/mL	8	100%
			4.5 $TCID_{50}$/mL	7	100%
		Guangdong-Moanan/SWL 1536/2019	0.33 $TCID_{50}$/mL	8	87.5%
			1 $TCID_{50}$/mL	8	100%
			3 $TCID_{50}$/mL	8	100%
		Michigan/272/2017 (H1N1)pdm09	0.17 $TCID_{50}$/mL	6	50%
			0.5 $TCID_{50}$/mL	6	100%
			1.5 $TCID_{50}$/mL	6	100%
		A/Iowa/53/2015 (H1N1)pdm09	0.33 $TCID_{50}$/mL	8	87.5%
			1 $TCID_{50}$/mL	8	100%
			3 $TCID_{50}$/mL	8	100%
		A/Bangladesh/3002/2015 (H1N1)pdm09	3.3 $CEID_{50}$/mL	8	62.5%
			10 $CEID_{50}$/mL	8	87.5%
			30 $CEID_{50}$/mL	8	100%
	H3N2	Switzerland/9715293/2013 (H3N2)	0.17 $TCID_{50}$/mL	8	87.5%
			0.5 $TCID_{50}$/mL	8	100%
			1.5 $TCID_{50}$/mL	8	100%
		Hong Kong/4801/2014 (H3N2)	0.15 $TCID_{50}$/mL	7	28.6%
			0.5 $TCID_{50}$/mL	8	100%
			1.5 $TCID_{50}$/mL	8	100%
		Kansas/14/2017 (H3N2)	2.67 $TCID_{50}$/mL	8	50%
			8 $TCID_{50}$/mL	8	87.5%
			24 $TCID_{50}$/mL	7	100%
		A/Wisconsin/04/2018 (H3N2)	3.3 $CEID_{50}$/mL	6	83.3%
			10 $CEID_{50}$/mL	6	100%
			30 $CEID_{50}$/mL	6	100%
		A/California/02/2014 (H3N2)	0.01 $TCID_{50}$/mL	8	85.7%
			0.03 $TCID_{50}$/mL	8	100%
			0.1 $TCID_{50}$/mL	7	100%
			0.33 $TCID_{50}$/mL	8	100%
			1 $TCID_{50}$/mL	8	100%
			3 $TCID_{50}$/mL	7	100%
	H2N2	A2/Japan/305/57 (H2N2)	10.87 pg/mL[1]	8	100%
			32.6 pg/mL[1]	8	87.5%
			97.8 pg/mL[1]	7	100%
	H5N2	A/Duck/Pennsylvania/10218/84 (H5N2)	8 pg/mL[1]	8	100%
			25 pg/mL[1]	8	100%
			75 pg/mL[1]	7	100%
	H7N9	A/Anhui/1/2013 (H7N9)	1:3E5[1]	8	50%
			1:1E5[1]	7	87.5%
			1:3.3E4[1]	8	100%
	H10N7	A/Chick/Germany/N/49 (H10N7)	22.67 pg/mL[1]	8	100%
			68 pg/mL[1]	8	100%
			204 pg/mL[1]	8	100%
Flu B	Victoria Lineage	Malaysia/2506/2004 (Victoria)	1 $TCID_{50}$/mL	8	100%
			3 $TCID_{50}$/mL	8	100%
			9 $TCID_{50}$/mL	8	100%
		Washington/02/2019 (Victoria)	2.5 $CEID_{50}$/mL	8	25.0%
			5 $CEID_{50}$/mL	8	87.5%
			15 $CEID_{50}$/mL	8	100%
		B/Maryland/15/2016 (Victoria)	0.01 $TCID_{50}$/mL	12	91.7%
			0.03 $TCID_{50}$/mL	8	100%
			0.1 $TCID_{50}$/mL	8	100%
			0.33 $TCID_{50}$/mL	16	100%
			1 $TCID_{50}$/mL	8	100%
			3 $TCID_{50}$/mL	8	100%

Preliminary Analysis					
Target	**Strain**		**Levels Tested**	**# Valid Results**	**% Pos**
Flu B (continued)	Yamagata Lineage	Wisconsin/1/2010 (Yamagata)	0.17 $CEID_{50}$/mL	8	75.0%
			0.5 $CEID_{50}$/mL	8	100%
			1.5 $CEID_{50}$/mL	8	100%
		B/Utah/09/2014 (Yamagata Lineage)	0.06 $CEID_{50}$/mL	8	25.0%
			0.19 $CEID_{50}$/mL	8	87.5%
			0.56 $CEID_{50}$/mL	7	85.7%
			1.7 $CEID_{50}$/mL	6	100%
			5 $CEID_{50}$/mL	6	100%
			15 $CEID_{50}$/mL	6	100%
		B/Oklahoma/10/2018 (NA D197N) (Yamagata)	0.33 $TCID_{50}$/mL	8	25.0%
			1 $TCID_{50}$/mL	8	87.5%
			3 $TCID_{50}$/mL	8	100%
RSV	RSVA	A (long)	0.67 pfu/ml	8	37.5%
			2 pfu/ml	8	100%
			6 pfu/ml	7	100%
	RSVB	B (9320)	0.03 pfu/mL	8	12.5%
			0.1 pfu/mL	8	87.5%
			0.3 pfu/mL	8	100%
SARS-CoV-2		USA/CA-Stanford-15_S02/2021 (Kappa, B1.617.1)	0.06 $TCID_{50}$/mL	8	0%
			0.17 $TCID_{50}$/mL	8	12.5%
			0.5 $TCID_{50}$/mL	8	37.5%
			1.5 $TCID_{50}$/mL	8	87.5%
			4.5 $TCID_{50}$/mL	8	100%
			13.5 $TCID_{50}$/mL	8	100%
		USA/CA_CDC_5574/2020 (Alpha, B.1.1.7)	0.006 $TCID_{50}$/mL	8	62.5%
			0.02 $TCID_{50}$/mL	8	87.5%
			0.06 TCI.D_{50}/mL	8	100%
			0.17 $TCID_{50}$/mL	7	100%
			0.5 $TCID_{50}$/mL	7	100%
			1.5 $TCID_{50}$/mL	7	100%
		Japan/TY7-503/2021 (Gamma, Brazil P.1)	0.002 $TCID_{50}$/mL	8	62.5%
			0.006 $TCID_{50}$/mL	8	100%
			0.02 $TCID_{50}$/mL	8	100%
			0.06 $TCID_{50}$/mL	8	100%
			0.17 $TCID_{50}$/mL	8	100%
			0.5 TCID50/mL	8	100%
		USA/PHC658/2021 (Delta, B.1.617.2)	0.001 $TCID_{50}$/mL	8	37.5%
			0.004 $TCID_{50}$/mL	8	87.5%
			0.013 $TCID_{50}$/mL	8	100%
			0.04 $TCID_{50}$/mL	8	100%
			0.11 $TCID_{50}$/mL	8	100%
			0.33 $TCID_{50}$/mL	4	100%
		Italy-INMI1	7.44 cp/mL[1]	8	37.5%
			22.33 cp/mL[1]	8	87.5%
			67 cp/mL[1]	8	100%
			200 cp/mL[1]	8	100%
			600 cp/mL[1]	8	100%
		Isolate Hong Kong/VM20001061/2020	7.44 cp/mL[1]	8	25.0%
			22.33 cp/mL[1]	8	87.5%
			67 cp/mL[1]	7	100%
			200 cp/mL[1]	7	100%
			600 cp/mL[1]	7	100%

[1]These variants were supplied with only a "total RNA" quantitation, which include both viral RNA and host cell RNA.

Table 10. Flu A, Flu B, RSV A, RSV B, and SARS-CoV-2 Strains – Confirmation of Reactivity Level

Confirmation					
Target	**Strain**		**Level**	**# Valid Results**	**% Pos**
Flu A	H1N1	Brisbane/02/2018	1.0 $TCID_{50}$/mL	23	91.3%
			1.5 $TCID_{50}$/mL	23	100%
		Guangdong-Moanan/SWL 1536/2019	0.5 $TCID_{50}$/mL	23	82.6%
			1.0 $TCID_{50}$/mL	24	100%
		Michigan/272/2017 (H1N1)pdm09	0.5 $TCID_{50}$/mL	24	100%
		A/Iowa/53/2015 (H1N1)pdm09	0.33 $TCID_{50}$/mL	24	85.7%
			0.67 $TCID_{50}$/mL	24	95.2%
		A/Bangladesh/3002/2015 (H1N1)pdm09	10 $CEID_{50}$/mL	24	100%
	H3N2	Switzerland/9715293/2013 (H3N2)	0.25 $TCID_{50}$/mL	24	87.0%
			0.5 $TCID_{50}$/mL	24	100%
		Hong Kong/4801/2014 (H3N2)	0.5 $TCID_{50}$/mL	23	91.3%
			1.0 $TCID_{50}$/mL	23	95.7%
		Kansas/14/2017 (H3N2)	12 $TCID_{50}$/mL	23	100%
		A/Wisconsin/04/2018 (H3N2)	5 $CEID_{50}$/mL	23	91.3%
			10 $CEID_{50}$/mL	23	100%
		A/California/02/2014 (H3N2)	0.01 $TCID_{50}$/mL	24	91.7%
			0.03 $TCID_{50}$/mL	24	100%
	H2N2	A2/Japan/305/57 (H2N2)	10.87 pg/mL[1]	24	100%
	H5N2	A/Duck/Pennsylvania/10218/84 (H5N2)	2 pg/mL[1]	24	83.3%
			4 pg/mL[1]	23	100%
			8 pg/mL[1]	23	100%
	H7N9	A/Anhui/1/2013 (H7N9)	1:3.3E4[1]	24	95.7%
	H10N7	A/Chick/Germany/N/49 (H10N7)	7.6 pg/mL[1]	23	73.9%
			22.67 pg/mL[1]	23	100%
Flu B	Victoria Lineage	Malaysia/2506/2004 (Victoria)	1 $TCID_{50}$/mL	23	95.7%
		Washington/02/2019 (Victoria)	5 $CEID_{50}$/mL	24	95.8%
			10 $CEID_{50}$/mL	24	100%
		B/Maryland/15/2016 (Victoria)	0.01 $TCID_{50}$/mL	23	83.3%
			0.03 $TCID_{50}$/mL	24	100%
	Yamagata Lineage	Wisconsin/1/2010 (Yamagata)	0.05 $CEID_{50}$/mL	24	100%
		B/Utah/09/2014 (Yamagata Lineage)	0.56 $TCID_{50}$/mL	24	87.0%
			1.5 $TCID_{50}$/mL	24	100%
		B/Oklahoma/10/2018 (NA D197N) (Yamagata)	0.75 $TCID_{50}$/mL	24	87.5%
			1.5 $TCID_{50}$/mL	20	95.0%
			3.0 $TCID_{50}$/mL	24	100%
RSV	RSVA	A (long)	2 pfu/mL	24	91.7%
			4 pfu/mL	24	95.8%
	RSVB	B (9320)	0.15 pfu/mL	24	100%
			0.3 pfu/mL	21	100%
SARS-CoV-2		USA/CA-Stanford-15_S02/2021 (Kappa, B1.617.1)	1.5 $TCID_{50}$/ml	24	100%
			3 $TCID_{50}$/ml	24	100%
			4.5 $TCID_{50}$/ml	24	100%
		USA/CA_CDC_5574/2020 (Alpha, B.1.1.7)	0.02 $TCID_{50}$/ml	24	95.8%
			0.06 $TCID_{50}$/ml	24	100%
		Japan/TY7-503/2021 (Gamma, Brazil P.1)	0.006 $TCID_{50}$/ml	24	95.8%
		USA/PHC658/2021 (Delta, B.1.617.2)	0.006 $TCID_{50}$/ml	24	87.5%
			0.013 $TCID_{50}$/ml	24	100%
SARS-CoV-2 (continued)		Italy-INMI1	22 cp/mL[1]	24	95.8%
			67 cp/mL[1]	24	100%
		Isolate Hong Kong/VM20001061/2020	22 cp/mL[1]	24	57.1%
			67 cp/mL[1]	24	100%

[1]These variants were supplied with only a "total RNA" quantitation, which include both viral RNA and host cell RNA.

The reactivity of the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay in detection of different clinical isolates of SARS-CoV-2 was demonstrated by performing an *in silico* analysis with the primers and probes of the assay against all the sequences available in GenBank (as of November 2021) using web-based NCBI Basic Local Alignment Search Tool (BLAST). The results show that the primers and probe for SARS-CoV-2 have 100% homology with over 98% of the sequences. Overall, the primers and probe have >95% homology to all sequences analyzed.

Intra-Lab Reproducibility

Intra-Lab Reproducibility for the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay was characterized by testing ten panels of Flu A, Flu B, RSV A, RSV B, or SARS-CoV-2 spiked individually at 2 levels [moderate positive (5x LoD) and low positive (2x LoD)] and one negative panel. The panels were tested across three lots of NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test strips manufactured under GMP, across two NeuMoDx Systems, and across six nonconsecutive days. Panel members were prepared in simulated nasopharyngeal swab specimens prepared with 3000 human epithelial cells per mL of Universal Viral Transport medium (UVT) and spiked a representative strain of Flu A, Flu B, RSV A, RSV B, and SARS-CoV-2. The NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strips and NeuMoDx Vantage Viral Lysis Buffer (VVLB) have been identified as the key test specific reagents capable of influencing the performance of the assay and therefore, the Pre-treated workflow was used in order to incorporate VVLB in the study. The standard deviation for Ct values within and across three lots of NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay test strips, two NeuMoDx Molecular Systems was ≤ 1.2 with coefficients of variation (CV) ≤ 4.0% for all targets demonstrating excellent reproducibility, *Table 11, 12, and 13*.

Table 11. Reproducibility of NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strips Across all Systems/Lots/Days

Target	Target Level	Valid N	% Positive	Avg Ct	SD	%CV
Flu A	Mod Pos	72	100%	31.21	0.59	1.9%
	Low Pos	72	100%	32.01	0.58	1.8%
Flu B	Mod Pos	72	100%	31.02	0.39	1.3%
	Low Pos	72	100%	31.88	0.56	1.7%
RSV A	Mod Pos	72	100%	29.71	0.95	3.2%
	Low Pos	72	100%	30.75	1.18	3.8%
RSV B	Mod Pos	72	100%	28.43	0.53	1.9%
	Low Pos	72	100%	29.45	0.56	1.9%
SARS-CoV-2	Mod Pos	72	100%	32.70	0.51	1.5%
	Low Pos	72	100%	33.68	0.56	1.7%
True Negative		72	0%	N/A	N/A	N/A

Table 12. Reproducibility of NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strips by each System

Panel		N0000096					N000012				
Target	Target Level	Valid N	% Positive	Avg Ct	SD	%CV	Valid N	% Positive	Avg Ct	SD	%CV
Flu A	Mod Pos	36	100%	31.37	0.66	2.1%	36	100%	31.05	0.46	1.5%
	Low Pos	36	100%	32.07	0.65	2.0%	36	100%	31.95	0.51	1.6%
Flu B	Mod Pos	36	100%	31.10	0.40	1.3%	36	100%	30.94	0.37	1.2%
	Low Pos	36	100%	31.84	0.57	1.8%	36	100%	31.91	0.55	1.7%
RSV A	Mod Pos	36	100%	29.94	0.97	3.2%	36	100%	29.49	0.89	3.0%
	Low Pos	36	100%	30.93	1.19	3.8%	36	100%	30.57	1.16	3.8%
RSV B	Mod Pos	36	100%	28.60	0.58	2.0%	36	100%	28.26	0.42	1.5%
	Low Pos	36	100%	29.60	0.53	1.8%	36	100%	29.29	0.56	1.9%
SARS-CoV-2	Mod Pos	36	100%	32.80	0.56	1.7%	36	100%	32.61	0.43	1.3%
	Low Pos	36	100%	33.83	0.64	1.9%	36	100%	33.52	0.42	1.2%
True Negative		36	0%	N/A	N/A	N/A	36	0%	N/A	N/A	N/A

Table 13. Reproducibility of NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strips by each Reagent Lot

Panel		Lot 1				Lot 2				Lot 3			
Target	Target Level	Valid N	Avg Ct	SD	%CV	Valid N	Avg Ct	SD	%CV	Valid N	Avg Ct	SD	%CV
Flu A	Mod Pos	24	31.06	0.38	1.2%	24	31.49	0.62	2.0%	24	31.08	0.65	2.1%
	Low Pos	24	32.02	0.59	1.8%	24	32.18	0.50	1.6%	24	31.82	0.61	1.9%
Flu B	Mod Pos	24	31.05	0.39	1.2%	24	31.08	0.47	1.5%	24	30.94	0.29	0.9%
	Low Pos	24	31.93	0.36	1.1%	24	32.01	0.77	2.4%	24	31.69	0.42	1.3%
RSV A	Mod Pos	24	29.04	0.71	2.4%	24	30.40	0.66	2.2%	24	29.69	0.94	3.2%
	Low Pos	24	31.53	0.50	1.6%	24	29.45	0.79	2.7%	24	31.25	0.87	2.8%
RSV B	Mod Pos	24	28.65	0.54	1.9%	24	28.29	0.52	1.8%	24	28.35	0.47	1.7%
	Low Pos	24	29.31	0.48	1.6%	24	29.46	0.64	2.2%	24	29.57	0.55	1.8%
SARS-CoV-2	Mod Pos	24	32.82	0.43	1.3%	24	32.70	0.56	1.7%	24	32.59	0.50	1.5%
	Low Pos	24	33.42	0.58	1.7%	24	33.80	0.57	1.7%	24	33.81	0.47	1.4%
True Negative		24	N/A	N/A	N/A	24	N/A	N/A	N/A	24	N/A	N/A	N/A

Clinical Performance

Clinical performance characteristics of the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay were determined using an internal retrospective method comparison study using residual nasopharyngeal (NP) swab specimens sourced from 4 geographically diverse clinical laboratory locations. Dilutions of clinical SARS-CoV-2 positive samples were also included in this study to demonstrate the clinical sensitivity near LoD.

Residual NP swab specimens from symptomatic patients were de-identified and given a unique ID number by the supplying laboratory, establishing a confidential list linking the patient ID to the de-identified specimens tested for study purposes. A total of 747 individual NP swab specimens were collected for this study. All specimens were processed through both the direct and pretreated workflows, ultimately producing 739 valid and 8 invalid results in the direct workflow and 736 valid and 11 invalid results in the pretreated workflow. Of these valid samples, 121 were exclusively dedicated to the assessment of Flu A, Flu B, and RSV targets. Flu A positive samples represent 54 of these specimens, wherein Flu B positive samples account for 34, and RSV positive samples for 33. Within this cohort of 121 samples, results for all 3 targets of interest were made available by the supplying clinical laboratories. As such, this cohort of positive samples also provided 67 negative Flu A results, 87 negative Flu B results, and 88 RSV-negative results. The aforementioned negative results were further supplemented by 59 clinical specimens that had comparator assay confirmed negative results for all 4 targets. Overall, 106 samples were identified as SARS-CoV-2 positive in both workflows. Clinical SARS-CoV-2 negatives were confirmed with a valid NeuMoDx result in 512 direct workflow samples and 509 pretreated workflow samples.

The test status of these samples was withheld from the operator to implement a “single blind study”. Results reported from the specific FDA- and CE-cleared, legally marketed molecular devices utilized by the laboratories for standard of care testing were used to perform the method comparison analysis.

Results of the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay provided a Clinical Sensitivity of 98.1% across both workflows for the Flu A target and a Clinical Specificity of 100% and 99.2% for the direct and pretreated workflows, respectively (*Table 14A*). Results for the Flu B target provided a Clinical Sensitivity and Clinical Specificity of 97.1% and 100%, respectively, for both workflows (*Table 14B*). Results for the RSV (undifferentiated) target provided a Clinical Sensitivity of 97% for both workflows and Clinical Specificity of 99.3 % and 98.6% in the direct and pretreated workflows, respectively.(*Table 14C*). Results for the SARS-CoV-2 target provided a Clinical Sensitivity of 97.2% for both workflows and a Clinical Specificity of 98.4% in the direct workflow and 98.2% in the pretreated workflow (*Table 14D*). The lower and upper limits of the 95% Confidence Intervals are presented in *Tables 14A, 14B, 14C,* and *14D* below and were calculated using the Wilson procedure with continuity correction.

Table 14A. Clinical Performance Summary – NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strip: Detection of **Flu A**
(a) Direct Workflow, and (b) Pretreated Workflow

(a) Direct Workflow

Flu A		FDA / CE Cleared Reference Test Result		
		POS	NEG	Total
NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Result	POS	53	0	53
	NEG	1	126	127
	Total	54	126	180
Clinical Sensitivity (Flu A) = 98.1% (88.8% – 99.9%)				
Clinical Specificity (Flu A) = 100% (96.3% – 100%)				

(b) Pretreated Workflow

Flu A		FDA / CE Cleared Reference Test Result		
		POS	NEG	Total
NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Result	POS	53	1	54
	NEG	1	125	126
	Total	54	126	180
Clinical Sensitivity (Flu A) = 98.1% (88.8% – 99.9%)				
Clinical Specificity (Flu A) = 99.2% (95.0% – 100%)				

Table 14B. Clinical Performance Summary – NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strip: Detection of **Flu B**
(a) Direct Workflow, and (b) Pretreated Workflow

(a) Direct Workflow

Flu B		FDA / CE Cleared Reference Test Result		
		POS	NEG	Total
NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Result	POS	33	0	33
	NEG	1	146	147
	Total	34	146	180
Clinical Sensitivity (Flu B) = 97.1% (82.9% – 99.8%)				
Clinical Specificity (Flu B) = 100% (96.8% – 100%)				

(b) Pretreated Workflow

Flu B		FDA / CE Cleared Reference Test Result		
		POS	NEG	Total
NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Result	POS	33	0	33
	NEG	1	146	147
	Total	34	146	180
Clinical Sensitivity (Flu B) = 97.1% (82.9% – 99.8%)				
Clinical Specificity (Flu B) = 100% (96.8% – 100%)				

Table 14C. Clinical Performance Summary – NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strip: Detection of **RSV** by (a) Direct Workflow, and (b) Pretreated Workflow

(a) Direct Workflow

RSV		FDA / CE Cleared Reference Test Result		
		POS	NEG	Total
NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Result	POS	32	1	33
	NEG	1	146	147
	Total	33	147	180
Clinical Sensitivity (RSV) = 97.0% (82.5% – 99.8%)				
Clinical Specificity (RSV) = 99.3% (95.7% – 100%)				

(b) Pretreated Workflow

RSV		FDA / CE Cleared Reference Test Result		
		POS	NEG	Total
NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Result	POS	32	2	34
	NEG	1	145	146
	Total	33	147	180
Clinical Sensitivity (RSV) = 97.0% (82.5% – 99.8%)				
Clinical Specificity (RSV) = 98.6% (94.7% – 99.8%)				

Table 14D. Clinical Performance Summary – NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Strip: Detection of **SARS-CoV-2** by (a) Direct Workflow, and (b) Pretreated Workflow

(a) Direct Workflow

SARS-CoV-2		FDA / CE Cleared Reference Test Result		
		POS	NEG	Total
NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Result	POS	103	8	111
	NEG	3	504	507
	Total	106	512	618
Clinical Sensitivity (SARS-CoV-2) = 97.2% (91.3% – 99.3%)				
Clinical Specificity (SARS-CoV-2) = 98.4% (96.8% – 99.3%)				

(b) Pretreated Workflow

SARS-CoV-2		FDA / CE Cleared Reference Test Result		
		POS	NEG	Total
NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Test Result	POS	103	9	112
	NEG	3	500	503
	Total	106	509	615
Clinical Sensitivity (SARS-CoV-2) = 97.2% (91.3% – 99.3%)				
Clinical Specificity (SARS-CoV-2) = 98.2% (96.5% – 99.1%)				

Analytical Specificity and Cross-Reactivity

The analytical specificity of the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 assay was evaluated by testing a panel of 47 organisms, consisting of 22 viral, 24 bacterial, and 1 yeast strain representing common respiratory pathogens or flora commonly present in the respiratory tract. Bacteria and yeast were tested at concentrations of ~6E6 CFU/mL or IFU/mL, except where otherwise noted. Viruses were tested at concentrations of 1E5 to 1E6 $TCID_{50}$/mL or copies/mL, except where otherwise noted. To confirm the potential cross reactivity between SARS-CoV-2 and the Coronavirus family (229E, OC43, NL63, MERS and SARS-1) along with *Legionella pneumophila*, additional replicates (> 20) were included to fulfill the MDCG requirement for SARS-CoV-2 in vitro diagnostic medical devices. Analytical specificity of the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 assay was 100% for Flu A, Flu B, RSV A, RSV B, and SARS-CoV-2.

HKU1 was another member of the Coronavirus family to be tested, however, due to the unavailability of the virus and genomic RNA, 4 replicates of synthetic material were tested. An *in-silico* analysis between the NeuMoDx SARS-CoV-2 primers and probes and HKU1 coronavirus genomes published in GenBank was also done to investigate the potential cross reactivity. A total of 57 sequences of HKU1 genomes were obtained from the NIH's NCBI Virus database. All HKU1 sequences had 3 or more mismatches to each of the NeuMoDx SARS-CoV-2 primer and probe. No close homology was detected. Therefore, no cross-reactivity is expected between Coronavirus HKU1 and NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay.

Table 15. Analytical Specificity Results

Organism	Concentration	Flu A	Flu B	RSV	SARS-CoV-2
Adenovirus Type 1	1E6 $TCID_{50}$/mL	-	-	-	-
Adenovirus Type 7	5E5 $TCID_{50}$/mL	-	-	-	-
Bordetella pertussis I176	10 ng/mL	-	-	-	-
Candida albicans	6E6 CFU/mL	-	-	-	-
Chlamydia pneumoniae	10 ng/mL	-	-	-	-
Corynebacterium xerosis	6E6 CFU/mL	-	-	-	-
EBV	1E6 $TCID_{50}$/mL	-	-	-	-
Escherichia coli	6E6 CFU/mL	-	-	-	-
Hemophilus influenzae	6E6 CFU/mL	-	-	-	-
HHV 6A	1E6 cp/mL	-	-	-	-
HHV 7	1E6 cp/mL	-	-	-	-
HHV 8	1E6 cp/mL	-	-	-	-
HSV 1	1E6 cp/mL	-	-	-	-
HSV 2	1E6 cp/mL	-	-	-	-
Human Coronavirus 229E	1E5 $TCID_{50}$/mL	-	-	-	-
Human Coronavirus HKU1	1E6 cp/mL	-	-	-	-
Human Coronavirus NL63	1E4 $TCID_{50}$/mL	-	-	-	-
Human Coronavirus OC43	1E5 $TCID_{50}$/mL	-	-	-	-
Human Enterovirus 68	1E5 $TCID_{50}$/mL	-	-	-	-
Human Metapneumovirus	1E4 $TCID_{50}$/mL	-	-	-	-
Human Parainfluenza Type 1	5E5 $TCID_{50}$/mL	-	-	-	-
Human Parainfluenza Type 2	5E5 $TCID_{50}$/mL	-	-	-	-
Human Parainfluenza Type 3	1E6 $TCID_{50}$/mL	-	-	-	-
Human Rhinovirus Type 1A	5E3 $TCID_{50}$/mL	-	-	-	-
Lactobacillus acidophilus	6E6 CFU/mL	-	-	-	-
Lactobacillus brevis	6E6 CFU/mL	-	-	-	-
Lactobacillus jensonii	6E6 CFU/mL	-	-	-	-
Lactobacillus lactis	6E6 CFU/mL	-	-	-	-
Legionella pneumophila	6E6 CFU/mL	-	-	-	-
Measles Virus	1E4 $TCID_{50}$/mL	-	-	-	-
MERS-Coronavirus EMC/2012	1E4 $TCID_{50}$/mL	-	-	-	-
Moraxella catarrhalis	6E6 CFU/mL	-	-	-	-
Mumps Virus	5E5 $TCID_{50}$/mL	-	-	-	-
Mycobacterium tuberculosis	6E6 CFU/mL	-	-	-	-
Mycoplasma pneumoniae	6E6 CFU/mL	-	-	-	-
Neisseria gonorrhoeae	6E6 CFU/mL	-	-	-	-
Neisseria meningitidis Sero A	6E6 CFU/mL	-	-	-	-
Neisseria meningitidis Sero B	6E6 CFU/mL	-	-	-	-
Neisseria meningitidis Sero C	6E6 CFU/mL	-	-	-	-
Neisseria meningitidis Sero D	6E6 CFU/mL	-	-	-	-
Pseudomonas aeruginosa	6E6 CFU/mL	-	-	-	-
SARS-Coronavirus	1E6 $TCID_{50}$/mL	-	-	-	-
Staphylococcus aureus	6E6 CFU/mL	-	-	-	-
Staphylococcus epidermidis	6E6 CFU/mL	-	-	-	-
Streptococcus pneumonia	6E6 CFU/mL	-	-	-	-

Organism	Concentration	Flu A	Flu B	RSV	SARS-CoV-2
Streptococcus pyogenes	6E6 CFU/mL	-	-	-	-
Streptococcus salivarius	6E6 CFU/mL	-	-	-	-
Flu A, Singapore/INIFMIH-16-0019/2016	3x LoD	+	-	-	-
Flu B, Florida/78/2015 (Victoria)	3x LoD	-	+	-	-
RSV A2	3x LoD	-	-	+	-
RSV B (WV/14617/85)	3x LoD	-	-	+	-
SARS-CoV-2, 1st WHO International Standard	3x LoD	-	-	-	+
Negative Control (No Pathogens)	N/A	-	-	-	-

Table 16. Analytical Specificity - Coronavirus family along with *Legionella pneumophila* (> 20 replicates tested)

Organism	Concentration	SARS-CoV-2
Human coronavirus NL63	1.00E+04 $TCID_{50}$/mL	-
SARS-Coronavirus-1	1.00E+06 pfu/mL	-
MERS-coronavirus EMC/2012	1.00E+04 $TCID_{50}$/mL	-
Human Coronavirus 229E	1.00E+05 $TCID_{50}$/mL	-
Human Coronavirus OC43	1.00E+05 $TCID_{50}$/mL	-
Legionella pneumophila	6.00E+06 CFU/mL	-
Positive control: SARS-CoV-2 First WHO Standard	3x LoD	+
Negative Control (No Pathogens)	N/A	-

Interfering Substances – Commensal Organisms

The NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay was tested for interference in the presence of non-target organisms (potentially present in the upper respiratory tract) by evaluating the assay performance at low levels (~3X LoD) of Flu A, Flu B, RSV A, RSV B, and SARS-CoV-2 in the presence of high concentrations of the organisms listed in *Table 15*, above. In addition, to confirm the potential interference between SARS-CoV-2 and the Coronavirus family (229E, OC43, NL63, MERS and SARS-1) along with *Legionella pneumophila* (*Table 16*), additional replicates (> 20) were included to fulfill the MDCG requirement for SARS-CoV-2 in vitro diagnostic medical devices. These samples were spiked with SARS-CoV-2 only at ~3X LoD for the interference portion of the study. 100% detection rate was observed for all the targets. Therefore, no interference on the detection of any target with any of the commensal organisms was observed.

Interfering Substances – Endogenous/Exogenous

The NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay was evaluated for susceptibility to interference caused by substances potentially associated with the collection of nasopharyngeal swab specimens. Residual clinical negative nasopharyngeal swab specimens were individually spiked with Flu A, Flu B, RSV A, RSV B, or SARS-CoV-2 at 3X LoD and processed in the presence and absence of the agents shown in *Table 17*. None of the substances included in the testing had an adverse effect on the assay performance for any of the targets.

Table 17. Substances Tested for Interference

Substance		Description/Active Ingredient	Concentration*
Exogenous	Neo-Synephrine	Phenylephrine	15% v/v
	Nasal Gel- Ayr Saline Nasal Gel	Sodium chloride with preservatives	15% v/v
	Homeopathic Allergy Relief- Similasan	Cardiospermum, Sabadilla, Luffa operculate, Galphimia glauca	15% v/v
	Nature's Bounty Zinc	Zinc Gluconate	0.1mg/mL
	Oral Anesthetic/Analgesic- Oragel	Benzocaine, Benzalkonium chloride	1% v/v
	Nasal Spray- Afrin	Oxymetazoline	15% w/v
	Nasal Spray- Zicam	*Luffa operculata*, *Galphimia glauca*, Histaminum hydrochloricum, Sulfur	15% v/v
	Nasal Corticosteroid- Flonase	Fluticasone	5% v/v
	Nasal Corticosteroid- Rhinocort	Budesonide	5% v/v
	Nasal Corticosteroid- Nasacort	Triamcinolone	5% v/v
	Nasal Corticosteroid- Dexamethasone	Dexamethasone	10 mg/mL
	Nasal Corticosteroid- Mometasone	Mometasone	10 mg/mL
	Nasal Corticosteroid- Beclomethasone	Beclomethasone	10 mg/mL
	Chloraseptic Throat Lozenge	Benzocaine, Menthol	2 mg/mL
	Antibiotic, Nasal Ointment	Mupirocin	10 mg/mL
	Relenza Antiviral Drug	Zanamivir	7.5 mg/mL
	Tamiflu Antiviral Drug	Oseltamivir	25mg/mL
	Antibiotic Systemic	Tobramycin	15 mg/mL

Substance		Description/Active Ingredient	Concentration*
Endogenous	Mucin	Purified Mucin Protein	2.5% w/v
	Human Blood	Blood	2% v/v

*Note: Concentrations shown are those used to saturate swabs before dosing contrived positive clinical samples with interfering substance. They are therefore representative of the level at the site of swab collection that can be tolerated.

Cross-contamination

The cross-contamination rate for the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay on the NeuMoDx Molecular 288 and 96 systems was determined by processing high positive and negative samples in an alternating "checkerboard" pattern. All samples consisted of simulated NP swab material, with positive samples spiked to ≥ 10^5 $TCID_{50}$/mL (or ≥ 10,000X LoD). Five sets of checkerboard testing was performed, ultimately producing a total of 60 negative replicates and 60 positive replicates on both the NeuMoDx 288 and 96 Molecular Systems. Across both system types, all 120 replicates of negative samples were accurately reported as negative, demonstrating the absence of cross-contamination during sample processing on the NeuMoDx Systems.

Turn Around Time

The Turn Around Time for processing 8 samples using the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay is determined to be ~85 minutes on N288 system and ~78 minutes on NeuMoDx 96 system for processing 4 samples.

Whole System Failure Rate

The Whole System Failure Rate for the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay was evaluated by testing 1 level of SARS-CoV-2 target at a concentration of ~3X LoD, prepared by spiking clinical negative nasopharyngeal swab specimens with the 1st WHO International Standard for SARS-CoV-2. A total of 200 replicates were processed using the Direct workflow on both the NeuMoDx 96 and 288 Molecular Systems (100 replicates per system). The failure rate was calculated at the percentage of false-negative results out of the total number of valid results obtained. The detection rate for the SARS-CoV-2 target in the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay was found to be 100% for both the NeuMoDx 96 and 288 Molecular Systems demonstrating a 0% failure rate across both systems.

System Robustness – Inhibition

The rate of inhibition was determined by calculating the Unresolved (sample process control not amplified in the absence of system error) rate across all the negative samples run throughout verification and validation studies. A total of 11 Unresolved results were obtained out of a total of 1221 negative samples processed, demonstrating a 0.9% inhibition rate for the NeuMoDx FluA/FluB/RSV/SARS-CoV-2 Assay.

REFERENCES

1. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th edition. HHS Publication No. (CDC) 21-1112, Revised December 2009.
2. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline – Fourth Edition. CLSI document M29-A4; May 2014.

TRADEMARKS

SYMBOL KEY

℞ only	Prescription use only		Do not re-use
	Manufacturer		Contains sufficient for <*n*> tests
IVD	*In vitro* diagnostic medical device		Consult instructions for use
EC REP	Authorized representative in the European Community		Caution
REF	Catalog number	CE	CE Mark
LOT	Batch code	CONT	Contains
	Use-by date	BIO	Contains biological material of animal origin
	Temperature limit		

NeuMoDx Molecular, Inc.
1250 Eisenhower Place
Ann Arbor, MI 48108, USA

Technical support / Vigilance reporting: support@qiagen.com

Patent: www.neumodx.com/patents

EC REP QIAGEN GmbH
QIAGEN Strasse 1
40724 Hilden
GERMANY
+49 2103 290

CE