NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Assay

Instructions For Use

For use under the Emergency Use Authorization (EUA) only

For in vitro diagnostic use

℞ only

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INTENDED USE

The NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay performed on the NeuMoDx 288 Molecular System and NeuMoDx 96 Molecular System (NeuMoDx Molecular System(s)), is a multiplex, in vitro real-time RT-PCR diagnostic test intended for simultaneous qualitative detection and differentiation of SARS-CoV-2, Influenza A virus, Influenza B virus, and/or Respiratory Syncytial Virus (RSV) RNA from nasopharyngeal (NP) or anterior nasal swab specimens collected in transport medium by a healthcare provider (HCP) from individuals suspected by a HCP of respiratory viral infection consistent with COVID-19. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza or RSV can be similar.

In the United States (US), testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform moderate or high complexity tests.

Results are for the identification and differentiation of RNA from SARS-CoV-2, Influenza A, Influenza B and/or RSV in humans. The NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay is not intended to detect Influenza C virus. RNA from SARS-CoV-2, Influenza A, Influenza B and/or RSV is generally detectable in nasopharyngeal and anterior nasal swab specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2, Influenza A, Influenza B and/or RSV RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. 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. Testing facilities within the U.S. and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.

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

The NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay is intended for use by qualified clinical laboratory personnel, specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY AND EXPLANATION OF THE ASSAY

NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay is a qualitative assay for the use on the NeuMoDx 96 and the NeuMoDx 288 instrument systems for the detection of the 2019 novel coronavirus (SARS-CoV-2), influenza A, influenza B, and/or RSV RNA in anterior nasal and nasopharyngeal swab samples. Nasopharyngeal or anterior nasal swab specimens are collected in Copan Universal Transport Medium (UTM-RT®) (Copan UTM-RT®, Copan, CA, USA), System, BD™ Universal Viral Transport System (UVT) (BD™ UVT, BD, NJ, USA), or Biologos Bio-VTM™ Viral Transport Media (VTM) (Bio-VTM™, Biologos LLC, IL, USA). The test uses an RNA Internal Sample Process Control (SPC2) that is added to each specimen during sample preparation and serves to monitor the entire sample preparation, reverse transcription and PCR amplification process. The NeuMoDx Flu A-B/RSV/SARS-CoV-2 Assay allows for two specimen processing workflows based on laboratory need, a direct workflow and a pre-treatment workflow. The NeuMoDx Molecular System automatically performs all the steps required to extract the target nucleic acid, prepare 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 Flu A-B/RSV/SARS-CoV-2 assay targets the conserved region of SARS-CoV-2 Nsp2 gene and regions in the M genes of influenza A, influenza B and respiratory syncytial virus genomes.

PRINCIPLES OF THE PROCEDURE

The NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Assay combines automated RNA extraction and amplification/detection of SARS-CoV-2, Flu A, Flu B, and/or RSV by real-time RT-PCR. Nasopharyngeal and anterior nasal swab samples are collected in the Copan UTM-RT® System, BD™ UVT System, or Biologos Bio-VTM™. 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 loaded 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 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 equal volume of NeuMoDx Vantage Viral Lysis Buffer (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 included 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 DNA 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 quantitative RT-PCR thermal cycler is directly proportional to the fluorophore released and can be correlated to the amount of target present.

TaqMan® probes are labeled with fluorophores at the 5' end and a dark quencher at the 3' end and are utilized to detect the viral targets. The fluorescent detection channel for each NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage 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).

Detection Channel

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

REAGENTS AND MATERIALS

Material Provided

REF	Contents	Units per package	Tests per unit	Tests per package
300900	**NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip** *Dried RT-PCR reagents containing Flu A-B/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
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 Tips (300 µL) with Filters**
235905	**Hamilton® CO-RE Tips (1000 µL) with Filters**

** Required only for direct processing of samples, without a pretreatment step. See "Instructions for Use" section below.*
*** Required only if a pretreatment step is desired prior to loading samples. See "Instructions for Use" section below.*

Swabs and Transport Media (Not Provided)

Sample Type	Recommended Collection Device	Recommended Swab
Nasopharyngeal or anterior Nasal Swab	3 mL Universal Transport Medium (Copan UTM-RT®, Copan, CA, USA), or 3 mL Universal Viral Transport System (BDTM UVT, BD, NJ, USA) or 3mL Bio-VTM™ Viral Transport Medium (Bio-VTMTM, Biologos LLC, IL, USA)	Flexible Minitip Nylon® Flocked Swab (Copan, CA, USA) or Flexible Minitip Flocked Swab (BD, NJ, USA)

Instrumentation Required (Not Provided)

NeuMoDx™ 288 Molecular System [REF 500100] or **NeuMoDx™ 96 Molecular System** [REF 500200]
NeuMoDx™ System Software version 1.8.3.5

WARNINGS AND PRECAUTIONS

- The NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Assay is for *in vitro* diagnostic use with NeuMoDx™ Systems under Emergency Use Authorization only.
- For Prescription Use Only.
- Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate or high complexity tests
- The NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Assay has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.

- The NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Assay has been authorized only for the detection and differentiation of nucleic acid from SARS-CoV-2, influenza A virus, influenza B virus, and/or Respiratory Syncytial Virus, not for any other viruses or pathogens.
- The emergency use of the NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Assay is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Results (positive and negative) for influenza and RSV should be interpreted with caution. If an influenza or RSV result is inconsistent with clinical presentation and/or other clinical and epidemiological information, FDA-cleared Influenza or RSV NAATs are available for confirmation if clinically indicated.
- Specimens should always be handled 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]
- Laboratories within the United States and its territories are required to report all results to the appropriate health authorities.
- 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. 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 transferring pipettes with aerosol barriers 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 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 SARS-CoV-2 Test Strip and NeuMoDx Extraction Plate, or the top surface of the NeuMoDx Lysis Buffer containers; handling of the consumables and reagents should be done by touching side surfaces only.
- Safety Data Sheets (SDS) are provided for each reagent (as applicable) at www.neumodx.com/client-resources.
- 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.
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state and local regulations.
- Use of the Flu A-B/RSV/SARS-CoV-2 Vantage Assay is limited to personnel who have been trained in the procedures of a molecular diagnostic assay and the NeuMoDx Molecular Systems.
- The instruments and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the positive controls or specimens must be controlled by good laboratory practices.

- Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination.
- Do not reuse.

PRODUCT STORAGE, HANDLING AND STABILITY

- NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strips are stable in the primary packaging through the stated expiration date on the immediate product label when stored at 4 to 28 °C.
- Do not use consumables and reagents past the stated expiration date.
- Do not use any test product if the primary or secondary packaging has been visually compromised.
- Do not reload any test product that has previously been loaded onto another NeuMoDx System.
- Once loaded, the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip may remain onboard the NeuMoDx System for 7 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 System.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

1. *Handle all specimens as if they are capable of transmitting infectious agents.*
2. Samples should be collected using the Copan UTM-RT® System, BD™ UVT System, or Bio-VTM™, using the validated nylon flocked swabs (see Swabs and Transport Media). In addition, flocked swabs, polyester and rayon swabs are acceptable swab types. Follow manufacturer instructions for collection, transport, and storage provided in the Copan UTM-RT® System, BD™ UVT System, or Bio-VTM™ System instructions for use as applicable.
    - Specimens collected in UVT, VTM, or equivalent can be stored up to 6 days at 2-8°C.
    - If refrigeration is not readily available, specimens should be transported on ice and stored at 2-8°C as soon as possible.
    - Specimens may remain on-board the NeuMoDx System for up to 12 hours prior to processing.
    - If unable to process specimens within 6 days, storage at -70 °C or colder is recommended.
    - If necessary, frozen specimens should be shipped using dry ice to maintain a frozen state until thawed for testing.

INSTRUCTIONS FOR USE

The NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay accommodates two different workflows, depending on user/laboratory preference:

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

-or-

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

Test Preparation – DIRECT Workflow for Direct Swab Samples

Note: Frozen specimens must be thawed completely and mixed well prior to processing.

1. Apply specimen barcode label to a specimen tube compatible with the NeuMoDx System as described under 2 and 3 below.
2. If testing the specimen in the primary collection tube, place the barcode-labeled tube into a 24-tube 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 compatible with the NeuMoDx System according to the volumes defined below:
   - Specimen Tuber Carrier (32-tube): 11 – 14 mm in diameter and 60 – 120 mm in height; minimum fill volume ≥ 550 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 Samples

Note: Frozen specimens must be thawed completely and mixed well prior to processing.

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 sample transport medium with an equal volume of NeuMoDx Vantage Viral Lysis Buffer (i.e., 1:1). Pretreatment can be performed in a secondary tube by combining an aliquot of the transport medium with an equal volume of NeuMoDx VVLB. The resulting mixture should meet the minimum volume requirements specified below.
2. Mix gently with pipette to ensure uniform distribution of NeuMoDx VVLB.
3. 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.
4. If using a secondary tube, transfer an aliquot of the transport medium to a barcoded specimen tube compatible with the NeuMoDx System and place into a Specimen Tube Carrier according to the minimum volumes defined below:
   - Specimen Tube Carrier (32-tube): 11 – 14 mm in diameter and 60 – 120 mm in height; minimum fill volume ≥ 700 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 Molecular System Operator's Manual; P/N 40600108

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

1. Populate the system carriers as necessary with the following consumables and use the touchscreen to load carrier(s) into the NeuMoDx System:
   - 1000 µL Pipette Tips
   - 300 µL Pipette Tips
   - NeuMoDx Cartridge
   - NeuMoDx Extraction Plate
   - NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip
   - NeuMoDx Lysis Buffer 2, as applicable based on workflow *(NOTE: remove foil seal from containers prior to loading)*
   - NeuMoDx Lysis Buffer 3, as applicable based on workflow *(NOTE: remove foil seal from containers prior to loading)*
2. Replace NeuMoDx Wash and NeuMoDx Release Reagents, and empty Priming Waste as necessary.
3. Empty Biohazard Waste Container (NeuMoDx 288 Molecular System only), Tip Waste Bin (NeuMoDx 96 Molecular System only), or Biohazard Waste Bin (NeuMoDx 96 Molecular System only) as necessary or when prompted by the NeuMoDx System software.
4. Populate one or more Test Strip Carrier(s) with NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip(s) and use the touchscreen to load the Test Strip Carrier(s) into the NeuMoDx System.
5. Load the test order onto the NeuMoDx System according to the workflow used for test preparation:
   - Untreated, neat swab samples prepared using the DIRECT workflow are tested by defining the sample as "**Transport Medium**".
   - Swab samples pretreated with VVLB using the PRETREATED workflow are tested by defining the specimen as "**User-Specified 1**"

If not defined in the test order, the Transport Medium specimen type (direct workflow), in a **Secondary Tube**, will be used as default.

6. Load the specimen(s) into a Specimen Tube Carrier and ensure caps and swabs (if applicable) are removed from all tubes.
7. 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 Flu A-B/RSV/SARS-CoV-2 Vantage Assay has only been evaluated for use on NeuMoDx Molecular Systems and with the reagents listed in the Material required section.
2. The NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay has been designed for detection of RNA from SARS-CoV-2, influenza A, influenza B, and/or RSV in clinician-collected nasopharyngeal, and anterior nasal swab samples collected with Copan UTM-RT® System, BD™ UVT System, or Biologos Bio-VTM™. Use of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay with other sample types has not been assessed and performance characteristics are unknown.
3. Anterior nasal swabs are considered acceptable specimen types for use with the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay but performance with this specimen type has not been established. Testing of anterior nasal swabs is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.
4. Reliable results depend on proper specimen collection, handling, and storage.
5. 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 Flu A-B/RSV/SARS-CoV-2 Vantage Assay.
6. If both the assay target(s) (SARS-CoV-2/Flu AB/RSV) 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. Deletions or mutations in the conserved regions targeted by the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay may affect detection and could lead to an erroneous result.
9. Presence of FluMist® nasal spray in nasopharyngeal specimens was not validated with NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay and could potentially interfere with Flu A-B/RSV/SARS-CoV-2 RNA detection and could lead to an erroneous result.
10. A positive result is indicative of the presence of SARS-CoV-2, influenza A, influenza B, SARS-CoV-2 and/or respiratory syncytial virus RNA but does not necessarily indicate the presence of viable, infectious SARS-CoV-2, influenza A, influenza B, and/or respiratory syncytial virus.
11. Negative results do not preclude infection with the SARS-CoV-2, influenza A, influenza B, and/or respiratory syncytial virus and should not be the sole basis of a patient treatment/management or public health decision. Follow up testing should be performed according to the current CDC recommendations.
12. Results from NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay should be used as an adjunct to clinical observations and other information available to the physician.
13. Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination.
14. Results (positive and negative) for influenza or RSV should be interpreted with caution. If an influenza or RSV result is inconsistent with clinical presentation and/or other clinical and epidemiological information, Results (positive and negative) for influenza or RSV should be confirmed by an FDA-cleared NAAT if clinically indicated.
15. The clinical performance has not been established in all circulating SARS-CoV-2 variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Assay Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website:

https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas

To assist clinical laboratories using the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay, the relevant Conditions of Authorization are listed below.

- Authorized laboratories[1] using the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay must perform the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay as outlined in the authorized Labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay are not permitted.
- Authorized laboratories that receive the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay must notify the relevant public health authorities of their intent to run the test prior to initiating testing.
- Authorized laboratories using the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories must collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and NeuMoDx Molecular Technical Support (techsupport@neumodx.com; 1-888-301-6639) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use the test in accordance with the authorized labeling.
- NeuMoDx Molecular, its authorized distributor(s) and authorized laboratories using the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] For ease of reference, the letter refers to, "laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform moderate and high complexity tests" as "authorized laboratories."

RESULTS

Available test results may be viewed or printed from the 'Results' tab in the Results window on the NeuMoDx System touchscreen. NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay results are automatically generated by the NeuMoDx System software using the decision algorithm and results processing parameters specified in the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay Definition File (Flu A-B-RSV SARS-CoV-2 ADF version 3.0.0 or higher). A NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay result may be reported as Negative, Positive, Indeterminate, No Result, or Unresolved based on the amplification status of the target and sample process control.

Criteria for a positive or negative call are specified in the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay Definition File (ADF) as installed on the NeuMoDx System. Results are reported based on the ADF decision algorithm, summarized in Table *1*, below.

Performance of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay for influenza A and B has not been established with a prospective clinical study. Results (positive and negative) for influenza should be confirmed by an FDA-cleared NAAT if clinically indicated.

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted and results must not be reported.

Table 1. NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay Results Interpretation

OVERALL RESULT	TARGET 1 (Flu B) FAM	TARGET 2 (Flu A) 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 < 28 AND EPR > 1.5 AND EP ≥ 600] OR (28 ≤ Ct ≤37 AND EP ≥ 600)	N/A	N/A	N/A	N/A	Flu B RNA Detected
	N/A	**AMPLIFIED** [5 ≤ Ct < 28 AND EPR > 2.0 AND EP ≥ 750] OR [28 ≤ Ct ≤ 37 AND EP ≥ 750]	N/A	N/A	N/A	Flu A 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 < 28 AND	**NOT AMPLIFIED** N/A **OR** (5 ≤ Ct < 28 AND	**NOT AMPLIFIED** N/A **OR**	**NOT AMPLIFIED** N/A **OR**	**AMPLIFIED** (24≤ Ct ≤31 AND	Flu A, Flu B, RSV, and SARS-CoV-2 RNA not detected

OVERALL RESULT	TARGET 1 (Flu B) FAM	TARGET 2 (Flu A) HEX	TARGET 3 (SARS-CoV-2) TX RED	TARGET 4 (RSV) Far Red	PROCESS CONTROL (SPC2) Red	INTERPRETATION
	EPR ≤ 1.5) **OR** (28 ≤ Ct ≤ 37 AND EP < 600) **OR** (Ct > 37)	EPR ≤ 2.0) **OR** (28 ≤ Ct ≤ 37 AND EP < 750) **OR** (Ct > 37)	(5 ≤ Ct < 25 AND EPR ≤ 1.5) **OR** (25 ≤ Ct ≤ 37 AND EP < 1200) **OR** (Ct > 37)	(5 ≤ Ct < 30 AND EPR ≤ 1.15) **OR** (30 ≤ Ct ≤ 37 AND EP < 1200) **OR** (Ct > 37)	EP ≥1800)	
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 Flu A-B/RSV/SARS-CoV-2 Vantage 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 with residual swab in viral transport medium 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 with residual swab in viral transport medium 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 with residual swab in viral transport medium 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 (P/N: 40600108) or the NeuMoDx 96 Molecular System Operator's User Manual (P/N: 40600317) for a list of error codes that may be associated with Invalid Results.

Quality Control

The Clinical Laboratory Improvement Amendments (CLIA) regulations specify that the laboratory is responsible for having 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, FDA-cleared or approved test system (42 CFR § 493.1256).

1. Control materials are not provided with the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay. However, the following control material were validated by NeuMoDx and are recommended. Controls must meet the same minimum volume specifications as clinical samples specified above based on the Specimen Tube Carrier size.

   - Positive Controls (1 mL per control):
     - 5 µL RSV Rapid Control Pack (Zeptometrix, Cat #: KZMC034)

- 5 µL NATrol Influenza A/B Positive Control (Zeptometrix, Cat #: MDZ046)
- Heat-inactivated SARS-CoV-2 virus (ATCC, VR-1986HK) at a final concentration of 1000 cp/mL
- BD™ Universal Viral Transport Medium (UVT) or equivalent to final volume of 1 mL

- Negative Control: Copan/BD™ Universal Viral Transport Medium (UVT, BD, NJ) or equivalent.

2. It is recommended that users process one set of positive and negative controls every 24 hours and prior to processing patient samples.
3. When processing User-Defined Controls, place the labeled controls in a specimen tube carrier and use the touchscreen to load the carrier into NeuMoDx System from the autoloader shelf. Once defined, the NeuMoDx System will recognize the barcodes and start processing controls.
4. 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. The primers and probe specific for the Sample Process Control (SPC2) are included in each NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip. This Sample Process Control allows the NeuMoDx System to monitor the efficacy of the RNA extraction and RT-PCR amplification processes.
5. 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.
6. The NeuMoDx System software continuously monitors on-board sensors and actuators to ensure a safe and effective operation of the System.
7. Multiple fluidic error recovery modes are implemented by active monitoring of aspiration and dispense 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.
8. 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.
9. 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.
10. A negative result reported for a positive control sample may indicate there is a reagent or NeuMoDx System related problem. Please refer to *NeuMoDx™ 288 or 96 Molecular System Operator's Manual* for troubleshooting tips.
11. If the User-Defined (External) Controls do not provide the expected results, it is recommended to repeat a set of positive and negative controls. Patient results should not be reported if controls do not give expected results.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The Analytical Sensitivity of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage 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 2*. First, a dilution series using model strains of each target in UVT were prepared with the Pretreated Workflow and then processed by the NeuMoDx System to determine a preliminary Limit of Detection (LoD) value. In the second part of testing, this preliminary LoD value was 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 3* while *Table 4* details the hit-rate confirmation for the N288 System and *Table 5* details the hit-rate confirmation for the N96 System.

Table 2. Strain Used for Each Target

Target/Strain	Source	Cat #	Lot #	Format
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	Virapur	N/A	B1904J	Live Crude
Flu A, Michigan/272/2017 pdm09 (H1N1)	IRR	FR-1615	70015567	Culture Fluid
Flu B, Colorado/6/2017 (Victoria)	IRR	FR-1592	70013310	Culture Fluid
Flu B, Florida/78/2015 (Yamagata)	ATCC	VR-1931	70020870	Culture Fluid
RSV A2	ATCC	VR-1540	60430286	Crude Culture
RSV B (WV/14617/85)	ATCC	VR-1400	70013461	Culture Fluid
SARS-CoV-2, Isolate USA-WA1/2020	ATCC	VR-1986HK	70034006	Heat inactivated virus
SARS-CoV-2, Isolate Italy-INMI1	BEI	NR-52498	70035261	genomic RNA

Table 3. Positive Detection Rates for Preliminary LoD Determination of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay

Target/Strain	Level	Unit	# Valid Results/Total (n/N)	# Positives	% Detection	Avg Ct	Ct SD
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	**0.5**	TCID$_{50}$/mL	10/10	10	**100%**	31.39	0.89
	0.25		10/10	9	90.0%	31.90	0.84
Flu A, Michigan/272/2017 pdm09 (H1N1)	**0.5**		10/10	10	**100%**	33.07	0.44
	0.25		10/10	8	80.0%	33.48	0.44
Flu B, Colorado/6/2017 (Victoria)	0.25		9/11	9	100%	31.00	0.13
	0.05		10/10	10	100%	32.56	0.33
	0.01		10/10	10	**100%**	33.52	0.45
Flu B, Florida/78/2015 (Yamagata)	**0.25**		10/10	10	**100%**	33.01	0.40
	0.1		10/10	9	90.0%	33.28	0.76
RSV A2	**0.5**		10/11	10	**100%**	31.31	0.27
	0.25		11/11	10	90.9%	31.52	0.40
RSV B (WV/14617/85)	0.25		10/10	10	100%	26.52	0.16
	0.05		9/10	9	**100%**	29.06	0.42
SARS-CoV-2, Isolate USA-WA1/2020	300	copies/mL	6/6	6	100%	33.73	0.30
	200		6/6	6	100%	34.06	0.69
	150		6/6	6	**100%**	33.77	0.39
	100		6/6	5	83.3%	33.20	0.38

Table 4. Positive Detection Rates for Confirmatory LoD Determination for the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay – N288, (a) Pretreated Workflow; (b) Direct Workflow

(a) Pretreated Workflow

Target/Strain	Level	# Valid Results/Total (n/N)	# Positives	% Detection	Avg Ct	Ct SD
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	**0.5 TCID$_{50}$/mL**	24/24	24	**100%**	31.98	1.14
Flu A, Michigan/272/2017 pdm09 (H1N1)	**0.5 TCID$_{50}$/mL**	23/26	23	**100%**	32.92	0.56
Flu B, Colorado/6/2017 (Victoria)	**0.01 TCID$_{50}$/mL**	24/24	24	**100%**	33.49	0.57
Flu B, Florida/78/2015 (Yamagata)	**0.25 TCID$_{50}$/mL**	24/24	24	**100%**	32.59	0.49
RSV A2	**0.25 TCID$_{50}$/mL**	21/22	21	**100%**	31.37	0.60
RSV B (WV/14617/85)	**0.05 TCID$_{50}$/mL**	22/22	22	**100%**	29.58	0.36
SARS-CoV-2, Isolate USA-WA1/2020	**150 copies/mL**	23/24	23	**100%**	33.95	0.33
SARS-CoV-2, Isolate Italy-INMI1	**150 copies/mL**	23/27	23	**100%**	33.89	0.57

(b) Direct Workflow

Target/Strain	Level	# Valid Results/Total (n/N)	# Positives	% Detection	Avg Ct	Ct SD
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	**0.5 $TCID_{50}$/mL**	24/24	24	**100%**	32.12	0.53
Flu A, Michigan/272/2017 pdm09 (H1N1)	**0.5 $TCID_{50}$/mL**	24/24	24	**100%**	32.60	0.33
Flu B, Colorado/6/2017 (Victoria)	**0.01 $TCID_{50}$/mL**	24/24	23	**95.8%**	33.31	0.45
Flu B, Florida/78/2015 (Yamagata)	**0.25 $TCID_{50}$/mL**	24/24	24	**100%**	32.46	0.33
RSV A2	0.25 $TCID_{50}$/mL	24/24	11	45.8%	30.41	4.74
	0.5 $TCID_{50}$/mL	24/24	9	37.5%	32.00	0.47
	1 $TCID_{50}$/mL	24/24	24	**100%**	31.35	1.37
RSV B (WV/14617/85)	**0.05 $TCID_{50}$/mL**	24/24	24	**100%**	30.31	0.66
SARS-CoV-2, Isolate USA-WA1/2020	**250 copies/mL**	24/24	23	**95.8%**	33.64	0.48
SARS-CoV-2, Isolate Italy-INMI1	**250 copies/mL**	23/24	23	**100%**	33.25	0.32

Table 5. Positive Detection Rates for Hit-Rate Confirmation of LoD for the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay – N96, (a) Pretreated Workflow; (b) Direct Workflow

(a) Pretreated Workflow

Target/Strain	Level	# Valid Results/Total (n/N)	# Positives	% Detection	Avg Ct	Ct SD
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	**0.5 $TCID_{50}$/mL**	24/24	23	**95.8%**	32.75	0.45
Flu A, Michigan/272/2017 pdm09 (H1N1)	**0.5 $TCID_{50}$/mL**	22/25	21	**95.5%**	32.61	0.61
Flu B, Colorado/6/2017 (Victoria)	**0.01 $TCID_{50}$/mL**	24/24	23	**95.8%**	33.08	0.85
Flu B, Florida/78/2015 (Yamagata)	**0.25 $TCID_{50}$/mL**	24/24	24	**100%**	32.67	0.56
RSV A2	**0.25 $TCID_{50}$/mL**	22/22	22	**100%**	31.41	0.46
RSV B (WV/14617/85)	**0.05 $TCID_{50}$/mL**	22/23	22	**100%**	30.06	0.74
SARS-CoV-2, Isolate USA-WA1/2020	**150 copies/mL**	23/24	22	**95.7%**	33.81	0.69
SARS-CoV-2, Isolate Italy-INMI1	**150 copies/mL**	24/24	23	**95.8%**	33.36	0.53

(b) Direct Workflow

Target/Strain	Level	# Valid Results/Total (n/N)	# POS	% Detection	Avg Ct	Ct SD
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	**0.5 $TCID_{50}$/mL**	24/24	23	**95.8%**	32.14	32.14
Flu A, Michigan/272/2017 pdm09 (H1N1)	**0.5 $TCID_{50}$/mL**	23/24	23	**100%**	32.55	32.55
Flu B, Colorado/6/2017 (Victoria)	**0.01 $TCID_{50}$/mL**	24/24	24	**100%**	32.95	32.95
Flu B, Florida/78/2015 (Yamagata)	**0.25 $TCID_{50}$/mL**	24/24	23	**95.8%**	31.72	31.72
RSV A2	0.25 $TCID_{50}$/mL	24/24	13	54.2%	29.92	29.92
	0.5 $TCID_{50}$/mL	24/24	5	20.8%	30.13	30.13
	1 $TCID_{50}$/mL	23/24	23	**100%**	31.32	31.32
RSV B (WV/14617/85)	**0.05 $TCID_{50}$/mL**	24/24	23	**95.8%**	30.43	30.43
SARS-CoV-2, Isolate USA-WA1/2020	**250 copies/mL**	23/26	23	**100%**	33.54	33.54
SARS-CoV-2, Isolate Italy-INMI1	**250 copies/mL**	23/23	22	**95.7%**	33.30	33.30

The levels accepted as the LoD values for the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay on the NeuMoDx Systems are summarized in *Table 6*.

The Limit of Detection of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay is claimed to be 0.5 $TCID_{50}$/mL for Flu A, 0.25 $TCID_{50}$/mL for Flu B, 1.0 $TCID_{50}$/mL for RSV A, 0.05 $TCID_{50}$/mL for RSV B and 250 copies/mL for SARS-CoV-2.

Table 6. Summary of Limit of Detection Study

Target	Strain	Limit of Detection		
		Pretreated Workflow	Direct Workflow	Unit
Influenza A (Flu A) – H3N2	Singapore/INIFMIH-16-0019/2016	0.5	0.5	$TCID_{50}$/mL
Influenza A (Flu A) – H1N1	Michigan/272/2017 pdm09	0.5	0.5	
Influenza B (Flu B) – Victoria lineage	Colorado/6/2017	0.01	0.01	
Influenza B (Flu B) – Yamagata lineage	Florida/78/2015	0.25	0.25	
RSV A	A2	0.25	1	
RSV B	(WV/14617/85)	0.05	0.05	
SARS-CoV-2	Isolate USA-WA1/2020	150	250	copies/mL
SARS-CoV-2	Isolate Italy-INMI1	150	250	

Co-formulated Limit of Detection

A co-formulated LoD study was performed for the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay run on the NeuMoDx 288 Systems using Direct and Pretreated workflows. Two pools containing the combinations of strains used in the LoD studies were prepared and tested. Analytes were spiked together into negative NP clinical matrix from individuals each at their respective 1X LoD concentration and tested with 20 individual replicates to demonstrate a minimum of 95% detection rate for each analyte. All targets were detected at a minimum of 95% positivity in these pools using both workflows.

Table 7. Summary of Co-formulated LOD Study for the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay run on the NeuMoDx 288 Systems using Direct and Pretreated workflows

Pool Targets + Levels	Workflow	Target	# Valid results (n/N)	# Pos	% Detection	Ct Avg	Ct SD
Pool A: Flu A Singapore: 0.5 TCID50/mL Flu B Colorado: 0.01 TCID50/mL RSV A: 0.25 TCID50/mL RSV B: 0.05 TCID50/mL SARS-CoV-2: 150 cp/mL	Pretreated	Flu A	20/20	20	100%	31.9	0.71
		Flu B	20/20	19	95.0%	32.8	0.59
		SARS-CoV-2	20/20	19	95.0%	33.3	0.79
		RSV	20/20	20	100%	30.9	1.10
Pool A: Flu A Singapore: 0.5 TCID50/mL Flu B Colorado: 0.01 TCID50/mL RSV A: 1 TCID50/mL RSV B: 0.05 TCID50/mL SARS-CoV-2: 250 cp/mL	Direct	Flu A	20/20	20	100%	31.6	0.34
		Flu B	20/20	19	95.0%	32.6	0.64
		SARS-CoV-2	20/20	20	100%	32.9	0.42
		RSV	20/20	20	100%	30.7	0.58
Pool B: Flu A Michigan: 0.5 TCID50/mL Flu B Florida: 0.25 TCID50/mL RSV A: 0.25 TCID50/mL RSV B: 0.05 TCID50/mL SARS-CoV-2: 150 cp/mL	Pretreated	Flu A	20/20	19	95.0%	31.5	0.84
		Flu B	20/20	20	100%	32.1	0.66
		SARS-CoV-2	20/20	19	95.0%	32.8	0.78
		RSV	20/20	20	100%	30.1	0.96

Pool B: Flu A Michigan: 0.5 TCID50/mL Flu B Florida: 0.25 TCID50/mL RSV A: 1 TCID50/mL RSV B: 0.05 TCID50/mL SARS-CoV-2: 250 cp/mL	Direct	Flu A	20/20	20	100%	31.9	0.33
		Flu B	20/20	20	100%	32.2	0.25
		SARS-CoV-2	20/20	20	100%	32.9	0.29
		RSV	20/20	20	100%	30.6	0.78

Analytical Reactivity

The analytical reactivity of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay was evaluated against multiple strains/isolates of Influenza A, Influenza B, Respiratory Syncytial Viruses and SARS-CoV-2. Viral strains/isolates were tested in minimum 20 replicates. A total of 24 Flu A strains, 6 Flu B strains, 3 RSV A isolates, 2 RSV B isolates, and 4 isolates of SARS-CoV-2 were tested (*Table 8)*.

Table 8. Flu A, Flu B, RSV A, RSV B, and SARS-CoV-2 Strains Tested

Target	Strain		Concentration	% Pos
Flu A	H1N1	Brisbane/02/2018	1 $TCID_{50}$/mL	95.5%
		California/07/2009	1 $TCID_{50}$/mL	100%
		California/07/2009 NYMC X-179A (H1N1)pdm09	18 $TCID_{50}$/mL	95.5%
		Louisiana/08/2013 pdm 09, AVR Reference Strain, M2: S31N, NA: H275Y	8 $TCID_{50}$/mL	100%
		New York/18/2009 (H1N1)pdm09	6 $TCID_{50}$/mL	100%
		Guangdong-Moanan/SWL 1536/2019	1 $TCID_{50}$/mL	100%
	H2N2	A2/Japan/305/57	32.6 pg/mL	100%
		Korea/426/68 (HA, NA) x A/PR/8/34	6.25 pg/mL	100%
	H3N2	Hong Kong/4801/2014	0.5 $TCID_{50}$/mL	100%
		Hong Kong/2671/2019	0.5 $TCID_{50}$/mL	100%
		Switzerland/9715293/2013	0.5 $TCID_{50}$/mL	100%
		Kansas/14/2017 (H3N2)	8 $TCID_{50}$/mL	100%
		Texas/50/2012 (H3N2)	4 $TCID_{50}$/mL	100%
		Wisconsin/15/2009 (H3N2)	0.5 $TCID_{50}$/mL	95.5%
	H5N1 - H5N3	chicken/Vietnam/NCVD-016/2008(H5N1)-PR8-IDCDC-RG12	1:50,000*	100%
		Egypt/N03072/2010(H5N1)-PR8-IDCDC-RG29	1;100,000*	100%
		Hubei/1/2010(H5N1)-PR8-IDCDC-RG30	1:10,000*	100%
		Duck/Pennsylvania/10218/84 (H5N2)	2.55 pg/mL	100%
		pheasant/New Jersey/1355/1998(H5N2)-PR8-IBCDC-4	1:50,000*	100%
		Duck/Singapore/645/97 (H5N3) V-331-0E5-271	24.8 pg/mL	100%
	H7N2, H7N7, H7N9	A/turkey/Virginia/4529/2002 (H7N2) x PR8-IBCDC-5	1:100,000*	95.5%
		A/mallard/Netherlands/12/2000(H7N7)/PR8-IBCDC-1, genomic RNA	1:100,000*	100%
		A/Anhui/1/2013 (H7N9)	1:100,000*	100%
	H10N7	A/Chick/Germany/N/49 (H10N7)	68 pg/mL	100%
Flu B	Victoria	Brisbane/60/2008	1 $TCID_{50}$/mL	100%
	Victoria	Malaysia/2506/2004	3 $TCID_{50}$/mL	100%
	Yamagata	Phuket/3703/2013	0.5 $TCID_{50}$/mL	95.2%
	N/A	Virginia/ATCC5/2012	0.02 pfu/mL	100%
	Victoria	Washington/02/2019	5 $TCID_{50}$/mL	100.0%
	Yamagata	Wisconsin/1/2010	0.05 $CEID_{50}$/mL	95.5%
RSV	RSV A	A (long)	2 pfu/mL	95.5%

Target	Strain		Concentration	% Pos
		A2001/3-12	8 $TCID_{50}$/mL	100%
		A2001/2-20	8 $TCID_{50}$/mL	100%
	RSV B	B, 9320	0.1 pfu/mL	100%
		B1	4 $TCID_{50}$/mL	100%
SARS-CoV-2		USA-IL1/2020	250 cp/mL	95.5%
		USA-AZ1/2020	250 cp/mL	100%
		USA-CA3/2020	250 cp/mL	100%
		Hong Kong/VM20001061/2020	250 cp/mL	100%

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

The analytical reactivity of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Assay in detection of different clinical isolates of SARS-CoV-2 was assessed by performing an *in silico* analysis with the primers and probes of the assay against all the sequences available in GenBank (as of August 12, 2020) 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.

In Silico Analysis

SARS-CoV-2

The ability of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Assay to detect different clinical isolates of SARS-CoV-2 was demonstrated by performing an *in-silico* analysis with the primers and probe of the assay target against all the sequences available in GenBank, using NCBI Basic Local Alignment Search Tool (BLAST). Nearly 10,000 sequences were used for the analysis, of which, 54 sequences were excluded due to either fragmented sequences or ambiguous nucleotides present in the sequences. As shown, the PCR primers and probe designed for the detection of SARS-CoV-2 in the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay had over 98% homology to all the 9943 valid sequences analyzed. Among those, over 98% sequences had 100% homology to the primers and probe. Less than 2% of sequences had one mismatch in either one of the primers, or the probe. More detailed analysis showed that over 50% of the mismatches were located at the 5' end of the primers or the probe, which had the least impact on the stability of the hybridization of the primers or probe to their targeted sequences. There were no instances of having one mismatch present in both the primers and probe at the same time in one genome sequence. Therefore, the impact of these mismatches on the sensitivity of the assay is very minimal. Thus, the *in silico* analysis illustrated that the primers and probe specific for SARS-CoV-2 in the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay showed > 95% homology to the SARS-CoV-2 isolates available on the GenBank (as of Aug 12, 2020).

Table 9. Primers and Probe Used in the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay for the SARS-CoV-2 Sequences – In silico BLAST results Summary

Total Seq analyzed, 9943	Forward Primer	Reverse Primer	Probe
# of Seq with 100% Homology	9790	9924	9913
% Seq with 100% Homology	98.5%	99.8%	99.7%
# of Seq with 96% Homology	153	19	30
% Seq with 96% Homology	1.5%	0.2%	0.3%
Total % of Seq with > 95% Homology	100%	100%	100%

An additional inclusivity study was performed (as of Feb 5, 2021) to cover the new SARS-CoV-2 variants from US, Brazil and South Africa. These results are summarized below.

UK Variant

The recently emerging UK variant, **B.1.1.7** contains a mutation in the **ORF1ab** region. This mutation is in the region of the *reverse* primer used in the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Assay and leads to a 96% match of the reverse primer. The mismatch, **C193T,** is located in the *middle of the primer* resulting in a G-T mismatch, rather at the 3' end of the primer and thereby is not expected to pose a significant threat to amplification efficiency of the SARS target in the NeuMoDx assay.

In general, there is empirical evidence in the literature that, in 5' nuclease assays such as those used by the NeuMoDx assay, a **G-T** mismatch results typically only *minor Ct shift* compared to other more severe type of mismatches such at **A–A**, **G–A**, **A–G**, and **C–C**, in PCR primers (Ralph Stadhouders et al, *The Effect of Primer-Template Mismatches on the Detection and Quantification of Nucleic Acids Using the 5' Nuclease Assay*. J Mol Diagn. 2010 Jan; 12(1): 109–117). The T_m of the primers, used in the NeuMoDx assay, was specifically designed to be a 2-3 degrees higher than the T_m normally recommended for qPCR to compensate for any potential single mismatches.

Based on the literature support, analysis and the robust design of the qPCR assay, it is expected that the single mismatch in *one of the primers,* observed in the UK variant may only cause a minor Ct shift. However, the impact on the detection of SARS-CoV-2 in symptomatic patients is expected to be insignificant.

Brazil Variant

The recent Brazil variant**, P.1 lineage** has 17 unique amino acid changes including one (**T733C**) in the ORF1ab region. However, this mutation is *outside* the targeting sequence of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Assay that targets the region 830-924 of RNA genome sequence (NC_045512). Consequently, this strain is not anticipated to impose any adverse effect on the performance of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Assay.

South Africa Variant

The South Africa variant (**501Y.V2**), also known as **20H/501Y.V2, B.1.351** lineage has a few mutations in the ORF1ab region. However, *none* of the mutations in the ORF1ab fall into the region between base pairs 830-924 which is the targeted region of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 assay. Consequently, this strain is not anticipated to impose any adverse effect on the performance of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Assay.

Analytical Specificity and Cross-Reactivity

The analytical specificity of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage 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 approximately 6E6 CFU/mL or IFU/mL, except where noted. Viruses were tested at concentrations of 1E5 to 1E6 $TCID_{50}$/mL or copy/mL, except where otherwise noted. Analytical specificity of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage assay was 100% for Flu A, Flu B, RSV A, RSV B and SARS-CoV-2 (*Table 10*).

Table 10. Analytical Specificity Results

Organism	Test Concentration	Flu A	Flu B	RSV A	RSV B	SARS-CoV-2
Adenovirus Type 1	1E6 $TCID_{50}$/mL	-	-	-	-	-
Adenovirus Type 7	1E6 $TCID_{50}$/mL	-	-	-	-	-
Bordetella pertussis I176	10 ng/mL	-	-	-	-	-
Candida albicans	6E6 CFU/mL	-	-	-	-	-

Organism	Test Concentration	Flu A	Flu B	RSV A	RSV B	SARS-CoV-2
Chlamydia pneumoniae	6E6 IFU/mL	-	-	-	-	-
Corynebacterium xerosis	6E6 CFU/mL	-	-	-	-	-
EBV	1E6 cp/mL	-	-	-	-	-
Escherichia coli	6E6 CFU/mL	-	-	-	-	-
Hemophilus influenzae	6E6 CFU/mL	-	-	-	-	-
HHV 6A	1E6 cp/mL	-	-	-	-	-
HHV 7	1E6 cp/mL	-	-	-	-	-
HHV8	1E6 cp/mL	-	-	-	-	-
HSV1	1E6 cp/mL	-	-	-	-	-
HSV2	1E6 cp/mL	-	-	-	-	-
Human Coronavirus 229E	1E5 $TCID_{50}$/mL	-	-	-	-	-
Human coronavirus HKU1	1E6 cp/mL	-	-	-	-	-
Human coronavirus NL63	1E5 $TCID_{50}$/mL	-	-	-	-	-
Human Coronavirus OC43	5E3 $TCID_{50}$/mL	-	-	-	-	-
Human Enterovirus 68	1E6 $TCID_{50}$/mL	-	-	-	-	-
Human Metapneumovirus	1E6 $TCID_{50}$/mL	-	-	-	-	-
Human Parainfluenza Type 1	1E5 $TCID_{50}$/mL	-	-	-	-	-
Human Parainfluenza Type 2	1E5 $TCID_{50}$/mL	-	-	-	-	-
Human Parainfluenza Type 3	1E6 $TCID_{50}$/mL	-	-	-	-	-
Human Rhinovirus Type 1A	1E5 $TCID_{50}$/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	1E5 $TCID_{50}$/mL	-	-	-	-	-
MERS-coronavirus EMC/2012	0.5 ng/mL	-	-	-	-	-
Moraxella catarrhalis	6E6 CFU/mL	-	-	-	-	-
Mumps Virus	1E5 $TCID_{50}$/mL	-	-	-	-	-
Mycobacterium tuberculosis	10 ng/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 pfu/mL	-	-	-	-	-
Staphylococcus aureus	1E6 CFU/mL	-	-	-	-	-
Staphylococcus epidermidis	6E6 CFU/mL	-	-	-	-	-
Streptococcus pneumonia	6E6 CFU/mL	-	-	-	-	-
Streptococcus pyogenes	6E6 CFU/mL	-	-	-	-	-
Streptococcus salivarius	6E6 CFU/mL	-	-	-	-	-
Flu A (Michigan/272/2017 pdm09 (H1N1)	3x LoD	+	-	-	-	-
Flu B, Florida/78/2015 (Yamagata)	3x LoD	-	+	-	-	-
RSV A, A2	3x LoD	-	-	+	-	-
RSV B (WV/14617/85)	3x LoD	-	-	-	+	-
SARS-CoV-2, USA-WA1/2020	3x LoD	-	-	-	-	+
Negative Control (No Pathogens)	N/A	-	-	-	-	-

Interfering Organisms (Non-Target)

The NeuMoDx Flu A-B/RSV/SARS-CoV-2 Assay was tested for potential 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 10*, above. No interference on the detection of any target was observed with any of the commensal organisms tested in this study.

Competitive Interference for Target Organisms: Flu A-B, RSV, and SARS-CoV-2

Competitive interference of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay was evaluated using panels of viral targets spiked in clinical negative NP swab specimens collected in UVT. Each panel contained one or two targets near their Limit of Detection (3-5X LoD) and a single target at ≥ 1000X of its LoD (≥1E5 copies/mL), representing the coinfected target. The presence of two to three viruses at varying concentrations in a single specimen had no effect on the analytical sensitivity, as shown in *Table 11*.

Table 11. *Summary of Competitive Interference Study*

Panel	Target	Target Conc.	Target Concentration ($TCID_{50}$/mL)	Target Concentration (Cp/mL)	Valid Results	# Pos	% Detection
1	Flu A	3X	1.5	2352	5	5	100%
	RSV A	3X	3	1740	5	5	100%
	Flu B	1000X	250	1.48E5	5	5	100%
2	Flu A	3X	1.5	2352	5	5	100%
	RSV A	5X	5	2900	5	5	100%
	SARS-CoV-2	1000X	N/A	2.5E5	5	5	100%
3	Flu A	3X	1.5	2352	5	5	100%
	SARS-CoV-2	3X	N/A	750	5	5	100%
	RSV A	1000X	1000	5.8E5	5	5	100%
4	Flu B	3X	0.75	446	5	5	100%
	RSV B	3X	0.15	ND*	5	5	100%
	Flu A	1000X	500	7.84E5	5	5	100%
5	Flu B	3X	0.75	446	5	5	100%
	RSV B	3X	0.15	ND	5	5	100%
	SARS-CoV-2	1000X	N/A	2.5E5	5	5	100%
6	Flu B	3X	0.75	446	5	5	100%
	RSV A	1000X	1000	5.8E5	5	5	100%
7	SARS-CoV-2	3X	N/A	750	5	5	100%
	Flu A	1000X	500	7.84E5	5	5	100%
8	SARS-CoV-2	3X	N/A	750	5	5	100%
	Flu B	1000X	250	1.48E5	5	5	100%
9	RSV A	3X	3	1740	5	5	100%
	Flu A	1000X	500	7.84E5	5	5	100%
10	RSV B	3X	0.15	ND	5	5	100%
	Flu B	1000X	250	1.48E5	5	5	100%

* ND = not determined

Interfering Substances – Endogenous/Exogenous

The NeuMoDx Flu A-B/RSV/SARS-CoV-2 Assay was evaluated for susceptibility to interference caused by substances potentially associated with the collection of nasopharyngeal or anterior nasal swab specimens. Residual clinical negative NP swab specimens in UVT were individually spiked with Flu A, Flu B, RSV A, RSV B, or SARS-CoV-2 at each organisms 3X LoD concentration and processed in the presence and absence of the agents shown in *Table 12*. Replicates of 3 were tested for each target for a total of 15 replicates per substance. All negative replicates returned a not-detected result and all positive replicates returned a positive result. None of the substances included in the testing had an adverse effect on the assay performance for any of the targets.

Table 12. Substances Tested for Interference

	Substance	Description/Active Ingredient	Concentration*
Exogenous	Neo-Synephrine	Phenylephrine	15% v/v
	Afrin Nasal Spray	Oxymetazoline	15% w/v
	Saline Nasal Spray	Sodium chloride with preservatives	15% v/v
	Zicam Nasal Spray	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	25 mg/mL
	Antibiotic, systemic	Tobramycin	1.5 mg/mL
Endogenou	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.

Specimen Storage Stability and Onboard Stability

Specimen storage stability was evaluated for nasopharyngeal (NP) swab specimens in UVT and VTM stored at 2-8˚C for up to 6 days. Positive specimens were contrived by spiking pooled residual deidentified clinical negative NP swab in UVT or VTM with each individual target at 3X LoD. Negative samples consisted of pooled residual negative NP swab in either UVT or VTM. Replicates of 10 positive specimens for each target and 10 negative specimens were tested at days 0, 4 (SARS-CoV-2 positive and negative specimens only), and 7.

Onboard specimen stability was evaluated following 4 days (SARS-CoV-2 samples only) and 7 days of storage at 2-8˚C. Replicates of 10 positive specimens for each target and 10 negative specimens were tested after being stored onboard the NeuMoDx Molecular System for 8 or 12 hours.

All positive and negative specimens were correctly identified using the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay after storage at 2-8˚C for 6 days followed by 12 hours onboard the NeuMoDx Molecular System. The study supports the recommended specimen storage conditions at refrigerated (2-8°C) temperatures for up to six days and for up to 12 hours when stored onboard the NeuMoDx Molecular Systems.

Collection Media Equivalency

The equivalency among the Copan UTM-RT® System, BDTM UVT System, or Biologos Bio-VTM™ were evaluated using panels of contrived samples containing a representative strain (or isolate) of Flu A, Flu B, RSV A, RSV B, or SARS-CoV-2 in pooled, negative NP swab specimens in the different collection media at near-LoD levels. The results showed no difference among swabs collected in the

different media tested when processed using the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay on the NeuMoDx Molecular Systems. These results demonstrated that swabs collected in Copan UTM-RT® System, BD™ UVT System, or Biologos Bio-VTM™ have no impact on the performance of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay.

Fresh vs. Frozen Matrix Equivalency

The equivalency between fresh and frozen NP swab samples was evaluated using panels of contrived samples containing a representative strain (or isolate) of Flu A, Flu B, RSV A, RSV B, or SARS-CoV-2 in pooled, negative NP swab specimens in UVT at near-LoD levels. The results showed no difference between fresh and frozen swab samples when processed using the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay on the NeuMoDx Molecular Systems. These results demonstrate that freezing of nasopharyngeal swabs collected in UVT has no impact on the performance of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay.

Cross-Contamination

The cross-contamination rate for the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay was determined by processing five runs of alternating high titer positive and negative samples on both the NeuMoDx 96 and NeuMoDx 288 Molecular Systems in a checkerboard configuration. Each target (Flu A, Flu B, RSV A, RSV B, and SARS-CoV-2) was processed individually as the representative "high" target for each run of 24 samples per System. All negative sample replicates were reported as negative, demonstrating no evidence of cross-contamination throughout sample processing on the NeuMoDx 96 and NeuMoDx 288 Molecular Systems.

Precision

Within-Lab Precision of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay was assessed on the NeuMoDx 96 and 288 Molecular Systems using a 16-member test panel prepared by individually spiking Flu A, Flu B, RSV A, RSV B, and SARS-CoV-2 targets at varying concentrations into sterile VTM. Within-Lab Precision was assessed using one NeuMoDx 96 Molecular System and two NeuMoDx 288 Molecular Systems over 12 days of testing, with two runs of testing per day. Detection rate was calculated for all targets in each panel member and a qualitative analysis was performed for the total number of replicates. Additionally, a nested ANOVA analysis was performed to quantify between-day, between-run, within-run, and total precision. The 16 panels consisted of three panels of each individual target at three levels: Weak Positive, Low Positive, and Moderate Positive. A True Negative panel was also processed in each run. For all 5 targets tested, Low Positive panels at 1X LoD achieved a detection rate of ≥95% and Moderate Positive panels at 5X LoD achieved a detection rate of ≥99% for their respective targets, demonstrating excellent precision at LoD and near-LoD levels. As expected, Weak Positive panels at 0.1X LoD achieved detection rates below 95%. Furthermore, the True Negative panel achieved a negative result rate of ≥99% across all assay targets. Additionally, low variability was seen across runs, days, and Systems.

Table 13: Qualitative Analysis of Within-Lab Precision

Panel ID	Panel Target	Panel/ Target Level	Target Conc	Performance Metric	Valid Results (n/N)	Correct Results	Correct Results – % Agreement (95% CI)
A	Flu A	Weak Pos	0.05 $TCID_{50}$/mL	Positive Rate	212/216	93	43.9% (37.1-50.8%)
B	Flu A	Low Pos	0.5 $TCID_{50}$/mL	Positive Rate	216/216	216	100% (98.3-100.0%)
C	Flu A	Mod Pos	2.5 $TCID_{50}$/mL	Positive Rate	216/216	216	100% (98.3-100.0%)
D	Flu B	Weak Pos	0.025 $TCID_{50}$/mL	Positive Rate	215/216	80	37.2% (30.7-44.0%)
E	Flu B	Low Pos	0.25 $TCID_{50}$/mL	Positive Rate	213/218	211	99.1% (96.7-99.9%)
F	Flu B	Mod Pos	1.25 $TCID_{50}$/mL	Positive Rate	216/217	216	100% (98.3-100.0%)

Panel ID	Panel Target	Panel/ Target Level	Target Conc	Performance Metric	Valid Results (n/N)	Correct Results	Correct Results – % Agreement (95% CI)
G	RSV A	Weak Pos	0.025 $TCID_{50}$/mL	Positive Rate	213/216	130	61% (54.1-67.6%)
H	RSV A	Low Pos	0.25 $TCID_{50}$/mL	Positive Rate	214/218	214	100% (98.3-100.0%)
I	RSV A	Mod Pos	1.25 $TCID_{50}$/mL	Positive Rate	216/216	215	99.5% (97.5-100.0%)
J	RSV B	Weak Pos	0.005 $TCID_{50}$/mL	Positive Rate	216/216	160	74.1% (67.7-79.8%)
K	RSV B	Low Pos	0.05 $TCID_{50}$/mL	Positive Rate	215/216	208	96.7% (93.4-98.7%)
L	RSV B	Mod Pos	0.25 $TCID_{50}$/mL	Positive Rate	214/216	213	99.5% (97.4-100.0%)
M	SARS-CoV-2	Weak Pos	15 copies/mL	Positive Rate	215/218	31	14.4% (10.0-19.8%)
N	SARS-CoV-2	Low Pos	150 copies/mL	Positive Rate	214/216	207	96.7% (93.4-98.7%)
O	SARS-CoV-2	Mod Pos	750 copies/mL	Positive Rate	214/217	214	100% (98.3-100.0%)
P	N/A	True Negative	N/A	Negative Rate	214/217	213	99.5% (97.4-100.0)

Table 14. Overall mean, standard deviation, and percent coefficient of variation for Ct values by positive panel member

Panel Level	Positivity Rate	Mean Ct	Between day		Between run		Within run		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%
Influenza A										
Weak Pos	44%	N/A	N/A		N/A		N/A		N/A	
Low Pos	100%	32	0.56	1.75	0.56	1.75	0.54	1.69	0.62	1.94
Mod Pos	100%	30.1	0.46	1.53	0.46	1.53	0.41	1.36	0.51	1.69
Influenza B										
Weak Pos	37%	N/A	N/A		N/A		N/A		N/A	
Low Pos	99%	33.7	0.63	1.87	0.62	1.84	0.6	1.78	0.64	1.90
Mod Pos	100%	32.4	0.46	1.42	0.46	1.42	0.44	1.36	0.47	1.45
RSV A										
Weak Pos	61%	N/A	N/A		N/A		N/A		N/A	
Low Pos	100%	31.2	0.57	1.83	0.56	1.79	0.55	1.76	0.59	1.89
Mod Pos	100%	30	0.5	1.67	0.5	1.67	0.46	1.53	0.52	1.73
RSV B										
Weak Pos	74%	N/A	N/A		N/A		N/A		N/A	
Low Pos	97%	31.2	0.57	1.83	0.57	1.83	0.53	1.70	0.58	1.86
Mod Pos	100%	29.4	1.6	5.44	1.6	5.44	1.59	5.41	1.6	5.44
SARS-CoV 2										
Weak Pos	14%	N/A	N/A		N/A		N/A		N/A	

Low Pos	97%	34.2	0.65	1.90	0.64	1.87	0.6	1.75	0.67	1.96
Mod Pos	100%	33.4	0.65	1.95	0.65	1.95	0.54	1.62	0.66	1.98

FDA Flu A-B/RSV/SARS-CoV-2 Reference Panel Testing

The sensitivity of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay for SARS-CoV-2 and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and instrument used were NeuMoDx Systems. The results are summarized in *Table 15* below.

Table 15. Summary of LoD Confirmation Result for the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swabs	$1.8x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

Clinical Performance

Clinical performance characteristics of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay were determined in a method comparison study using residual nasopharyngeal (NP) swab specimens sourced from two geographically diverse clinical laboratory locations.

Residual NP swab specimens in UVT from symptomatic patients were first tested by clinical laboratories as part of standard patient care using FDA-cleared tests for Flu and RSV and an FDA authorized highly sensitive comparator test for SARS-CoV2. The results generated from the comparator tests were used to compare the results generated by the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay. Among the de-identified residual clinical NP swab specimens, 51 were identified as Flu A positive, 30 were identified as Flu B positive, 30 were identified as RSV A/B (undifferentiated) positive, and 32 specimens were identified as SARS-CoV-2 positive by the clinical laboratories. Additionally, 50 individual specimens were identified as negative for Flu A, Flu B, and RSV targets and another 50 individual specimens were identified as SARS-CoV-2 negative by the clinical laboratories. Operators were blinded to the prior sample result. A total of 486 individual NP swab specimens were tested randomized with both the Direct and Pretreated Workflows. Results are summarized in *Tables 16A, 16B, 16C,* and *16D* below. The 95% Confidence Intervals were calculated using the Wilson procedure with continuity correction.

Results of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay provided Positive and Negative Agreements of 100% for both workflows for the Flu A target (*Table 16A*).

Results for the Flu B target provided Positive and Negative Agreements of 96.7% and 98%, respectively, for both workflows (*Table 16B*).

Results for the RSV (undifferentiated) target provided Positive Agreement of 100% for both workflows while the Negative Agreement was determined to be 98% for the Direct Workflow and 100% for the Pretreated Workflow (*Table 16C*).

Results for the SARS-CoV-2 target provided Positive Agreement of 100% and Negative Agreement of 98% for both workflows (*Table 16D*). The study included 22% of low positive samples as defined by being within 3 cycles of the mean Ct value at the Comparators LoD.

Table 16A. Clinical Performance Summary – NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip: Detection of Flu A by (a) Direct Workflow, and (b) Pretreated Workflow

(a) Direct Workflow

Flu A		FDA Cleared Comparator Test Result		
		POS	NEG	Total
NeuMoDx Flu A-B/RSV/SARS-CoV-2	POS	50	0	50
	NEG	0	50	50
	Total	50	50	100
PPA: 100% (95% CI 91.1% - 100%)				
NPA: 100% (95% CI 91.1% - 100%)				

(b) Pretreated Workflow

Flu A		FDA Cleared Comparator Test Result		
		POS	NEG	Total
NeuMoDx Flu A-B/RSV/SARS-CoV-2	POS	51	0	51
	NEG	0	50	50
	Total	51	50	101
PPA: 100% (95% CI 91.1% - 100%)				
NPA: 100% (95% CI 91.1% - 100%)				

Table 16B. Clinical Performance Summary – NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip: Detection of Flu B by (a) Direct Workflow, and (b) Pretreated Workflow

(a) Direct Workflow

Flu B		FDA Cleared Comparator Test Result		
		POS	NEG	Total
NeuMoDx Flu A-B/RSV/SARS-CoV-2	POS	29	1	30
	NEG	1	49	50
	Total	30	50	80
PPA: 96.7% (95% CI 80.9% - 99.8%)				
NPA: 98.0% (95% CI 88.0% - 99.9%)				

(b) Pretreated Workflow

Flu B		FDA Cleared Comparator Test Result		
		POS	NEG	Total
NeuMoDx Flu A-B/RSV/SARS-CoV-2	POS	29	1	30
	NEG	1	49	50
	Total	30	50	80
PPA: 96.7% (95% CI 80.9% - 99.8%)				
NPA: 98.0% (95% CI 88.0% - 99.9%)				

Table 16C. Clinical Performance Summary – NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip: Detection of RSV A/B by (a) Direct Workflow, and (b) Pretreated Workflow

(a) Direct Workflow

RSV A/B		FDA Cleared Comparator Test Result		
		POS	NEG	Total
NeuMoDx Flu A-B/RSV/SARS-CoV-2	POS	30	1	31
	NEG	0	49	49
	Total	30	50	80
PPA: 100% (95% CI 85.9% - 100%)				
NPA: 98.0% (95% CI 87.9% - 99.9%)				

(b) Pretreated Workflow

RSV A/B		FDA Cleared Comparator Test Result		
		POS	NEG	Total
NeuMoDx Flu A-B/RSV/SARS-CoV-2	POS	30	0	30
	NEG	0	50	50
	Total	30	50	80
PPA: 100% (95% CI 85.9% - 100%)				
NPA: 100% (95% CI 91.1% - 100%)				

Table 16D. Clinical Performance Summary – NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip: Detection of SARS-CoV-2 by (a) Direct Workflow, and (b) Pretreated Workflow

(a) Direct Workflow

SARS-CoV-2		FDA EUA Comparator Test Result		
		POS	NEG	Total
NeuMoDx Flu A-B/RSV/SARS-CoV-2	POS	32	1	33
	NEG	0	49	49
	Total	32	50	82
PPA: 100% (86.7% - 100%)				
NPA: 98.0% (87.9% - 99.9%)				

(b) Pretreated Workflow

SARS-CoV-2		FDA EUA Comparator Test Result		
		POS	NEG	Total
NeuMoDx Flu A-B/RSV/SARS-CoV-2	POS	32	1	33
	NEG	0	49	49
	Total	32	50	82
PPA: 100% (86.7% - 100%)				
NPA: 98.0% (87.9% - 99.9%)				

Finally, analysis of invalid results in the clinical study was performed and summarized in Table 17 below.

Table 17. Summary of Invalid Results Rate in the Clinical Study for Individual Direct and Pretreated Workflows and Combined

	Direct		Pretreated		Combined	
Result Type	**Total**	**Rate**	**Total**	**Rate**	**Total**	**Rate**
Unresolved	0	0%	0	0%	0	0%
Indeterminate	1	0.41%	0	0%	1	0.21%
No Result	1	0.41%	0	0%	1	0.21%
Total Invalid	2	0.82%	0	0%	2	0.41%

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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.

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SYMBOLS KEY

℞ only	Prescription use only	↙	Temperature limit
↺	Manufacturer	⇆	Do not re-use
⌴	*In vitro* diagnostic medical device	⇪	Contains sufficient for <*n*> tests
◢	Authorized representative in the European Community	↓	Consult instructions for use
↑	Catalog number	⇪	Caution
→	Batch code	⇆	Biological risks
⇇	Use-by date	⇆	CE Mark

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REF 300900 NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip

Product Information Card (PIC)

NeuMoDx™ molecular

For use under FDA Emergency Use Authorization only in the United States and its territories

CE IVD ℞ only

This PIC is not a full Instruction For Use

Electronic version of the Instructions for Use (IFU) and Safety Data Sheets (SDS) are available at www.neumodx.com/client-resources

The NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Assay has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.

This product has been authorized only for the detection and differentiation of nucleic acid from SARS-CoV-2, influenza A virus, influenza B virus, and/or Respiratory Syncytial Virus, not for any other viruses or pathogens.

The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

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