NeuMoDx™ SARS-CoV-2 Assay

Instructions For Use

For use under FDA Emergency Use Authorization within the United States and Territories

IVD *For in vitro diagnostic use*

℞ only

REF 300800 NeuMoDx™ SARS-CoV-2 Test Strip

CONTENTS

INTENDED USE

The NeuMoDx™ SARS-CoV-2 Assay performed on the NeuMoDx™ 288 Molecular System and NeuMoDx™ 96 Molecular System (NeuMoDx Molecular System(s)), is a real-time RT-PCR diagnostic test intended for the qualitative detection of SARS-CoV-2 RNA from nasal, nasopharyngeal and oropharyngeal swabs in transport medium and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider (HCP). Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform moderate or high complexity tests.

This test is also for use with saliva specimens that are collected in a healthcare setting under the supervision of a healthcare provider (HCP) using the NeuMoDx Saliva Collection Kit from individuals suspected of COVID-19 as determined by an HCP due to symptoms.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 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. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.

The NeuMoDx™ SARS-CoV-2 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™ SARS-CoV-2 Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY AND EXPLANATION OF THE TEST

NeuMoDx SARS-CoV-2 Assay is a real-time reverse transcription PCR performed on the NeuMoDx Molecular systems. The NeuMoDx System automatically performs all the steps required to extract the target nucleic acid, prepare the isolated RNA for real-time reverse transcription polymerase chain reaction (RT-PCR) and, if present, amplify and detect target sequences of the non-structural protein 2 (Nsp2) gene and the N gene, both specific to the SARS-CoV-2 genome.

PRINCIPLES OF THE PROCEDURE

The NeuMoDx SARS-CoV-2 Assay combines automated RNA extraction and amplification/detection by real-time RT-PCR. Nasopharyngeal, oropharyngeal, or nasal swab samples are collected in the Copan UTM-RT® System or BD™ UVT System. Saliva specimens are collected into NeuMoDx™ Saliva Collection Kit. There are two workflows available for swab specimen preparation with the NeuMoDx SARS-CoV-2 Assay. The direct workflow allows for the swab collection tube or an aliquot of the transport medium in a secondary tube to be loaded onto the NeuMoDx System for processing without further intervention. Alternatively, the swab sample medium is pretreated with NeuMoDx Viral Lysis Buffer before being placed on the NeuMoDx System for processing. For the saliva specimen, the operator loads the primary specimen stabilization tube containing stabilized saliva directly on the NeuMoDx System. The NeuMoDx System automatically begins processing by aspirating an aliquot of the swab sample matrix or the stabilized saliva and mixing it with NeuMoDx Lysis Buffer and the reagents contained in the NeuMoDx™ Extraction Plate. The NeuMoDx System automates and integrates RNA extraction and concentration, PCR 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 using the separately available NeuMoDx™ reagents. 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 RT-PCR mix containing all the elements necessary for amplification of the SARS-CoV-2 and SPC2 targets. This enables simultaneous amplification and detection of both target and SPC2 in one reaction. 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 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. A TaqMan probe labeled with a FAM fluorophore (470/510 nm) is used to detect the Nsp2 region of the SARS-CoV-2 genome and a TaqMan probe labeled with a HEX fluorophore (530/555 nm) is used to detect the N gene of the SARS-CoV-2 genome. For detection of the SPC2, the TaqMan probe is labeled with a Far-Red fluorophore (680/715 nm). The NeuMoDx System software monitors the fluorescent signal emitted by the TaqMan probes at the end of each amplification cycle. When amplification is complete, the NeuMoDx System software analyzes the data and reports results as POSITIVE, NEGATIVE, INDETERMINATE, NO RESULT, or UNRESOLVED.

REAGENTS AND MATERIALS

Material Provided

REF	Contents	Units per package	Tests per unit	Tests per package
300800	**NeuMoDx™ SARS-CoV-2 Test Strip** *Dried RT-PCR reagents containing SARS-CoV-2 and SPC2 specific TaqMan® probes and primers*	6	16	96

Materials Required but Not Provided (Available Separately from NeuMoDx)

REF	Contents
100100	**NeuMoDx™ Cartridge**
100200	**NeuMoDx™ Extraction Plate**
400100	**NeuMoDx™ Wash Reagent**
400200	**NeuMoDx™ Release Reagent**
400500 (*Optional**)	**NeuMoDx™ Lysis Buffer 2**
400600**	**NeuMoDx™ Lysis Buffer 3**
401600 (*Optional**)	**NeuMoDx™ Viral Lysis Buffer**
235903	**Hamilton® CO-RE Tips (300 µL) with Filters**
235905	**Hamilton® CO-RE Tips (1000 µL) with Filters**

**Required if a pretreatment step is desired for offboard lysis prior to loading of swab samples. Also required for processing of saliva specimens. See section "Instructions for Use" below.*

***Required only for processing of direct swab samples. See section "Instructions for Use" below.*

Swab and Transport Media (Not Provided)

Contents
3mL/1mL Universal Transport Medium (UTM®, Copan Diagnostic Inc, CA) or Universal Viral Transport System (BD™ UVT, BD Diagnostic, NJ)
Flexible Minitip Size Nylon® Flocked Swab (Copan Diagnostic Inc, CA) Flexible minitip flocked swab (BD Diagnostics, MD)

Saliva Collection Material (Available Separately from NeuMoDx)

REF	Contents	Units per package
100500	**NeuMoDx™ Saliva Collection Kit** *Contains (1) NeuMoDx™ Saliva Collection Vial, (1) NeuMoDx™ Specimen Stabilization Tube with 1 mL NeuMoDx™ saliva stabilization buffer, and (1) disposable transfer pipette (sufficient for the collection of one sample per kit; refer to the instructions for use for details: P/N 40600441)*	*96*

Instrumentation Required (Not Provided)

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

⚠ ⊗ WARNINGS AND PRECAUTIONS

- For Prescription Use Only.
- The NeuMoDx™ SARS-CoV-2 Assay is for *in vitro* diagnostic use with NeuMoDx™ Systems.
- For Use under FDA Emergency Use Authorization only.
- The NeuMoDx™ SARS-CoV-2 Assay has not been FDA cleared or approved but has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories.
- The emergency use of the NeuMoDx™ SARS-CoV-2 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.
- 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™ SARS-CoV-2 Assay has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- Testing of saliva specimens is limited to patients with symptoms of COVID-19 using the NeuMoDx Saliva Collection Kit.
- Do not re-use.
- 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 reagents or consumables 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 available 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 NeuMoDx SARS-CoV-2 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.

PRODUCT STORAGE, HANDLING, AND STABILITY

- NeuMoDx SARS-CoV-2 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 SARS-CoV-2 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

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

Nasopharyngeal and nasal specimens

Specimens should be collected using the Copan UTM-RT® System or BD™ UVT System using the validated nylon flocked swabs (see materials not provided). 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 instructions for use:

- After collection, the specimen should be stored at 2-25 °C and processed within 48 hours.
- If delivery and processing exceed 48 hours, specimens should be transported in dry ice and once in laboratory frozen at -70°C or colder.

Saliva specimens

Specimens should be collected and stabilized using NeuMoDx™ Saliva Collection Kit. *For detailed saliva sample collection and stabilization instructions, refer to the NeuMoDx™ Saliva Collection Kit [REF 100500] IFU (P/N 40600441), which can be found at www.neumodx.com/client-resources.*

After mixing the saliva with the stabilization buffer, check the volume in the Specimen Stabilization Tube. If the total volume is below the fill line, add molecular grade water to bring the total volume to the fill line.

- Stabilized saliva should be transported on ice packs.
- The stabilized saliva can be tested directly on the NeuMoDx System or stored for later testing as follows:
  - Saliva samples should be added to the stabilization tube of the saliva collection kit within two hours of collection. Saliva specimens can be stored for up to 2 hours at ambient conditions prior to mixing with NeuMoDx™ Stabilization Buffer (SSB).
  - Stabilized saliva can be stored for up to 48 hours at ambient conditions and up to 7 days at 2-8°C. Specimen should be allowed to reach room temperature before testing.
  - Stabilized saliva can be stored for 12 hours onboard the NeuMoDx Molecular Systems.

INSTRUCTIONS FOR USE

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

Workflow 1: DIRECT – swab specimen in transport medium and saliva in stabilization buffer are loaded directly onto the NeuMoDx System in primary collection tube or secondary specimen tubes

-or-

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

Test Preparation – DIRECT Workflow for Direct Swab and Saliva Samples

Note: Bring swab samples to room temperature (15 to 30 °C) before processing.

1. Apply specimen barcode label to a specimen tube compatible with the NeuMoDx System as described under 4 and 5 below.
2. If testing the specimen in the primary collection tube (Swab specimens) or Specimen Stabilization Tube (Saliva specimens), place the barcoded tube into a Specimen Tube Carrier and ensure the cap and/or swab are removed prior to loading onto the NeuMoDx System.
3. Alternatively, an aliquot of the transport medium or the Stabilized Saliva may be transferred to a barcoded secondary tube and placed into a 32-tube Specimen Tube Carrier. If using a secondary tube, transfer an aliquot of the transport medium or the Stabilized Saliva to the barcoded specimen tube compatible with the NeuMoDx System according to the volumes defined below:
4. *For swab specimens:*
   - 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
5. *For Stabilized Saliva specimens:*
   - Specimen Tuber Carrier (32-tube): 11 – 14 mm in diameter and 60 – 120 mm in height; minimum fill volume ≥ 800 µL
   - Low Volume Specimen Tube Carrier (32-tube): 1.5 mL conical bottom microcentrifuge tube; minimum fill volume ≥ 700 µL

Test Preparation – PRETREATED Workflow for Pretreated Swab Samples

Note: Bring swab samples to room temperature (15 to 30 °C) before processing.

WARNING: Pretreatment of swab samples with NeuMoDx 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 Viral Lysis Buffer (i.e., 1+1). 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 Viral Lysis Buffer. The resulting mixture should meet the minimum volume requirements specified below.

2. Mix gently with pipette to ensure uniform distribution of NeuMoDx Viral Lysis Buffer.
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 lysate to the barcoded specimen tube compatible with the NeuMoDx System according to the minimum 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

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 SARS-CoV-2 Test Strip
    - NeuMoDx Lysis Buffer 2 *(NOTE: remove foil seal from containers prior to loading)*
    - NeuMoDx Lysis Buffer 3 *(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. Load the specimen tube(s) into Specimen Tube Carrier, and ensure caps and swabs (if applicable) are removed from all specimen tubes.
5. Place the Specimen Tube carrier on the Autoloader shelf and use the touchscreen to load carrier into the system. This will initiate processing of test(s).
6. 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 using the PRETREATED workflow are tested by defining the specimen as "**UserSpecified1**"
    - Stabilized Saliva using the DIRECT workflow are tested by defining the specimen as "**UserSpecified2**"
7. Populate one or more Test Strip Carrier(s) with NeuMoDx SARS-CoV-2 Test Strip(s) and use the touchscreen to load the Test Strip Carrier(s) into the NeuMoDx System.
8. Load the specimen(s) into a Specimen Tube Carrier and ensure caps are removed from all tubes.
9. 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

- The NeuMoDx SARS-CoV-2 Assay has only been evaluated for use on NeuMoDx Molecular Systems.
- The NeuMoDx SARS-CoV-2 Assay has been designed for detection of SARS-CoV-2 RNA in nasopharyngeal or oropharyngeal swab samples collected with Copan UTM-RT System (UTM-RT®) or BD™ Universal Viral Transport System (UVT)), or saliva samples collected using the NeuMoDx Saliva Collection Kit. Use of the NeuMoDx SARS-CoV-2 Assay with other sample types has not been assessed and performance characteristics are unknown.
- Nasal and mid-turbinate nasal swabs and bronchoalveolar lavage specimens are considered acceptable specimen types for use with the NeuMoDx SARS-CoV-2 but performance with these specimen types has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) 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.
- Reliable results are dependent on proper specimen collection, handling, and storage.
- Erroneous results could occur from improper specimen collection, handling, storage, technical error, or specimen tube mix-up. Incorrect saliva volume in the Specimen Stabilization Tube may reduce the sensitivity of the test. 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 SARS-CoV-2 Assay.
- Deletions or mutations in the regions targeted by the NeuMoDx SARS-CoV-2 Assay may affect detection and could lead to an erroneous result.
- Presence of Crest® Pro-Health Advanced Gum Protection Toothpaste in saliva specimens may interfere with SARS-CoV-2 RNA detection and could lead to a false negative result.
- A positive result is indicative of the presence of SARS-CoV-2 RNA but does not necessarily indicate the presence of infectious SARS-CoV-2.
- Negative results do not preclude infection with the SARS-CoV-2 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.
- Results from NeuMoDx SARS-CoV-2 Assay should be used as an adjunct to clinical observations and other information available to the physician.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The NeuMoDx™ SARS-CoV-2 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 SARS-CoV-2 Assay, the relevant Conditions of Authorization are listed below.

- Authorized laboratories[1] using the NeuMoDx SARS-CoV-2 Assay must include with result reports of the NeuMoDx SARS-CoV-2 Assay, 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 SARS-CoV-2 Assay must perform the NeuMoDx SARS-CoV-2 Assay as outlined in the NeuMoDx SARS-CoV-2 Assay Instructions for Use. 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 SARS-CoV-2 Assay are not permitted.
- Authorized laboratories that receive the NeuMoDx SARS-CoV-2 Assay must notify the relevant public health authorities of their intent to run the test prior to initiating testing.

- Authorized laboratories using the NeuMoDx SARS-CoV-2 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 SARS-CoV-2 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, this letter will refer to, "United States (U. S.) laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate or 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. A test result is called Positive (POS), Negative (NEG), Indeterminate (IND), No Result (NR) or Unresolved (UNR) based on the amplification status of the target and the Sample Process Control (SPC2).

Criteria for a positive or negative call are specified in the NeuMoDx SARS-CoV-2 Assay Definition File (ADF) as installed on the NeuMoDx System. Results for swab and saliva specimens are reported based on the ADF decision algorithm, summarized in *Tables 1 and 2*, respectively, below.

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

Table 1. NeuMoDx SARS-CoV-2 Assay Results Interpretation – Swab Specimens

OVERALL RESULT	TARGET 1 (Nsp2-gene) FAM	TARGET 2 (N-gene) HEX	PROCESS CONTROL (SPC2) Far Red	Interpretation
POSITIVE	**AMPLIFIED** [5 ≤ Ct < 20 AND EPR ≥ 1.2 AND EP ≥ 700] OR (20 ≤ Ct ≤40 AND EP ≥700)	N/A	N/A	SARS-CoV-2 RNA detected**
	N/A	**AMPLIFIED** (5 ≤ Ct < 20 AND EPR ≥1.5) AND EP ≥ 1000] OR (20 ≤ Ct ≤ 40 AND EP >1000)		
NEGATIVE	**NOT AMPLIFIED** N/A **OR** (5 ≤ Ct < 20 AND EPR <1.2) **OR** (20 ≤ Ct ≤ 40 AND EP <700) **OR** (Ct > 40)	**NOT AMPLIFIED** N/A **OR** (5 ≤ Ct < 20 AND EPR <1.5) **OR** (20 ≤ Ct ≤ 40 AND EP <1000) **OR** (Ct > 40)	**AMPLIFIED** (24≤ Ct ≤33 AND EP ≥1000)	SARS-CoV-2 RNA not detected
IND*	**NOT AMPLIFIED/System Errors Noted, Sample Processing Completed**			All target results were invalid; retest sample
NR*	**NOT AMPLIFIED/System Errors Noted, Sample Processing Aborted**			Sample processing was aborted; retest sample
UNR*	**NOT AMPLIFIED/No System Errors Noted**			All target results were invalid; retest sample

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

****A re-test may be performed if desired in the event of only one of the two SARS-CoV-2 target being amplified.**

Table 2. NeuMoDx SARS-CoV-2 Assay Results Interpretation -Saliva Specimens

OVERALL RESULT	TARGET 1 (Nsp2-gene) FAM	TARGET 2 (N-gene) HEX	PROCESS CONTROL (SPC2) Far Red	Interpretation
POSITIVE	**AMPLIFIED** [5 ≤ Ct < 28 AND EP ≥600 AND EPR >1.2] **OR** [28 ≤ Ct ≤ 40 AND EP ≥600]	N/A	N/A	SARS-CoV-2 RNA detected**
	N/A	**AMPLIFIED** [5 ≤ Ct < 28 AND EP ≥675 AND EPR >1.2] OR [28 ≤ Ct ≤ 40 AND EP ≥675]		
NEGATIVE	**NOT AMPLIFIED** N/A **OR** [5 ≤ Ct < 28 AND EPR ≤1.2] **OR** [28 ≤ Ct ≤ 42 AND EP <600] **OR** [Ct >40]	**NOT AMPLIFIED** N/A **OR** [5 ≤ Ct < 28 AND EPR ≤1.2] **OR** [28 ≤ Ct ≤ 42 AND EP <675] **OR** [Ct >40]	**AMPLIFIED** (24≤ Ct ≤33 AND EP ≥1000)	SARS-CoV-2 RNA not detected
IND*	**NOT AMPLIFIED/System Errors Noted, Sample Processing Completed**			All target results were invalid; retest sample
NR*	**NOT AMPLIFIED/System Errors Noted, Sample Processing Aborted**			Sample processing was aborted; retest sample
UNR*	**NOT AMPLIFIED/No System Errors Noted**			All target results were invalid; retest sample

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

****A re-test may be performed if desired in the event of only one of the two SARS-CoV-2 target being amplified.**

A positive result may be reported for samples yielding a differential amplification status, such that only one of the targets—Target 1 (Nsp2 gene) or Target 2 (N gene)—amplifies. This may occur due to 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in one of the target regions, or 3) other factors. In the case of a positive test where only one of the targets amplifies, repeat testing may be considered if the SPC2 control is negative. If the repeat result remains the same, additional confirmation testing should be conducted if clinically indicated.

Invalid Results

If both the SARS-CoV-2 targets and the SPC2 target do not amplify, the NeuMoDx SARS-CoV-2 Assay performed on the NeuMoDx System an invalid result will be reported. The invalid result 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. In the event a repeat test yields the same type of invalid result, it may be beneficial to obtain a new sample. If a different type of invalid result is obtained, proceed with testing the sample for a third time if specimen volume allows. If you systematically encounter an increased number of invalid samples.

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.

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 Sars-CoV-2 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.

   For Swab and BAL Specimens the following controls are recommended

   - Positive Control:
     - Purified SARS-CoV-2 genomic RNA (Cat# VR-1986D, ATCC, Manassas, VA, USA) at final concentration of 5E3 cp/mL
     - Heat-inactivated SARS-CoV-2 (Cat# VR-1986HK, ATCC, Manassas, VA, USA) at final concentration of 5E3 cp/mL
     - 5 µL of NATtrol™ SARS-CoV-2 (recombinant) Stock (contains only N gene, Catalog# 0831042, ZeptoMetrix, Buffalo, NY, USA) in 1 mL BD™ UVT medium.
   - Negative Control: Copan/BD™ UVT media or equivalent.

   For Saliva Specimens the following controls are recommended

   - Positive Control: Dilute any of the following material into a mixture of molecular grade water and SSB at a ratio of 1:1.67 water/SSB (v/v):
     - Purified SARS-CoV-2 genomic RNA (Cat# VR-1986D, ATCC, Manassas, VA, USA) at final concentration of 5E3 cp/mL
     - Heat-inactivated SARS-CoV-2 (Cat# VR-1986HK, ATCC, Manassas, VA, USA) at final concentration of 5E3 cp/mL
     - NATtrol™ SARS-CoV-2 (Recombinant) Stock (contains only N gene, Cat#0831042, Zeptometrix, Buffalo, NY, USA) at 1:20 dilution.
   - Negative control: 0.6 mL molecular grade water added to 1 mL saliva stabilization buffer (SSB), or at ratio of 1:1.67 water/SSB (v/v).
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 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. The primers and probe specific for the Sample Process Control (SPC2) are included in each NeuMoDx SARS-CoV-2 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 a specimen contamination problem. Please refer to NeuMoDx™ *288 or 96 Molecular System Operator's Manual* for 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.

PERFORMANCE CHARACTERISTICS

Specimen Stability

The stability of saliva in the NeuMoDx Saliva Stabilization Buffer was determined by using a contrived panel of low positive samples prepared by spiking gamma-irradiated SARS-CoV-2 virus in the mixture of pooled saliva specimens with SSB at a ratio of 1:1.67 (v/v), and negative sample panel using the pooled negative saliva with SSB mixture. Enough volume was dispensed into barcoded secondary tubes to allow for a maximum of 4 or 5 tests from each tube.

The contrived positive saliva panels were stored at ambient temperature for 24 and 48 hours after mixing with SSB. A set of six (6) replicates of the positive and negative panels were processed at each timepoint. Following the initial testing, the saliva panels were stored for 7 days at 2-8°C. One set of six replicates of positive and negative samples was stored at 2-8°C for 7 days immediately after dispensing into secondary tubes. After storage for 7 days, each set of specimens was immediately processed on the NeuMoDx Molecular System before being left onboard the system's worktable for a total of 12 hours. Additional testing was performed after 8 and 12 hours onboard storage. A total of 48 replicates were processed for this study.

Table 3. Saliva in SSB Stability Results using the NeuMoDx SARS CoV-2 Assay on NeuMoDx Molecular System

Timepoint	SARS-CoV-2 Positive Panel				Negative Panel		
	# Sample Tested	# Valid Result	% Amplified (N gene)	% Amplified (Nsp2 gene)	# Sample Tested	# Valid Result	% Amplified
0 Days (T0)	6	6	100%	100%	6	6	0%
7 Days 2-8°C	6	6	100%	100%	6	6	0%
7 Days 2-8°C + 8h	6	6	100%	100%	6	6	0%
7 Days 2-8°C + 12h	6	6	100%	100%	6	6	0%
24h Ambient	6	5[1]	100%	100%	6	6	0%
24h + 7 Days 2-8°C	6	6	100%	100%	6	5[1]	0%
24h + 7 Days 2-8°C + 8h	6	6	100%	100%	6	6	0%
24h + 7 Days 2-8°C + 12h	6	5[1]	100%	100%	6	6	0%
48h Ambient	6	6	100%	100%	6	6	0%
48h + 7 Days 2-8°C	6	6	100%	100%	6	6	0%
48h + 7 Days 2-8°C + 8h	6	6	100%	100%	6	6	0%
48h + 7 Days 2-8°C + 12h	6	6	100%	100%	6	6	0%

[1] One sample had an UNR result.

As shown in Table 1, 100% detection rate was observed from the initial run (Time 0) and at the 24-hour, 48-hour, and 7-day timepoints for saliva samples in SSB. Three samples had Unresolved results.

- All valid SARS-CoV-2 Positive samples correctly reported Positive results after ambient storage and storage at 2-8°C for 7 days.
- Furthermore, 100% detection was observed up to 12 hours onboard the system. All positive samples correctly reported Positive results for valid SARS CoV-2 Positive samples after storage onboard the system for 8 and 12 hours.

This demonstrates that saliva specimens in SSB are stable for at least 48 hours when stored at ambient temperature, 7 days when stored at 2-8°C, and for up to 12 hours when stored onboard the NeuMoDx Molecular System.

Analytical Sensitivity – Nasopharyngeal Swab Samples

The limit of detection (LoD) of the NeuMoDx SARS-CoV-2 Assay was determined by testing a dilution series of pooled negative clinical nasopharyngeal swab samples (Nylon Flocked Swab collected in UTM [Copan Diagnostic Inc, CA] or VTM [BD, NJ]) spiked with SARS-CoV-2 genomic RNA (BEI Resources NR-52285) and processed using the both DIRECT and PRETREATED workflows. At least twenty replicates of each dilution were evaluated across both NeuMoDx Systems for each workflow. The LoD was determined to be **150 copies/mL**.

Table 4. Detection Rate and Limit of Detection for SARS-CoV-2 on NeuMoDx 96 Molecular System: Pretreated Workflow

SARS-CoV-2 LoD: N96, Pretreated Workflow								
Target Level	Valid results	Nsp2-gene Positive		Nsp2-gene Detection Rate	N-gene Positive		N-gene Detection Rate	Both Targets Amplified Rate
		n	Mean Ct		n	Mean Ct		
250 cp/mL	22	22	31.7	100%	22	30.9	100%	100%
150 cp/mL	20	20	31.5	100%	20	31.0	100%	100%
50 cp/mL	24	0	n/a	0%	22	31.8	91.7%	0%
Negative	30	0	n/a	0.0%	0	n/a	0.0%	0.0%
N96 LoD: 150 cp/mL [lowest target level demonstrating >95% detection rate of both targets]								

Table 5. Detection Rate and Limit of Detection for SARS-CoV-2 on NeuMoDx 288 Molecular System: Pretreated Workflow

SARS-CoV-2 LoD: N288, Pretreated Workflow								
Target Level	Valid results	Nsp2-gene Positive		Nsp2-gene Detection Rate	N-gene Positive		N-gene Detection Rate	Both Targets Amplified Rate
		n	Mean Ct		n	Mean Ct		
250 cp/mL	21	21	32.1	100%	21	31.4	100%	100%
150 cp/mL	26	26	31.7	100%	26	31.2	100%	100%
50 cp/mL	21	11	32.2	52.4%	20	32.2	95.2%	52.4%
Negative	20	0	n/a	0%	0	n/a	0%	0%
N288 LoD: 150 cp/mL [lowest target level demonstrating >95% detection rate of both targets]								

Table 6. Detection Rate and Limit of Detection for SARS-CoV-2 on NeuMoDx 96 Molecular System: Direct Workflow

SARS-CoV-2 LoD: N96, Direct Workflow								
Target Level	Valid results	Nsp2-gene Positive		Nsp2-gene Detection Rate	N-gene Positive		N-gene Detection Rate	Both Targets Amplified Rate
		n	Mean Ct		n	Mean Ct		
400 cp/mL	24	23*	32.4	95.8%	24	31.1	100.0%	95.8%
250 cp/mL	24	24	33.0	100.0%	24	31.7	100.0%	100.0%
150 cp/mL	24	24	33.4	100.0%	24	32.4	100.0%	100.0%
50 cp/mL	24	12	32.6	50.0%	18	32.8	75.0%	41.7%**
Negative	22	0	n/a	0.0%	0	n/a	0.0%	0.0%
N96 LoD: 150 cp/mL [lowest target level demonstrating >95% detection rate of both targets]								

*This sample additionally displayed weak SPC2 amplification, and the lack of amplification was believed to be an artifact of system processing. This is supported by a 100% detection rate at the same target concentration in RPT-8505B (Clinical Evaluation). Additionally, for this study a 100% detection rate was achieved at the lower 250 cp/mL and 150 cp/mL concentrations.

** Ten of 24 samples had both targets detected at 50 cp/mL, for an overall positivity rate of 41.7%

Table 7. Detection Rate and Limit of Detection for SARS-CoV-2 on NeuMoDx 288 Molecular System: Direct Workflow

SARS-CoV-2 LoD: N288, Direct Workflow								
Target Level	Valid results	Nsp2-gene Positive		Nsp2-gene Detection Rate	N-gene Positive		N-gene Detection Rate	Both Targets Amplified Rate
		n	Mean Ct		n	Mean Ct		
400 cp/mL	24	24	32.8	100.0%	24	31.7	100.0%	100.0%
250 cp/mL	24	24	33.0	100.0%	24	32.0	100.0%	100.0%
150 cp/mL	22	21	33.5	95.5%	22	32.4	100.0%	95.5%
50 cp/mL	24	20	34.3	83.3%	24	33.4	100.0%	83.3%
Negative	24	0	n/a	0.0%	0	n/a	0.0%	0.0%
N288 LoD: 150 cp/mL [lowest target level demonstrating >95% detection rate of both targets]								

Analytical Sensitivity –Saliva Samples

The limit of detection (LoD) of the NeuMoDx SARS-CoV-2 Assay using saliva samples was evaluated by testing a dilution series of pooled negative saliva samples (mixed with NeuMoDx™ Saliva Stabilization Buffer at 1:1.67 saliva to buffer ratio) spiked with γ-irradiated SARS-CoV-2 virus (BEI Resources NR-52287) or SARS-CoV-2 genomic RNA (BEI Resources NR-52285) and processed using Direct workflow. At least five replicates at each dilution were evaluated around the expected LoD, followed by the confirmatory processing of at least twenty replicates at the lowest levels that gave all positive results. The LoD for genomic RNA and γ-irradiated virus were respectively determined to be **50 copies/mL** and **0.0075 TCID50/mL**.

Table 8. Detection Rates and Preliminary Limit of Detection with γ-Irradiated SARS-CoV-2 in Saliva

SARS-CoV-2 LoD; γ-Irradiated SARS-CoV-2 Virus in Saliva									
Target Level	Valid results	Nsp2-gene Positive		Nsp2-gene Detection Rate	N-gene Positive		N-gene Detection Rate	Nsp2 + N-gene Detection Rate	Overall Positive Rate
		n	Mean Ct		n	Mean Ct			
0.01 TCID50/mL	5	5	32.8	100%	5	32.6	100%	100%	100%
0.005 TCID50/mL	5	5	34.0	100%	5	33.1	100%	100%	100%
0.0025 TCID50/mL	10	4	33.5	40%	5	32.7	50%	30%*	60%
Preliminary LoD – γ-Irradiated Virus: 0.005 TCID50/mL [lowest target level demonstrating >95% detection rate of both targets]									
*Three of ten (3/10) samples had both targets detected at 0.0025 TCID50/mL									

Table 9. Detection Rates and Preliminary Limit of Detection with SARS-CoV-2 gRNA in Saliva

SARS-CoV-2 LoD; SARS-CoV-2 Genomic RNA in Saliva									
Target Level	Valid results	Nsp2-gene Positive		Nsp2-gene Detection Rate	N-gene Positive		N-gene Detection Rate	Nsp2 + N-gene Detection Rate	Overall Positive Rate
		n	Mean Ct		n	Mean Ct			
100 cp/mL	5	5	32.7	100%	5	31.8	100%	100%	100%
50 cp/mL	5	5	33.3	100%	5	32.5	100%	100%	100%
40 cp/mL	10	6	34.4	60%	9	33.1	90%	60%*	90%
25 cp/mL	10	4	34.1	40%	9	33.0	90%	40%**	90%
Preliminary LoD – gRNA: 50 cp/mL [lowest target level demonstrating >95% detection rate of both targets]									
*Six of ten (6/10) samples had both targets detected at 40 cp/mL **Four of ten (4/10) samples had both targets detected at 25 cp/mL									

Table 10. Detection Rates and Limit of Detection Confirmation with γ-Irradiated SARS-CoV-2 in Saliva

SARS-CoV-2 LoD; γ-Irradiated SARS-CoV-2 Virus in Saliva										
System	Target Level	Valid results	Nsp2-gene Positive		Nsp2-gene Detection Rate	N-gene Positive		N-gene Detection Rate	Nsp2 + N-gene Detection Rate	Overall Positive Rate
			n	Mean Ct		n	Mean Ct			
N288	0.0075 TCID50/mL	20	20	33.5	100%	20	33.3	100%	100%	100% (20/20)
N96	0.0075 TCID50/mL	20	20	33.4	100%	20	33.3	100%	100%	100% (20/20)
N288	0.005 TCID50/mL	20	18	33.7	90%	18	33.0	90%	85%*	90% (19/20)
N96	0.005 TCID50/mL	20	15	34.3	75%	16	33.8	80%	65%**	80% (18/20)
N288 LoD: 0.0075 TCID50/mL [lowest target level demonstrating >95% detection rate of both targets] **N96 LoD: 0.0075 TCID50/mL [lowest target level demonstrating >95% detection rate of both targets]**										
*Eighteen (18) of twenty (20) samples had both targets detected on N288 **Thirteen (13) of twenty (20) samples had both targets detected on the N96										

Table 11. Detection Rates and Limit of Detection Confirmation with SARS-CoV-2 gRNA in Saliva

SARS-CoV-2 LoD; SARS-CoV-2 Genomic RNA in Saliva										
System	Target Level	Valid results	Nsp2-gene Positive		Nsp2-gene Detection Rate	N-gene Positive		N-gene Detection Rate	Nsp2 + N-gene Detection Rate	Overall Positive Rate
			n	Mean Ct		n	Mean Ct			
N288	50 cp/mL	20	20	34.3	100%	20	33.9	100%	100%	100% (20/20)
N96	50 cp/mL	20	19	33.9	95%	19	33.8	95%	95%*	95% (19/20)
N288 LoD: 50 cp/mL [lowest target level demonstrating >95% detection rate of both targets] **N96 LoD: 50 cp/mL [lowest target level demonstrating >95% detection rate of both targets]**										
*Nineteen (19) of twenty (20) samples had both targets detected on the N96										

Inclusivity

The inclusivity of the NeuMoDx SARS-CoV-2 Assay was evaluated by *in silico* analysis aligning the assay primers and probes to all curated full length SARS-CoV-2 sequences (approximately 9800) in the NCBI database as of 05 January 2021. The regions of the test's primers and probes were compared by *in silico* analysis to verify sequence homology with circulating SARS-CoV-2 strains. The NeuMoDx SARS-CoV-2 Assay had 100% homology to 99% and 98% of sequence analyzed for the N gene and Nsp2 gene, respectively. Less than 1.5% of the sequences had one mismatch in either primer or probe regions. None of the mismatches are present in the same viral genome at the same time so that the dual target design of the test mitigates the risk of false negative results.

Sequence analysis has also been conducted to evaluate the impact of the new emerging SARS-CoV-2 variant in UK (SARS-CoV-2 VOC 202012/01) and South Africa (501Y.V2) on the sensitivity of the NeuMoDx SARS-CoV-2 assay. The three mutations, K417N, E484K, and N501Y, reported in the South Africa 501Y.V2 variant are in the spike protein which is not the target region for the NeuMoDx SARS-CoV-2 assay and will therefore not impact the sensitivity of the NeuMoDx SARS-CoV-2 assay. Neither variant has substitutions or mismatches in the primers and probe for N gene of the NeuMoDx SARS-CoV-2 assay. With the exception of one mutation, the 23 nucleotide substitutions in the viral genome of VOC 202012/01 are outside the tests target sequences. and do not impact the binding

of the S and Nsp primers and probes. One of the substitutions, C913T, resides in the reverse primer for Nsp2 target. However, this substitution is not unique to the VOC 202012/01 variant, has been present in other SARS-CoV-2 sequences submitted to the GenBank prior to the emerging of the UK variant, and should be tolerated by the robust design of the assay due to its location in the primer. There are no substitutions or mismatches in the primers and probe for the NeuMoDx SARS-CoV2 assay that are expected to result in false negative results.

Cross-reactivity/Microbial Interference

The NeuMoDx SARS-CoV-2 Assay was evaluated i*n silico* for possible cross-reactions with the microorganisms shown in *Table 12* by individually aligning the primers and probes of the NeuMoDx SARS-CoV-2 Assay to sequences in the NCBI database. None of the sequences analyzed showed homology for the primers or probe of the Nsp2 gene (Target 1). *Haemophilus influenzae* (CP000672.1) showed 80% homology to the forward primer of the N gene (Target 2) but had no significant homology to the reverse primer and probe. Similarly, SARS coronavirus (AY345986.1) showed homology for the forward primer and probe of the N gene but no significant homology for the reverse primer. *Pseudomonas aeruginosa* (CP000438.1) showed homology for the forward SPC2 primer but not for either of the SARS-CoV-2 targets. The *in silico* analysis therefore showed no probable cross-reactivity to any of the sequences evaluated. Further wet testing was done to confirm that *H. influenzae* and *P. aeruginosa* posed no risk of cross-reactivity or microbial interference with the primer and probe sets of the NeuMoDx SARS-CoV-2 Assay. Results are presented in *Tables 13* and *14*.

Table 12. *In Silico* Analysis for Cross-Reactive Organisms

Organism	NCBI GenBank Accession Number(s)	Organism	NCBI GenBank Accession Number(s)
Human coronavirus 229E	KF514433.1	Influenza B	MK969560.1
	KF514432.1	Enterovirus	JF896312.1
Human coronavirus OC43	KX344031.1	Respiratory syncytial virus	JN032120.1
	KF530099.1	Rhinovirus	NC_001490.1
Human coronavirus HKU1	KF430201.1	*Chlamydia pneumoniae*	NZ_LN847241.1
	MH940245.1	*Haemophilus influenzae*	CP000672.1
Human coronavirus NL63	KF530114.1	*Legionella pneumophila*	CP015928.1
	KF530113.1	*Mycobacterium tuberculosis*	AP018036.1
SARS coronavirus	AY686863.1	*Streptococcus pneumoniae*	CP027540.1
		Streptococcus pyogenes	AE009949.1
MERS coronavirus	MH013216.1	*Bordetella pertussis*	CP011448.1
Adenovirus	AC_000017.1	*Mycoplasma pneumoniae*	CP039772.1
Human Metapneumovirus (hMPV)	KJ627437.1	*Pneumocystis jirovecii* (PJP)	MH010446.1
Parainfluenza virus 1	KX639498.1	*Candida albicans*	NC_018046.1
Parainfluenza virus 2	KM190939.1	*Pseudomonas aeruginosa*	CP000438.1
Parainfluenza virus 3	KF530243.1	*Staphylococcus epidermis*	KY750253.1
Parainfluenza virus 4	KF483663.1	*Streptococcus salivarius*	CP020451.2
Influenza A	MH798556.1		

Table 13. Cross Reactivity and Interference Testing for *H. Influenzae*

SAMPLE		Valid results	N gene (HEX)			Nsp2 gene (FAM)			SPC2 (Q-705)
			Positive	% Positive	Avg Ct	Positive	% positive	Avg Ct	Avg Ct
Cross Reactivity	Neat UVT (Control Negative)	3	0	0%	N/A	0	0%	N/A	27.7
	UVT+H. Influenzae (7.2E6 CFU/mL)	3	0	0%	N/A	0	0%	N/A	28.3
Interference	Neat UVT + SARS-CoV-2 RNA (750 copies/mL) (Control Positive)	3	3	100%	32.03	3	100%	34.05	27.8
	UVT+H. Influenzae (7.2E6 CFU/mL) + SARS-CoV-2 RNA (750 copies/mL)	3	3	100%	32.45	3	100%	33.98	27.7

Table 14. Cross Reactivity and Interference Testing for *P. aeruginosa*

SAMPLE		Valid results	N gene (HEX)			Nsp2 gene (FAM)			SPC2 (Far Red)
			Positive	% Positive	Avg Ct	Positive	% positive	Avg Ct	Avg Ct
Cross-reactivity	UVT+P. aeruginosa (1^E6 CFU/mL)	3	0	0%	N/A	0	0%	N/A	27.5
Interference	Neat UVT Control Positive	3	3	100%	30.3	3	100%	32.0	26.9
	UVT + P. aeruginosa (1^E6 CFU/mL) + SARS-CoV-2 RNA (450 copies/mL)	3	3	100%	30.4	3	100%	32.0	27.0

Interfering Substances – Nasopharyngeal Swab Samples

The NeuMoDx 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 spiked with SARS-CoV-2 genomic RNA (BEI Resources NR-52285) at 5X LoD and processed in the presence and absence of the agents shown below in *Table 15*. No substances included in the testing had an adverse effect on the assay performance.

Table 15. Substances Tested for Interference – Nasopharyngeal Swab Samples

	Substance	Concentration*
Endogenous	Mucin	0.5% (w/v)
	Blood (human)	2% (v/v)
Exogenous	Afrin® Original (oxymetazoline)	15% (v/v)
	Zicam® Cold Remedy Nasal Spray	5% (v/v)
	Flonase® Allergy Relief (fluticasone)	5% (v/v)
	Beclomethasone	10 mg/mL
	Mupirocin	11.4 mg/mL
	Relenza® (zanamivir)	5.25 mg/mL
	Tamiflu® (oseltamivir)	7.5 mg/mL
	Tobramycin	1.8 mg/mL

*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.

Interfering Substances – Saliva Samples

The NeuMoDx SARS-CoV-2 Assay was evaluated for susceptibility to interference caused by substances potentially associated with the collection of saliva specimens. Pooled negative saliva was spiked with γ-irradiated SARS-CoV-2 virus (BEI Resources NR-52287) at 10X LoD, prepared with the NeuMoDx Saliva Collection Kit, and processed in the presence and absence of the agents shown below in *Table 16*. No substances included in the testing had an adverse effect on the assay performance at the given concentrations.

Table 16. Substances Tested for Interference – Saliva Samples

	Substance	Concentration
Endogenous	Whole Blood	1% v/v
Exogenous	Altoids™ (Spearmint)	2% w/v
	Aspirin™	1% w/v
	LISTERINE® Ultra-clean Antiseptic Mouthwash	1% v/v
	Halls™ Cough Drop (Mentho-Lyptus)	1% w/v
	Crest° Pro-Health Advanced Gum Protection	0.001% w/v*
	Wal-Tussin® DM Max Cough Syrup	1% v/v

*Concentration of this substance is reported as a result of a dose response study from 0.1%, where it was shown to be inhibitory.

Clinical Performance

a. Nasopharyngeal Swab Specimens

The performance of the NeuMoDx SARS-CoV-2 Assay was also evaluated using clinical specimens. Leftover deidentified clinical nasopharyngeal (NP) swab specimens from symptomatic patients were collected with flocked minitip swabs into 3 mL BD Universal Viral Transport Medium (BD UVT). The specimens were submitted for SARS-CoV-2 testing to two external testing sites which performed the Comparator testing of these specimens with tests previously authorized by the U.S. FDA for emergency use. Testing with the NeuMoDx SARS-CoV-2 Assay was performed at one internal and one external testing site. A total of 40 samples were processed using the NeuMoDx SARS-CoV-2 Assay. Some samples were tested at both, the N288 and the N96 NeuMoDx Systems and employing both PRETREATED and DIRECT workflows. Results of the NeuMoDx SARS-CoV-2 Assay were in complete agreement with the comparator assay results for all clinical samples tested in this method comparison study (Tables 17 and 18).

Table 17. Qualitative Method Comparison Results for the NeuMoDx SARS-CoV-2 Assay on NeuMoDx Molecular Systems v. Reference Tests – PRETREATED Workflow

N96 and N288 Pretreated		Comparator Assay(s)		
		Pos	Neg	Total
NeuMoDx SARS-CoV-2 Assay	Pos	25	0	25
	Neg	0	15	15
	Total	25	15	40
Clinical sensitivity 100% (95% CI 86.6-100%)				
Clinical specificity 100% (95% CI 79.5-99.9%)				

Table 18. Qualitative Method Comparison Results for the NeuMoDx SARS-CoV-2 Assay v. Reference Tests – DIRECT Workflow (**a)** on the NeuMoDx 288 Molecular System (N288) and (**b)** on the NeuMoDx 96 Molecular System (N96)

(a)

N288 Direct		Comparator Assay(s)		
		Pos	Neg	Total
NeuMoDx SARS-CoV-2 Assay	Pos	10	0	10
	Neg	0	9	9
	Total	10	9	19
Clinical sensitivity 100% (95% CI 72.1-99.9%)				
Clinical specificity 100% (95% CI 69.9-99.9%)				

(b)

N96 Direct		Comparator Assay(s)		
		Pos	Neg	Total
NeuMoDx SARS-CoV-2 Assay	Pos	5	0	5
	Neg	0	6	6
	Total	5	6	11
Clinical sensitivity 100% (95% CI 56.4-99.9%)				
Clinical specificity 100% (95% CI 60.8-99.9%)				

b. Saliva Specimens

The performance of the NeuMoDx SARS-CoV-2 Assay with saliva samples (prepared using the NeuMoDx™ Saliva Collection Kit) was evaluated comparing paired NP swab and saliva specimens using 61 deidentified paired saliva and nasopharyngeal (NP) swab specimens collected from the same individual. Saliva specimens were collected using the NeuMoDx™ Saliva Collection Kit, while the NP swab specimens were collected with flocked minitip swabs into 3 mL BD Universal Viral Transport Medium (BD UVT). All saliva specimens were tested using the emergency authorized NeuMoDx SARS-CoV-2 Assay. Nasopharyngeal swab (NP) specimens were processed using emergency use authorized comparator tests. Testing was performed at one internal site on a combination of N288 and N96 NeuMoDx Systems. Overall, > 95% positive and negative concordance to reference test results for NP swab specimens was demonstrated for the NeuMoDx SARS-CoV-2 Assay using saliva specimens, as detailed in *Table 19*.

Table 19. Qualitative Method Comparison Results for the NeuMoDx SARS-CoV-2 Assay with Saliva Specimens v. NP Swab Specimens

SARS-CoV-2 results		Comparators (NP swab)		
		Pos	Neg	Total
NeuMoDx SARS-CoV-2 Assay (Saliva)	Pos	31	0	31
	Neg	0	30	30
	Total	31	30	61
Positive Percent Agreement 100% (88.98%, 100.0%)				
Negative Percent Agreement 100% (88.65%, 100.0%)				

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity 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 the *Table 20*.

Table 20: Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swabs	$5.4x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

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

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.

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SYMBOL KEY

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

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