REF 200300 NeuMoDx™ CT/NG Test Strip

℞ only

CAUTION: For US Export Only

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

For insert updates, go to: www.neumodx.com/client-resources
For detailed instructions, refer to the NeuMoDx™ 288 Molecular System Operator's Manual; P/N 40600108
For detailed instructions, refer to the NeuMoDx™ 96 Molecular System Operator's Manual; P/N 40600317

INTENDED USE

The NeuMoDx CT/NG Assay, as performed on the NeuMoDx™ 96 Molecular System and NeuMoDx™ 288 Molecular System, is an automated, qualitative *in vitro* nucleic acid amplification test for the direct detection and differentiation of *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (NG) DNA in urogenital specimens. The assay utilizes real-time polymerase chain reaction (PCR) for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA in clinician-collected vaginal swab specimens, self-collected vaginal swab specimens (collected in a clinical setting) and endocervical swab specimens, all collected using a polyester tip swab with a plastic applicator in a universal transport medium (Universal Transport Medium, UTM-RT®, Copan Diagnostics, CA, USA, or BD™ Universal Viral Transport System, BD™ UVT, Becton, Dickinson and Company, MD, USA or equivalent), cervical specimens collected in PreservCyt® solution (Hologic, Inc, MA, USA), and male and female urine. The NeuMoDx™ CT/NG Assay is intended to be used as an aid in the diagnosis of chlamydial and gonococcal urogenital disease in both symptomatic and asymptomatic individuals.

SUMMARY AND EXPLANATION

To test a urine specimen using the NeuMoDx CT/NG Assay, a urine sample is collected in a standard urine collection cup with no preservatives or additives. To prepare for testing, an aliquot of the urine is dispensed into a secondary tube compatible with the NeuMoDx System and loaded onto the NeuMoDx System in designated sample carriers to begin processing. For each sample, a 550µL aliquot of the urine is mixed with NeuMoDx™ Lysis Buffer 2 and the NeuMoDx System automatically performs all the steps required to extract the target nucleic acid, prepare the isolated DNA for real-time PCR amplification, and if present, amplify and detect the targets of amplification (sections of the *targeted* gene sequences of the CT and NG chromosomes and plasmids).

To test a swab specimen using the NeuMoDx CT/NG Assay, an endocervical swab sample or a clinician or self-collected vaginal swab sample must be collected using a polyester tip swab with plastic applicator in 3 mL of Universal Transport Medium (UTM-RT, UVT) or equivalent. The swab sample may be tested directly from the primary transport medium tube or an aliquot dispensed into a secondary tube compatible with the NeuMoDx System and loaded onto the NeuMoDx System using the appropriate sample carrier to begin processing. If a sample has been frozen, it is recommended to pre-heat the thawed sample at 85 °C for 5-10 minutes prior to testing. For each sample, a 400µL aliquot of the swab media is mixed with NeuMoDx™ Lysis Buffer 2 and the NeuMoDx System automatically performs all the steps required to extract the target nucleic acid, prepare the isolated DNA for real-time PCR amplification, and if present, amplify and detect the targets of amplification (sections of the *targeted* gene sequences of the CT and NG chromosomes and plasmids).

To test a cytology specimen using the NeuMoDx CT/NG Assay, a ThinPrep® Pap Test is collected by a clinician according to the manufacturer's instructions. Following processing on a ThinPrep® Processor, an aliquot of the PreservCyt® solution should be dispensed into a secondary tube compatible with the NeuMoDx System and loaded onto the NeuMoDx System using the appropriate sample carrier to begin processing. It is required to bring the specimen to room temperature prior to processing. For each sample, a 550µL aliquot of the PreservCyt liquid is mixed with NeuMoDx™ Lysis Buffer 2 and the NeuMoDx System automatically performs all the steps required to extract the target nucleic acid, prepare the isolated DNA for real-time PCR amplification, and if present, amplify and detect the targets of amplification (sections of the *targeted* gene sequences of the CT and NG chromosomes and plasmids).

The NeuMoDx CT/NG Assay includes a DNA Sample Process Control (SPC1) to help monitor for the presence of potential inhibitory substances and NeuMoDx System or reagent failures that may be encountered during the extraction and amplification processes.

Chlamydia trachomatis and *Neisseria gonorrhoeae* infections are two of the most common sexually transmitted infections worldwide. In the United States, over 1.6 million new cases of chlamydia and 470,000 gonorrhea were diagnosed in 2016, the highest number ever according to the latest report by the Centers for Disease Control and Prevention (CDC) (CDC, 2017).[1]

Chlamydiae are non-motile, gram-negative, obligate intracellular bacteria. The *Chlamydia trachomatis* species is comprised of fifteen serovars (A, B, Ba, C, D, E, F, G, H, I, J, K, L1, L2 and L3) that can cause disease in humans.[2] Serovars D through K are the major cause of genital chlamydial infections in men and women.[2] *C. trachomatis* can cause non-gonococcal urethritis, epididymitis, proctitis, cervicitis, acute salpingitis, and Pelvic Inflammatory Disease (PID).[3-6] Chlamydial infections are often asymptomatic in both males and females. Children born to infected mothers are at significantly higher risk for inclusion conjunctivitis and chlamydial pneumonia.[7,8] Untreated infection can result in PID, which is a major cause of infertility, ectopic pregnancy, and chronic pelvic pain.[5] Data from randomized controlled trials of chlamydia screening suggest that screening programs can lead to a reduction in the incidence of PID.[9-12] As with other inflammatory STDs, chlamydial infection could facilitate the transmission of HIV infection.[13] In addition, pregnant women infected with chlamydia can pass the infection to their infants during delivery, potentially resulting in ophthalmia neonatorum, which can lead to blindness and pneumonia. Because of the large burden of disease and risks associated with infection, CDC recommends annual chlamydia screening for all sexually active women younger than age 25 years and women ≥ 25 years at increased risk for infection (e.g., women with new or multiple sex partners).[14]

Neisseria gonorrhoeae is the causative agent of gonorrheal disease. *N. gonorrhoeae* are non-motile, gram-negative diplococci. The most common site of *N. gonorrhoeae* infection is the urogenital tract. NG infections tend to cause a stronger inflammatory response than *C. trachomatis* but are typically asymptomatic in women until complications such as PID develop.[15] PID can lead to tubal infertility, ectopic pregnancy, and chronic pelvic pain. In men, the majority of urethral infections cause urethritis with painful urination or dysuria with penile

discharge (usually symptomatic) and, less commonly, epididymitis or disseminated gonococcal infection.[15] In addition, epidemiologic and biologic studies provide strong evidence that gonococcal infections facilitate the transmission of HIV infection.[13] The CT/NG Assay utilizes real-time PCR to detect a region of the multi-copy opacity gene on the *Neisseria gonorrhoeae* chromosome.

Historically, culture for *C. trachomatis* and *N. gonorrhoeae* was the "gold standard" for detection of CT/NG. However, culture methods require the viability of the organisms to be maintained during transport and storage. Culture methods for CT are difficult to standardize, technically demanding, expensive, labor intensive and relatively insensitive. Culture methods for conventional diagnosis of NG infection can have good clinical sensitivity but require isolation of the organism on selective media and are highly dependent on proper specimen handling. Improper specimen storage and transport can result in the loss of organism viability and yield false negative results. In addition, poor sampling technique, toxic sampling materials, and the inhibition of growth by components of body secretions can also result in false negative results. These drawbacks make culture methods less ideal to implement as routine screening tests. Multiple non-culture laboratory tests, including nucleic acid amplification test (NAAT) methods have been developed for the detection of chlamydia and gonorrhea. Since 2002, improvements in NAAT technologies along with the use of less invasive specimen collection have enabled significant adaptation of NAATs in diagnosis of CT and NG. Nucleic acid amplification testing is now the only recommended method among the non-culture methods for routine laboratory use in CT/NG testing by CDC since 2014.[16] The CT/NG Assay utilizes real-time PCR to detect two distinct regions in *Chlamydia trachomatis,* one targeting the helicase gene present in the multiple copy cryptic plasmid and one targeting the outer membrane gene of the CT chromosome. As such, the detection of CT is not affected by the recent mutation identified in the 23S region of the CT chromosome, or by the deletion in the plasmid in the nvCT identified in Sweden in 2006.

PRINCIPLES OF THE PROCEDURE

The NeuMoDx CT/NG Assay combines the technologies of DNA extraction and amplification/detection by real-time PCR. Specimens are collected in conventional urine specimen collection cups, swab specimen collection tubes (UTM-RT, UVT or equivalent), or PreservCyt® Liquid (a ThinPrep® Pap Test). The NeuMoDx System automatically aspirates an aliquot of the urine, swab, or cytology specimen to mix with NeuMoDx Lysis Buffer 2 and the extraction reagents contained in the NeuMoDx™ Extraction Plate to begin processing. The NeuMoDx System automates and integrates DNA extraction and concentration, reagent preparation, and nucleic acid amplification and detection of the target sequence using real-time PCR. The included Sample Process Control (SPC1) helps monitor for the presence of potential inhibitory substances as well as system, process, or reagent failures. No operator intervention is necessary once the specimen is loaded onto the NeuMoDx System.

The NeuMoDx Systems use a combination of heat, lytic enzyme, and extraction reagents to perform cell lysis, DNA extraction and removal of inhibitors. The released nucleic acids are captured by paramagnetic particles. The particles, with the bound nucleic acids, are loaded into the NeuMoDx™ Cartridge where the unbound, non-DNA components are further washed away with NeuMoDx™ Wash Reagent and the bound DNA is eluted using NeuMoDx™ Release Reagent. The NeuMoDx System then uses the eluted DNA to rehydrate proprietary NeuDry™ amplification reagents containing all the elements necessary for amplification of the CT and NG targets and a section of the SPC1 sequence. This enables simultaneous amplification and detection of both target(s) and control DNA sequences. After reconstitution of the dried PCR reagents, the NeuMoDx System dispenses the prepared PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Amplification and detection of the control and target (if present) DNA sequences occur in the PCR chamber. The NeuMoDx Cartridge, including the PCR chamber, is designed to contain the amplicon following real-time PCR, thereby essentially 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 for 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, resulting in the quencher molecule quenching the fluorescence emitted by the fluorophore via FRET (Förster Resonance Energy Transfer).

TaqMan® probes are designed to 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 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 thermal cycler is directly proportional to the fluorophore released.

A TaqMan® probe labeled with a fluorophore (Excitation: 490 nm & Emission: 521 nm) at the 5' end and a dark quencher at the 3' end is used to detect NG DNA and a TaqMan® probe labeled with a fluorophore (Excitation: 590 nm & Emission: 610 nm) at the 5' end and a dark quencher at the 3' end is used to detect CT DNA. For detection of the Sample Process Control, the TaqMan® probe is labeled with an alternate fluorescent dye (Excitation: 535 nm & Emission: 556 nm) at the 5' end, and a dark quencher at the 3' end. The NeuMoDx System monitors the fluorescent signal emitted by the TaqMan® probes at the end of each amplification cycle. When amplification is complete, the NeuMoDx System analyzes the data and reports a final qualitative result (POSITIVE/NEGATIVE/INDETERMINATE/UNRESOLVED/NO RESULT).

REAGENTS / CONSUMABLES

Material Provided

REF	Contents	Units per package	Tests per unit	Tests per package
200300	**NeuMoDx™ CT/NG Test Strip** *Dried real-time PCR reagents containing CT/NG-specific TaqMan® probes and primers with Sample Process Control-specific TaqMan® probe and primers.*	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**
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**

Swab and Transport Media (Not Provided)

Sample Type	Recommended Medium	Recommended Collection Device
Vaginal or Endocervical Swab	3mL Universal Transport Medium (Copan UTM-RT®) **or** 3mL Universal Viral Transport System (BD™ UVT)	Flexible Minitip Nylon® Flocked Swab (Copan) **or** Flexible Minitip Flocked Swab (BD)
Cytology Specimen	PreservCyt® Solution liquid Pap specimen	Broom-type or endocervical brush/ plastic spatula combination

Instrumentation Equipment Required but Not Provided

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

⚠ ⊗ WARNINGS AND PRECAUTIONS

- The NeuMoDx™ CT/NG Test Strip is for *in vitro* diagnostic use with NeuMoDx™ Systems only.
- Do not use the consumables or reagents 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.
- Do not use urine collected in containers with preservatives. The NeuMoDx CT/NG Assay has not been validated for use with preservatives.
- Swab specimens should be collected using a polyester swab with a plastic applicator. Remove swab from transport media prior to testing. The NeuMoDx CT/NG Assay has not been validated for use with other swab types.
- Do not collect swab specimens in transport media other than UTM-RT, UVT, or equivalent. The NeuMoDx CT/NG Assay has not been validated for use with other transport media.
- Cytology specimens should be collected by a clinician and according to the ThinPrep® Pap Test sample collection instructions. ThinPrep® Pap Tests are collected into PreservCyt® Liquid.
- Do not collect cytology specimens into media other than PreservCyt® Liquid. The NeuMoDx CT/NG Assay has not been validated for use with other cytology preservatives.
- Cytology specimens should be brought to room temperature before testing on NeuMoDx™ Systems. For specimens kept at 4°C with 1 mL aliquoted into a daughter tube, a 30-minute incubation at room temperature is recommended. For full ThinPrep containers (~20 mL PreservCyt) kept at 4°C, a 40-minute incubation at room temperature is recommended.
- Minimum specimen volume is dependent on the tube size and specimen tube carrier as defined below:
  - **Specimen Tube Carrier (32-tube)**: ≥ 700 µL of specimen is required when using secondary tubes appropriate for the 32-tube Specimen Tube Carrier; Volume below the specified minimum may result in a "Quantity Not Sufficient" error.
  - **Specimen Tube Carrier (24-tube)**: ≥ 2mL of specimen is required when using primary tubes, or ≥1.1 mL of specimen is required when using secondary tubes appropriate for the 24-tube Specimen Tube Carrier. Volume below the specified minimum may result in a "Quantity Not Sufficient" error.
  - **Low Volume Specimen Tube Carrier (32-tube)**: ≥ 650 µL of urine or cytology specimen or ≥ 550 µL of swab specimen is required when using secondary tubes appropriate for the 32-tube Low Volume Specimen Tube Carrier. Volume below the specified minimum may result in a "Quantity Not Sufficient" error.

- Performing a CT/NG test on urine or swab specimens more than 7 days old may produce invalid or erroneous results when using the NeuMoDx CT/NG Test Strip.
- Performing a CT/NG test on a cytological specimen more than 30 days old (when stored at 2°C- 30°C) may produce invalid or erroneous results (see ThinPrep® Pap Test manufacturer's recommendation).
- Avoid microbial and deoxyribonuclease (DNase) contamination of reagents. The use of sterile DNase-free disposable transferring pipettes is recommended. 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 Biohazardous Waste Container 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 CT/NG Test Strip, the 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 CT/NG Test Strip or NeuMoDx Extraction Plate, or the top surface of the NeuMoDx Lysis Buffer 2; 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 kit reagents are being handled.
- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in *Biosafety in Microbiological and Biomedical Laboratories*[17] and in CLSI Document M29-A3.[18]
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state and local regulations.
- Do not reuse.

PRODUCT STORAGE, HANDLING, AND STABILITY

- NeuMoDx CT/NG Test Strips are stable in the primary packaging through the stated expiration date on the immediate product label when stored between 15 - 28°C. NeuMoDx CT/NG Test Strip's shelf life is 24 months from the date of manufacture.
- 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.
- Once loaded, the NeuMoDx CT/NG Test Strip may remain onboard the NeuMoDx System for 14 days. Remaining shelf life of loaded test strips is tracked by the software and reported to the user in real time. Removal of a test strip that has been in use beyond its allowable period will be prompted by the System.

SPECIMEN COLLECTION, TRANSPORT, AND STORAGE

- The NeuMoDx CT/NG Test Strip has been tested using female and male neat urine specimens, clinician and self-collected vaginal swab specimens, endocervical swab specimens, and PreservCyt Liquid from ThinPrep Pap Tests. Swab specimens should be taken using a polyester tip swab with plastic applicator (UTM-RT, UVT, or equivalent). ThinPrep Pap Tests should be collected according to manufacturer recommendation. Performance with other specimen types other than those mentioned have not been evaluated.
- Collected urine specimens should be kept between 2 - 8 °C during transport.
- Collected swab specimens should be kept at the temperature recommended in the swab collection kit during transport.
- Urine and swab specimens should be stored between 2 - 8 °C for no longer than 7 days prior to testing and a maximum of 24 hours at room temperature.
- Cytology specimens may be stored at 2 - 30°C for up to 30 days and used according to manufacturer (Hologic, Inc, MA, USA) recommendation.

INSTRUCTIONS FOR USE

Specimen Collection/Transport

1. First catch urine (recommended by CDC[16]) should be collected into urine collection cups with no preservatives. If possible, the patient should not urinate for at least 1 hour prior to specimen collection.
2. Clinician and self-collected vaginal swabs, as well as endocervical swabs, should be collected into following instructions provided by the manufacturer with the swab collection device.
3. Cytology specimens should be collected by a clinician following instructions provided by the manufacturer with the ThinPrep® Pap Test collection kit.
4. If swab and/or urine specimens are not tested within 24 hours, they should be stored between 2 - 8 °C for up to 7 days prior to testing. Cytology specimens may be stored at 2 - 30 °C for up to 30 days according to manufacturer (Hologic, Inc, MA, USA) recommendation.

Test Preparation – *Urine Specimen*

1. Apply specimen barcode label to a specimen tube compatible with the NeuMoDx System.
2. Gently swirl the urine specimen in the parent container to achieve uniform distribution.
3. Using a different transfer pipette or pipette tip for each specimen, transfer an aliquot of urine to the barcoded specimen tube compatible with the NeuMoDx System.

Test Preparation – *Swab Specimen*

1. Apply specimen barcode label to a specimen tube compatible with the NeuMoDx System. The primary swab collection tube may be labeled and placed directly into the 24-tube specimen carrier. Alternatively, an aliquot of the swab medium may be transferred to a secondary tube for processing on the NeuMoDx System.
2. Briefly vortex the swab specimen in the parent container to achieve uniform distribution.
3. If testing the swab specimen in the primary collection tube, place the barcode-labeled tube into a 24-Tube Specimen Tube Carrier and ensure that the cap is removed prior to loading on to the NeuMoDx System.
4. If using a secondary tube, transfer an aliquot of the swab specimen to the barcoded specimen tube compatible with the NeuMoDx System.

Test Preparation – *Cytology Specimen*

1. Apply specimen barcode label to a specimen tube compatible with the NeuMoDx System.
2. Gently swirl the PreservCyt Liquid to achieve uniform distribution. The NeuMoDx CT/NG Assay has only been validated with post-processed ThinPrep® liquid cytology specimens.
3. Using a different transfer pipette or pipette tip for each specimen, transfer an aliquot of PreservCyt to the barcoded specimen tube compatible with the NeuMoDx System.

NeuMoDx System Operation

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

1. Load the test order onto the NeuMoDx System according to the desired specimen type (Urine, Transport Medium, or Cytology) and tube type. If not defined in the test order, the **Urine** specimen type in a **Secondary Tube** will be used as default.
2. Populate one or more NeuMoDx Test Strip Carrier(s) with NeuMoDx CT/NG Test Strip(s) and use the touchscreen to load the Test Strip Carrier(s) into the NeuMoDx System.
3. If prompted by the NeuMoDx System software, add the necessary required consumables to the NeuMoDx System consumable carriers and use the touchscreen to load carrier(s) into the NeuMoDx System.
4. If prompted by the NeuMoDx System software, replace NeuMoDx Wash Reagent, NeuMoDx Release Reagent, empty the Priming Waste, Biohazard Waste Container (NeuMoDx 288 only), Tip Waste Bin (NeuMoDx 96 only) or Biohazard Waste Bin (NeuMoDx 96 only), as appropriate.
5. Load the specimen tube(s) into an appropriate Specimen Tube Carrier, and ensure caps are removed from all specimen tubes.
6. Place the Specimen Tube Carrier on the autoloader shelf and use the touchscreen to load carrier into the NeuMoDx System. This will initiate processing of the specimen(s) loaded for the tests identified.

LIMITATIONS

- The NeuMoDx CT/NG Test Strip can only be used on NeuMoDx Systems.
- The performance of the NeuMoDx CT/NG Test Strip has been established with male and female urine specimens, self-collected and clinician-collected vaginal swabs, endocervical swab specimens, and PreservCyt liquid cytology specimens. Use of the NeuMoDx CT/NG Test Strip with other clinical sources has not been assessed and performance characteristics are unknown for other specimen types.
- Because detection of CT and NG is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
- Erroneous test results could occur from improper specimen collection, handling, storage, technical error, or sample mix-up. In addition, false negative results could occur because the number of organisms in the specimen is below the analytical sensitivity of the test.
- Testing is limited to use by personnel trained on the use of the NeuMoDx System.
- If the Sample Process Control does not amplify and the NeuMoDx CT/NG test result is Negative, an invalid result (Indeterminate or Unresolved) will be reported and the test should be repeated.
- A positive test result does not necessarily indicate the presence of viable organisms. It is, however, presumptive for the presence of CT and/or NG DNA.
- While there are no known strains/isolates of NG lacking the *Opacity* genes, the occurrence of such a strain could lead to an erroneous result using the NeuMoDx CT/NG Test Strip.

- The NeuMoDx CT/NG test incorporates both genomic and a plasmid (cryptic plasmid) targets for CT to ensure accurate detection of all strains. However, an erroneous result could occur if CT strains/isolates do not have a cryptic plasmid as well as the porin protein gene in the genome.
- Mutations in primer/probe binding regions may affect detection using the NeuMoDx CT/NG Assay.
- Results from NeuMoDx CT/NG test should be used as an adjunct to clinical observations and other information available to the physician. The test is not intended to differentiate carriers of CT and/or NG DNA from those with chlamydial and/or or gonococcal disease.
- Test results may be affected by concurrent antibiotic therapy as CT and NG DNA may continue to be detected following antimicrobial therapy.
- Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination of specimens.

RESULTS

NeuMoDx Molecular Systems

Available results may be viewed or printed from the 'Results' tab in the Results window on the NeuMoDx System touchscreen. NeuMoDx CT/NG Assay results are automatically generated by the NeuMoDx System software using the decision algorithm and results processing parameters specified in the NeuMoDx CT/NG Assay Definition File (ADF). A test result may be reported as Positive, Negative, Indeterminate (IND), No Result (NR), or Unresolved (UNR) based on the amplification status of the target and the Sample Process Control (SPC1).

Criteria for a positive or negative call is specified in the NeuMoDx System CT/NG Assay Definition File (ADF) as installed on the system by NeuMoDx. Results are reported based on the ADF decision algorithm, summarized below in *Table 1*.

Table 1. Summary of NeuMoDx CT/NG Test Decision Algorithm

RESULT	CT and/or NG TARGETS	PROCESS CONTROL (SPC1)
Positive	Amplified	N/A
Negative	Not Amplified	Amplified
Indeterminate†	Not Amplified, System Error Detected, Sample Processing Completed	
No Result*†	Not Amplified, System Error Detected, Sample Processing Aborted	
Unresolved†	Not Amplified, No System Error Detected	

*No Result flag is only reported on NeuMoDx System software versions 1.8 and higher
†The NeuMoDx System is equipped with automatic Rerun/Repeat capability that the end user can choose to use to ensure that IND/UNR/NR result is automatically reprocessed to minimize delays in result reporting.

Invalid Results

If a NeuMoDx CT/NG Assay performed on the NeuMoDx System fails to produce a valid result, it will be reported as Indeterminate (IND), No Result (NR), or Unresolved (UNR) based on the type of error that occurred.

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

If a NeuMoDx CT/NG Assay performed on the NeuMoDx System fails to produce a valid result and sample processing is aborted prior to completion, it will be reported as No Result (NR).

NOTE: Upon receiving an invalid (IND/UNR/NR) result, the user may perform an optional step to heat the sample for 5-10 minutes at 85°C *prior* to repeating the assay.

Quality Control

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

1. External (User-Defined) control materials will not be provided by NeuMoDx Molecular, Inc. Appropriate controls must be chosen and validated by the laboratory. The NeuMoDx Software (version 1.8 and above) allow multiple specimen types to be assigned to the same set of controls. Alternatively, a separate set of controls can be defined for each specimen type. The External Controls must meet the same minimum volume specifications as clinical samples specified above based on the tube/specimen carrier size. The user may define the specific barcodes per Positive and Negative Control and per matrix.

2. Recommended: 10 µL of AcroMetrix™ CT/NG Positive Control (Thermo Fisher Scientific REF 967146) diluted in 1 mL CT/NG negative urine or a commercially available urine chemistry control as the urine matrix control, in 1mL UTM-RT as the swab matrix control, or in 1mL PreservCyt as the cytology matrix control using the 32-tube Specimen Tube Carrier. If 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. The NeuMoDx System will recognize the barcodes and start processing controls unless adequate regents or consumables required for testing are not loaded.
3. The primers and probe specific for Sample Process Control 1 (SPC1) are included in each NeuMoDx CT/NG Test Strip. This Sample Process Control allows the NeuMoDx System to monitor the efficacy of the DNA extraction and PCR amplification processes.
4. A positive test result reported for a negative control sample indicates a specimen contamination problem. Please refer to NeuMoDx™ *288 or 96 Molecular System Operator's Manual* for Troubleshooting tips.
5. 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

Clinical Performance in Urine Specimens

Clinical performance characteristics of the NeuMoDx CT/NG Assay were determined through an internal retrospective method comparison study using residual urine specimens sourced from three (3) geographically diverse laboratory locations.

Residual urine specimens were de-identified and given a unique ID number by clinical laboratories, establishing a confidential list linking the patient ID to the de-identified specimens tested for study purposes. A total of 388 pre-screened specimens provided from three clinical laboratories were tested. Among the 388 samples, 90 samples were identified as CT positive and 53 samples were identified as NG positive by the clinical laboratories. Some specimens tested positive for both CT and NG, indicating a dual or co-infection. The test status of these samples was withheld from the operator to implement a "single blind study." Results reported from the specific FDA-cleared and CE-marked, legally marketed molecular devices utilized by the laboratories for standard of care testing were used to perform the method comparison analysis.

Results of the NeuMoDx CT/NG test provided a Clinical Sensitivity of 96.7% for the CT target and 98.1% for the NG target, both reported at 95%CI. The Clinical Specificity from the study was determined to be 99.7% for both CT and NG, again using 95% CI. The lower and upper limits of the 95% confidence interval (CI) presented in *Tables 2A* and *2B* below were calculated using the Wilson procedure with continuity correction.

Table 2A.
Clinical Performance Summary in Urine – NeuMoDx 288
NeuMoDx CT/NG Test Strip Detection of *C. trachomatis*

CT (urine specimens)		FDA / CE Reference Test Result		
		POS	NEG	Total
NeuMoDx CT/NG Test	POS	87	1	*88*
	NEG	3	297	*300*
	Total	*90*	*298*	*388*
Clinical Sensitivity (CT) = 96.7% (89.9 - 99.1)				
Clinical Specificity (CT) = 99.7% (97.8 - 99.9)				

Table 2B.
Clinical Performance Summary in Urine – NeuMoDx 288
NeuMoDx CT/NG Test Strip Detection of *N. gonorrhoeae*

NG (urine specimens)		FDA / CE Reference Test Result		
		POS	NEG	Total
NeuMoDx CT/NG Test	POS	51	1	*52*
	NEG	1	335	*336*
	Total	*52*	*336*	*388*
Clinical Sensitivity (NG) = 98.1% (88.4 - 99.9)				
Clinical Specificity (NG) = 99.7% (98.1 - 99.9)				

Additional testing was performed on the NeuMoDx 96 Molecular System using a reduced number of residual clinical urine samples. As with the previous testing performed on the NeuMoDx 288, results obtained from the NeuMoDx 96 were compared to the results reported by the FDA-cleared and CE-marked assays utilized by source laboratories for the standard of care testing. The 208 valid results are summarized with the 95% CI in *Table 2C*, below.

Table 2C. Clinical Performance Summary in Urine – NeuMoDx 96
NeuMoDx CT/NG Test Strip Detection of *C. trachomatis* and *N. gonorrhoeae*

Performance Summary (NeuMoDx CT/NG Assay on the NeuMoDx 96 Molecular System compared to FDA/CE Reference Test Result)	
CT	**NG**
Sensitivity: 92.8% (83.2 – 97.3)	Sensitivity: 92.8% (83.2 – 97.3)
Specificity: 99.3% (95.4 – 99.9)	Specificity: 99.3% (95.4 – 99.9)

Based on the population, performance of the NeuMoDx CT/NG Assay on the NeuMoDx 288 Molecular System and the reduced number of clinical samples tested on the NeuMoDx 96, the anticipated clinical sensitivity is a value within the two-sided 95% CI of (86.9% - 100%) for CT and (90.6% - 100%) for NG. The anticipated clinical specificity for both targets is a value within the two-sided 95% CI of (98.6% - 100%). The clinical performance of the NeuMoDx CT/NG Assay as demonstrated by the additional testing performed on the NeuMoDx 96 Molecular System was within the expected values as shown in the summary table above.

Clinical Performance in Swab Specimens

The clinical performance of the NeuMoDx CT/NG Assay in testing swab specimens collected in UVT was verified with an internal verification study using a combination of prospectively collected clinical specimens and residual clinical specimens from two (2) geographically diverse laboratory locations. Positive, contrived samples were used in addition to other clinical specimens due to the relatively low prevalence rate of CT and NG targets in swab specimens.

Prospective and residual swab specimens were de-identified and assigned a unique ID number by the external clinical laboratories from which they were sourced, establishing a confidential (and masked to NeuMoDx) link of the patient ID to the de-identified specimens tested for study purposes. A total of 110 vaginal swabs and 121 endocervical swabs provided from two clinical laboratories were tested. Of the swab specimens, 38 were identified as CT positive and 9 were identified as NG positive. An additional 48 vaginal and 48 endocervical swabs pre-screened to be *negative* for CT and NG were spiked to create the contrived samples (due to low prevalence of CT and NG) for a total of an additional 96 positive specimens. Among these positive samples, some specimens were positive for CT only, NG only, or both CT and NG targets. Results reported from the specific FDA-cleared and CE-marked, legally marketed molecular device by the source laboratories, or *expected* results for the contrived samples were used to perform the comparison analysis.

Results of the clinical method comparison study provided estimates of Clinical Sensitivity (100%) and Clinical Specificity (99.6%) for the CT target, and estimates of Clinical Sensitivity (100%) and Clinical Specificity (98.7%) for the NG target. Furthermore, Clinical Sensitivity and Clinical Specificity were very similar between the two swab types. For the endocervical swab matrix, results of the test gave estimates of Clinical Sensitivity (100%) and Clinical Specificity (99.2%) for the CT target, and Clinical Sensitivity (100%) and Clinical Specificity (99.1%) for the NG target. For the vaginal swab matrix, results of the test gave estimates of Clinical Sensitivity (100%) and Clinical Specificity (100%) for the CT target, and Clinical Sensitivity (100%) and Clinical Specificity (98.1%) for the NG target. The lower and upper limits of the 95% confidence interval (CI) presented in *Tables 3A* and *3B* below were calculated using the Wilson procedure with continuity correction.

Table 3A. Clinical Performance Summary in Swab (Endocervical & Vaginal) – NeuMoDx 288 & 96 Molecular Systems, NeuMoDx CT/NG Test Strip Detection of *C. trachomatis*

CT (swab specimens)		**FDA/CE Reference Test Result**		
		POS	**NEG**	**Total**
NeuMoDx CT/NG Test	**POS**	62	1	63
	NEG	0	263	*263*
	Total	62	264	326
Clinical Sensitivity (CT) = 100% (92.7-100)				
Clinical Specificity (CT) = 99.6% (97.6-100)				

Table 3B. Clinical Performance Summary in Swab (Endocervical & Vaginal) – NeuMoDx 288 & 96 Molecular Systems, NeuMoDx CT/NG Test Strip Detection of *N. gonorrhoeae*

NG (swab specimens)		**FDA/CE Reference Test Result**		
		POS	**NEG**	**Total**
NeuMoDx CT/NG Test	**POS**	103	3	106
	NEG	0	220	*220*
	Total	103	223	326
Clinical Sensitivity (NG) = 100% (95.5-100)				
Clinical Specificity (NG) = 98.7% (95.8-99.7)				

Clinical Performance in Cytology Specimens

Clinical performance characteristics of the NeuMoDx CT/NG Assay were determined through an internal retrospective method comparison study using residual PreservCyt liquid cytology specimens sourced from a single clinical laboratory.

Residual cytology specimens were de-identified and given a unique ID number by clinical laboratories, establishing a confidential list linking the patient ID to the de-identified specimens tested for study purposes. A total of 83 pre-screened specimens provided from the clinical laboratory were tested. Thirty additional NG positive samples were contrived from residual negative samples, for a total of 113 samples tested. Among the 113 samples evaluated, 30 samples were identified as CT positive and 33 samples (30 of these were contrived) were identified as NG positive by the clinical laboratory. No specimens tested positive for both CT and NG. The test status of these samples was withheld from the operator to implement a "single blind study." Results reported from the specific FDA-cleared and CE-marked, legally marketed molecular devices utilized by the laboratories for standard of care testing were used to perform the method comparison analysis.

Results of the NeuMoDx CT/NG test provided a Clinical Sensitivity of 100% for the CT target and 97.0% for the NG target, both reported at 95% confidence interval (CI). The Clinical Specificity from the study was determined to be 100% for both CT and NG, again using 95% CI. The lower and upper limits of the 95% CI presented in *Tables 4A* and *4B* below were calculated using the Wilson procedure without continuity correction.

Table 4A. Clinical Performance Summary for Cytology Specimens– NeuMoDx 288 & 96 Molecular Systems
NeuMoDx CT/NG Test Strip Detection of *C. trachomatis*

CT (cytology specimens)		**FDA / CE Reference Test Result**		
		POS	**NEG**	**Total**
NeuMoDx CT/NG Test	**POS**	30	0	*30*
	NEG	0	53	*53*
	Total	*30*	*53*	*83*
Clinical Sensitivity (CT) = 100% (88.7 - 100)				
Clinical Specificity (CT) = 100% (93.2 - 100)				

Table 4B. Clinical Performance Summary for Cytology Specimens – NeuMoDx 288 & 96 Molecular Systems
NeuMoDx CT/NG Test Strip Detection of *N. gonorrhoeae*

NG (cytology specimens)		**FDA / CE Reference Test Result**		
		POS	**NEG**	**Total**
NeuMoDx CT/NG Test	**POS**	32	0	*32*
	NEG	1	80	*81*
	Total	*33*	*80*	*113*
Clinical Sensitivity (NG) = 97.0% (84.7 - 99.5)				
Clinical Specificity (NG) = 100% (95.4 - 100)				

Analytical Sensitivity – Urine Specimens

The Limit of Detection of the NeuMoDx CT/NG Assay was determined with clinical negative urine spiked with either Acrometrix™ CT control (Serovar D) or AcroMetrix™ NG control at the levels indicated in the tables below. The tests were conducted with 10 replicates at each level on three days across two NeuMoDx 288 Molecular Systems using 3 lots of reagents (20 replicates/lot and 60 total). Detection rates are depicted in *Tables 5A* and *5B*. The limit of detection of CT was determined to be 4.5 EB/mL and LoD of NG was 0.22 cells/mL based on a Probit style analysis. Additional testing was performed with a reduced number of samples on the NeuMoDx 96 Molecular System, where Probit style analysis determined the LoD to be 7 EB/mL for CT and 0.3 cells/mL for NG.

The Limit of Detection of the NeuMoDx CT/NG Assay is claimed to be 6 EB/mL for CT and 5 cells/mL for NG based on the results of the Interference Study shown later.

Table 5A. Positive detection rates for CT in urine used in LoD Study for the NeuMoDx CT/NG Test Strip

CT (EB/mL)	n	# Positive	% Positive	LoD (Probit)
32	60	60	100%	**4.5 EB/mL**
16	60	60	100%	
8	60	60	100%	
4	59	54	91.5%	
2	60	38	63.3%	
0	60	0	0%	

Table 5B. Positive detection rates for NG in urine used in LoD Study for the NeuMoDx CT/NG Test Strip

NG (cells/mL)	n	# Positive	% Positive	LoD (Probit)
10	58	58	100%	**0.22 Cells/mL**
5	60	60	100%	
1	60	60	100%	
0.3	59	58	98.3%	
0.1	59	37	63.8%	
0	59	0	0%	

Probit style analysis of the data in the above tables was used to determine the LoD of the CT Target to be 4.5 EB/mL, and the LoD of the NG Target to be 0.22 cells/mL [*Figure 1*].

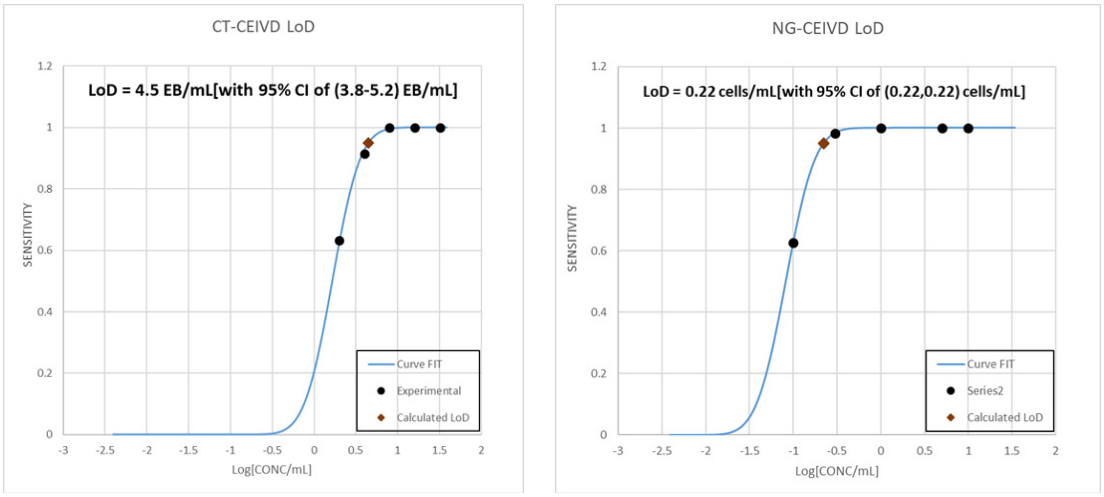

Figure 1. Probit style analysis to determine the LoD of the NeuMoDx CT/NG Assay using NeuMoDx CT/NG Test Strips.

Analytical Sensitivity – Swab Specimens

The Limit of Detection of the NeuMoDx CT/NG Assay was determined with clinical negative endocervical and vaginal swabs spiked with either Acrometrix™ CT control (Serovar D) or AcroMetrix™ NG control at the levels indicated in the tables below. Results were analyzed using the hit-rate method and the level at which 95% or above was detected was also accepted as the Limit of Detection in swab. Detection rates are depicted in *Tables 6A* and *6B*. The limit of detection of CT was determined to be 20 EB/mL and LoD of NG was 5 cells/mL based on ≥95% detection rate. Testing was performed on both NeuMoDx 288 and 96 Systems.

Table 6A. Positive Detection Rate for CT in Swab used in LoD Study for the NeuMoDx CT/NG Assay

CT (EB/mL)	n	# Positive	% Positive	LoD (Hit Rate)
Vaginal Swab				**20 EB/mL**
30	48	48	100%	
20	48	48	100%	
0	0	48	0%	
Endocervical Swab				
30	48	48	100%	
20	48	48	100%	
0	0	48	0%	

Table 6B. Positive Detection Rate for NG in Swab used in LoD Study for the NeuMoDx CT/NG Assay

NG (Cells/mL)	n	# Positive	% Positive	LoD (Hit Rate)
Vaginal Swab				5 cells/mL
9	48	48	100%	
5	48	47	98%	
0	0	48	0%	
Endocervical Swab				
9	48	48	100%	
5	48	48	100%	
0	0	48	0%	

Analytical Sensitivity – Cytology Specimens

The Limit of Detection of the NeuMoDx CT/NG Assay was determined with clinical negative PreservCyt spiked with either Acrometrix™ CT control (Serovar D) or AcroMetrix™ NG control at the levels indicated in the tables below. Results were analyzed using the hit-rate method and the level at which 95% or above was detected was accepted as the Limit of Detection. Detection rates are depicted in *Tables 7A* and *7B*. The limit of detection of CT was determined to be 15 EB/mL and LoD of NG was 5 cells/mL based on ≥95% detection rate. Testing was performed on both NeuMoDx 288 and 96 Systems.

Table 7A. Positive Detection Rate for CT in Cytology Specimens used in LoD Study for the NeuMoDx CT/NG Assay

CT (EB/mL)	n	# Positive	% Positive	LoD (Hit Rate)
15	40	40	100%	15 EB/mL
0	40	0	0%	

Table 7B. Positive Detection Rate for NG in Cytology Specimens used in LoD Study for the NeuMoDx CT/NG Assay

NG (cells/mL)	n	# Positive	% Positive	LoD (Hit Rate)
5	40	40	100%	5 cells/mL
0	40	0	0%	

Detection of Variants

The analytical sensitivity of NeuMoDx CT/NG Assay was further confirmed with 14 different CT serovars and 11 NG clinical isolates. Testing was performed using the CT serovars and NG isolates listed below in *Table 8*. CT or NG target at either ~1X or ~2X LoD level was spiked into negative urine specimens prior to testing. At least 95% detection was obtained at levels close to LoD and 100% detection was observed for both CT and NG variants at levels close to 2X LoD, indicating no significant difference in detection of relevant CT serovars and a representative set of NG isolates.

Table 8. CT/NG Serotypes Tested

CT Serotype	Detection Rate (%)		NG Clinical Isolate	Detection Rate (%)	
	6 EB/mL	12 EB/mL	[ATCC #]	0.25 cells/mL	0.5 cells/mL
A	N/A	100	49981	100	100
B		100	31426	100	100
Ba		100	31407	100	100
C		100	27633	N/A	100
LGV I		100	9793		100
LGV II		100	43070		100
LGV III		100	51109		100
E	100	100	35542		100
F	95	100	35541		100
G	95	100	49498		100
H	100	100	49926		100
I	95	100			
J	100	100			
K	100	100			

Analytical Specificity

A total of 113 culture isolates or DNA from organisms potentially cohabiting or phylogenetically similar to either CT or NG were evaluated for possible cross reactivity when testing with the NeuMoDx CT/NG Test Strip. Organisms were prepared in pools of 5 to 6 organisms each and tested at a high concentration. Most of the organisms were spiked into CT/NG negative urine at approximately 1 x 10^6 CFU/mL, except some organisms from commercial sources in which high copies of DNA (10 ng/mL) were spiked into CT/NG negative urine. No cross reactivity was observed with any of the pathogens tested in this study. The list of organisms tested is shown in *Table 9* on following page.

Table 9. List of Pathogens Used to Demonstrate Analytical Specificity

Bacteria	Bacteria	Bacteria
Achromobacter xerosis	*Fusobacterium nucleatum*	*Peptostreptococcus anaerobius*
Acinetobacter baumannii	*Gardnerella vaginalis*	*Peptostreptococcus productus*
Acinetobacter calcoaceticus	*Gemella haemolysans*	*Plesiomonas shigelloides*
Acinetobacter Iwoffii	*Haemophilus ducreyi*	*Propionibacterium acnes*
Actinomyces israelii	*Haemophilus influenzae*	*Proteus mirabilis*
Aerococcus viridans	*Kingella dentrificans*	*Proteus vulgaris*
Aeromonas hydrophila	*Kingella kingae*	*Providencia stuartii*
Alcaligenes faecalis	*Klebsiella oxytoca*	*Pseudomonoas aeruginosa*
Arcanobacterium pyogenes	*Klebsiella pneumoniae*	*Pseudomonas fluorescens*
Bacillus subtilis	*Lactobacillus acidophilus*	*Pseudomonas putida*
Bacteroides fragilis	*Lactobacillus brevis*	*Rahnella aquatilis*
Bacteroides ureolyticus	*Lactobacillus jensenii*	*Rhodospirillum rubrum*
Bifidobacterium adolescentis	*Lactobacillus lactis*	*Saccharomyces cerevisiae*
Bifidobacterium breve	*Legionella pneumophila*	*Salmonella minnesota*
Brevibacterium linens	*Listeria monocytogenes*	*Salmonella typhimurium*
Campylobacter jejuni	*Micrococcus luteus*	*Serratia marcescens*
Candida albicans	*Moraxella catarrhalis*	*Staphylococcus saprophyticus*
Candida glabrata	*Moraxella lacunata*	*Staphylococcus aureus*
Candida parapsilosis	*Moraxella osloensis*	*Staphylococcus epidermidis*
Candida tropicalis	*Morganella morganii*	*Streptococcus agalactiae*
Chlamydia pneumoniae	*Mycobacterium smegmatis*	*Streptococcus bovis*
Chromobacterium violaceum	*Mycoplasma genitalium*	*Streptococcus mitis*
Citrobacter freundii	*Mycoplasma hominis*	*Streptococcus mutans,* Serogroup A
Clostridium perfringens	*Neisseria meningitidis,* Serogroup A	*Streptococcus pneumoniae*
Corynebacterium genitalium	*Neisseria meningitidis,* Serogroup B	*Streptococcus pyogenes*
Corynebacterium xerosis	*Neisseria meningitidis,* Serogroup C	*Streptococcus salivarius*
Cryptococcus neoformans	*Neisseria meningitidis,* Serogroup D	*Streptococcus sanguinis*
Deinococcus radiodurans	*Neisseria meningitidis,* Serogroup Y	*Streptomyces griseus*
Derxia gummosa	*Neisseria meningitidis, Serogroup* W135	*Trichomonas vaginalis*
Eikenella corrodens	*Neisseria cinerea*	*Ureaplasma urealyticum*
Elizabethkingia miricola	*Neisseria elongata*	*Vibrio parahaemolyticus*
Enterobacter aerogenes	*Neisseria flavescens*	*Weissella paramesenteroides*
Enterobacter cloacae	*Neisseria lactamica*	*Yersinia enterocolitica*
Enterococcus avium	*Neisseria mucosa*	**Viruses**
Enterococcus faecalis	*Neisseria sicca*	Cytomegalovirus
Entercoccus faecium	*Neisseria subflava*	Herpes simplex virus I
Erwinia herbicola	*Neisseria perflava*	Herpes simplex virus II
Escherichia coli	*Neisseria polysaccharea*	Human papillomavirus 16

Interfering Substances - Commensal Organisms

The NeuMoDx CT/NG Test Strip was tested for interference in the presence of non-target organisms (co-habiting in the urogenital tract) by evaluating the performance of the NeuMoDx CT/NG Assay at low levels of CT and NG on the NeuMoDx 288 Molecular System. The same panel of 113 organisms [*Table 9*] used for assessing cross-reactivity was used for this study. The organisms were pooled into groups of 5-6 in CT/NG negative urine specimens and spiked with 18 EB/mL of CT purified elementary bodies and 0.75 cells/mL of NG cellular control. No interference was observed with any of the commensal organisms with the exception of the detection of NG target at low levels (3X LoD) being adversely affected in the presence of high levels of CT target (>1.0 x 10^6 EB/mL). In this case, high CT affected the detection of NG at concentrations below 20X LoD (~5 cells/mL), and consequently, the limit of detection in the presence of the high CT target background would be 5 cells/mL.

Interfering Substances - Endogenous and Exogenous Substances Encountered in CT/NG Clinical Urine Specimens
The following potentially interfering moieties were individually spiked into urine specimens [*Table 10*]: blood (7%), urine analytes, protein, glucose, urobilinogen, pH 4 (acidic), pH 9 (alkaline), leukocytes (1.0 x 10^6 cells/mL). All agents were tested for potential interference in the absence and presence of CT and NG (at 3X and 10X LoD). No interference was observed with any of the tested substances.

Table 10. Exogenous and Endogenous Interfering Agents Tested in Urine Specimens

	Interfering Substance
Endogenous	Bilirubin, ~ 10 mg/dL
	Glucose, 1000 mg/dL
	pH 4
	pH 9
	Protein (albumin), 50 mg/mL
	Blood, 7%
	Leukocytes (PBMC), 1E6 cells/mL
Exogenous	*Talcum Powder, 0.1%

** Initially, 2 of the 3 NG samples tested at 3x LoD did not amplify in the presence of talcum powder but performed as expected upon re-test.*

Interfering Substances - Endogenous and Exogenous Substances Encountered in CT/NG Clinical Swab Specimens
The following potentially interfering moieties were individually spiked into clinical endocervical and vaginal swab specimens [*Table 11*]: blood (10%), mucin, PBMCs (1.0 x 10^5 cells/mL), progesterone, Monistat® 1, Vagisil® Moisturizer, K-Y™ Jelly Personal Lubricant, Yeast-Gard Advanced™ Douche, and Seminal Fluid. All agents were tested for potential interference in the presence of CT and NG (@3X and 10X LOD). No interference was observed with any of the substances at the levels listed below.

Table 11. Exogenous and Endogenous Interfering Agents Tested in Swab Specimens

	Interfering Substance
Endogenous	Blood, 10%
	*Mucin, ~13.5 mg/mL
	PBMCs, 1E5 Cells/mL
Exogenous	Progesterone, ~7 mg/mL
	Monistat® 1, ~22 mg/mL
	Vagisil® Moisturizer, ~7 mg/mL
	K-Y Jelly Personal Lubricant, ~43mg/mL
	Yeast-Gard Advanced™ Douche, ~32mg/mL
	Seminal Fluid, ~13.5 mg/mL

** Mucin dosed from a 0.8% stock*

Interfering Substances - Endogenous and Exogenous Substances Encountered in CT/NG Clinical Cytology Specimens
The following potentially interfering moieties were individually spiked into clinical PreservCyt specimens [*Table 12*]: blood (10%), mucin, PBMCs (1.0 x 10^5 cells/mL), Yeast-Gard Advanced™ Douche, Seminal Fluid, progesterone, Vagisil® Anti-Itch Cream, Clotrimazole Vaginal Cream, Preparation H® Cream, Monistat® 1, Abreva® Cold Sore Cream, Vagisil® Moisturizer, K-Y™ Jelly Personal Lubricant, Delfen Contraceptive Foam, and Metronidazole Vaginal Cream. All agents were tested for potential interference in the presence of CT and NG at 10X LoD. No interference was observed with any of the substances at the levels listed below.

Table 12. Exogenous and Endogenous Interfering Agents Tested in Cytology Specimens

	Interfering Substance
Endogenous	Blood, 10% v/v
	Mucin, 0.25% w/v
	PBMCs, 1E5 Cells/mL
Exogenous	Yeast Gard Douche, 5% v/v
	Seminal Fluid, 5% v/v
	Progesterone, 5.6 mg/mL
	Vagisil Anti Itch Cream, 4.2 mg/mL
	Clotrimazole Vaginal Cream, 5.6 mg/mL
	Preparation H, 10.9 mg/mL
	Monistat 1, 5.6 mg/mL
	Abreva Cold Sore Cream, 7 mg/mL
	Vagisil Moisturizer, 5.6 mg/mL
	KY Jelly Personal Lubricant, 11.8 mg/mL
	Delfen Contraceptive Foam, 5.6 mg/mL
	Metronidazole Vaginal Cream, 18 mg/mL

Within Lab Precision

Within lab Precision of the NeuMoDx CT/NG Assay was verified by following a controlled test plan over 12 non-consecutive days using three different instruments and multiple operators. Each instrument (NeuMoDx™ 288 Molecular System) performed two sample sets per day, alternating between operators and two different lots of reagents which were shared across instruments. A sample set was defined as three replicates tested for each of the five different levels (True Negative, Low Negative, Moderate Negative, Low Positive and Moderate Positive) for a total of 15 specimens per set per system. Specimens were prepared using pooled, screened healthy donor urine specimens. A total of 72 sample sets (1080 tests) were analyzed in this study. Results are presented in *Tables 13 - 15*.

Table 13. Within Lab Precision Summary

Sample	Levels Tested		Replicates /set	Samples / day (across 3X Systems)	Samples / 12 days total
	Chlamydia trachomatis EB/mL	*Neisseria gonorrhoeae* cells/mL			
Moderate Positive (MP) *8X LoD*	48	2.0	3	18	216
Low Positive (LP) *2.5X LoD*	15	0.625	3	18	216
Moderate Negative (MN) *1:10 dilution of 1X LoD*	0.6	0.025	3	18	216
Low Negative (LN) *1:100 dilution of 1X LoD*	0.06	0.0025	3	18	216
True/Blank Negative (TN) *0 Target*	0	0	3	18	216
Total samples tested				**90**	**1080**

Table 14A. CT Target: Qualitative Results from Within-Lab Precision Study (Across Instruments)

Sample	Instrument 1	Instrument 2	Instrument 3	Overall
	Percent Positive	Percent Positive	Percent Positive	Percent Positive
MP	100% (72/72)	100% (72/72)	100% (72/72)	100% (216/216)
LP	100% (72/72)	100% (72/72)	100% (72/72)	100% (216/216)
MN	19.4% (14/72)	25% (18/72)	26.4% (19/72)	23.6% (51/216)
LN	1.4% (1/72)	1.4% (1/72)	1.4% (1/72)	1.4% (3/216)
TN	0% (0/72)	0% (0/72)	0% (0/72)	0% (0/216)

Table 14B. NG Target: Qualitative Results from Within-Lab Precision Study (Across Instruments)

Sample	Instrument 1	Instrument 2	Instrument 3	Overall
	Percent Positive	Percent Positive	Percent Positive	Percent Positive
MP	100% (72/72)	100% (72/72)	100% (72/72)	100% (216/216)
LP	100% (72/72)	100% (72/72)	98.6% (71/72)	100% (216/216)
MN	20.8% (15/72)	23.6% (17/72)	16.7% (12/72)	20.3% (44/216)
LN	0% (0/72)	2.8% (2/72)	0% (0/72)	0.9% (2/216)
TN	0% (0/72)	0% (0/72)	0% (0/72)	0% (0/216)

Table 15A. CT Target: Quantitative Parameter Analysis from Within Lab Precision (Across Instruments)

Sample	Instrument 1			Instrument 2			Instrument 3			Overall		
	Ave Ct	Std Dev	% CV*	Ave Ct	Std Dev	% CV	Ave Ct	Std Dev	% CV	Ave Ct	Std Dev	% CV*
MP	31.23	0.67	2.1%	31.34	0.44	1.4%	31.28	0.69	2.2%	31.28	0.61	2.0%
LP	32.52	0.62	1.9%	32.34	0.53	1.6%	32.52	0.68	2.1%	32.46	0.62	1.9%
MN	N/A											
LN												
TN												

Table 15B. NG Target: Quantitative Parameter Analysis from Within Lab Precision (Across Instruments)

Sample	Instrument 1			Instrument 2			Instrument 3			Overall		
	Ave Ct	Std Dev	% CV*	Ave Ct	Std Dev	% CV	Ave Ct	Std Dev	% CV	Ave Ct	Std Dev	% CV*
MP	30.76	0.31	1.0%	30.83	0.30	1.0%	30.91	0.31	1.0%	30.83	0.31	1.0%
LP	31.86	0.42	1.3%	31.85	0.43	1.4%	31.95	0.65	2.0%	31.89	0.51	1.6%
MN	N/A											
LN												
TN												

Carry-over and Cross-contamination

Potential sample carry-over and cross-contamination studies were performed on the NeuMoDx 288 Molecular System using the NeuMoDx CT/NG Test Strip for both the urine and cytology matrices. Both studies were executed in two parts, and first evaluated the impact on CT and NG negative specimens of being interspersed with specimens containing high CT and NG target. The positive and negative specimens were loaded onto the NeuMoDx System such that each negative specimen was adjacent to a high positive specimen. The second part of this study processed all negative specimens immediately following a run which had processed all high CT and NG concentration specimens. No contamination was seen in negative specimens integrated with high level specimens, or in negative specimens that followed specimens with high concentrations of CT and NG, demonstrating the lack of any carry over and/or cross-contamination. Additional testing was performed on the NeuMoDx 96 Molecular System and results were confirmed, as there was no evidence of any carry over or cross-contamination.

Fresh versus Frozen Specimen Equivalence

Testing was performed to demonstrate specimen matrix equivalency between fresh and frozen neat urine, vaginal and endocervical swab specimens. Clinical urine samples and prospective vaginal and endocervical swabs were procured and screened for CT and NG. Negative specimens were spiked with CT elementary bodies and NG cells at 2X LoD (urine) and 3X LoD (swab) of the NeuMoDx CT/NG Assay. Each sample was then divided equally into two aliquots, one of which was tested immediately and the second after a single freeze/thaw cycle at -20 °C. Results from fresh versus frozen urine and swab specimens were compared for equivalency by regression analysis. The data demonstrated excellent equivalency between fresh and frozen urine and fresh and frozen swab specimens.

Effectiveness of Control

The efficacy of the sample process control included in the NeuMoDx CT/NG Test Strip to report any process step failures or inhibition affecting NeuMoDx CT/NG Test performance was assessed on the NeuMoDx 288 Molecular System. The conditions tested are representative of critical process step failures that could potentially occur during sample processing and *may not be detected* by the onboard sensors that monitor the performance of the NeuMoDx System. Effectiveness of control was evaluated by simulating failure of various sample process flow steps to mimic a potential system error and by spiking specimen with a known inhibitor to observe the effect of inefficient inhibitor mitigation on detection of the Sample Process Control (see *Table 16*). In instances where the processing errors did not adversely impact the performance of the Sample Process Control (NO WASH/NO WASH BLOWOUT), the test was repeated with specimens containing low levels of CT and NG (near LoD) to confirm the process error also had NO adverse effect on the detection of CT or NG Target as well. *Table 16* summarizes the results of the efficacy of control verification test.

Table 16. Effectiveness of Control Data Summary

Condition	Expected Result	Observed Result
Normal Processing	Negative	Negative
Normal Processing + Inhibitor	Unresolved	Unresolved
No Wash Reagent	Unresolved or Negative	Negative
No Wash Blowout	Unresolved or Negative	Negative
No Release Reagent	Indeterminate	Indeterminate
No PCR Master Mix Reagents	Indeterminate	Indeterminate

On-board Sample Stability of Urine Specimens

CT and NG negative urine specimens were spiked with 2 levels of CT and NG target and processed with an equal number of negative specimens using the NeuMoDx CT/NG Assay. At the end of processing, all the positive and negative specimen tubes were left on the system worktable for a total of 24 hours. Additional testing was performed on the specimen tubes left on-board the system worktable at 4 hours, 8 hours, and 24 hours past the initial testing time point. The expected result at all time points was POSITIVE (for the appropriate target) for all the urine specimens spiked with CT or NG target and NEGATIVE (for both targets) in the urine specimens that were not spiked with target. Complete concordance with expected result was observed at all time points, including the 24-hour time point, demonstrating an on-board stability of 24 hours for testing with the NeuMoDx CT/NG Assay. Results summarized in *Table 17* below.

Table 17. On-board Sample Stability Data Summary in Urine

On-Board Specimen Stability, Urine		T_0	4 hr	8 hr	24 hr
		% Agreement	% Agreement	% Agreement	% Agreement
NG positive ATCC-31426	10 cells/mL	100%	100%	100%	100%
	20 cells/mL	100%	100%	100%	100%
CT positive ATCC_VR-879	10 EB/mL	100%	100%	100%	100%
	20 EB/mL	100%	100%	100%	100%
Negative		100%	100%	100%	100%

On-board Sample Stability of Swab Specimens

CT and NG negative endocervical and vaginal specimens were spiked with 2 levels of CT and NG target and processed with an equal number of negative specimens using the NeuMoDx CT/NG Assay. At the end of processing, all the positive and negative specimen tubes were left on the system worktable for a total of 24 hours. Additional testing was performed on the specimen tubes left on-board the system worktable at 4 hours, 8 hours, and 24 hours past the initial testing time point. The expected result at all time points was POSITIVE (for the appropriate target) for all the swab specimens spiked with CT or NG target and NEGATIVE (for both targets) in the swab specimens that were not spiked with target. Complete concordance with expected result was observed at all time points, including the 24-hour time point, demonstrating an on-board stability of 24 hours for testing with the NeuMoDx CT/NG Assay. Results summarized in *Tables 18A* and *18B* below.

Table 18A. On-board Sample Stability Data Summary in Endocervical Swab

On-Board Specimen Stability, Endocervical Swab		T_0	4 hr	8 hr	24 hr
		% Agreement	% Agreement	% Agreement	% Agreement
NG positive ATCC-31426	15 cells/mL	100%	100%	100%	100%
	50 cells/mL	100%	100%	100%	100%
CT positive ATCC_VR-879	60 EB/mL	100%	100%	100%	100%
	200 EB/mL	100%	100%	100%	100%
Negative		100%	100%	100%	100%

Table 18B. On-board Sample Stability Data Summary in Vaginal Swab

On-Board Specimen Stability, Vaginal Swab		T_0	4 hr	8 hr	24 hr
		% Agreement	% Agreement	% Agreement	% Agreement
NG positive ATCC-31426	15 cells/mL	100%	100%	100%	100%
	50 cells/mL	100%	100%	100%	100%
CT positive ATCC_VR-879	60 EB/mL	100%	100%	100%	100%
	200 EB/mL	100%	100%	100%	100%
Negative		100%	100%	100%	100%

On-board Sample Stability of Cytology Specimens

CT and NG negative cytology specimens were spiked with individual- target at 3x LoD for each target (45 EB/mL for CT and 15 cells/mL for NG, Acrometrix) and processed with an equal number of negative specimens using the NeuMoDx CT/NG Assay. At the end of processing, all the positive and negative specimen tubes were left on the system worktable for a total of 24 hours. Additional testing was performed on the specimen tubes left on-board the system worktable at 4 hours, 8 hours, and 24 hours past the initial testing time point. The expected result at all time points was POSITIVE (for the appropriate target) for all the cytology specimens spiked with CT or NG target and NEGATIVE (for both targets) in the cytology specimens that were not spiked with target. Complete concordance with expected result was observed at all time points, including the 24-hour time point, demonstrating an on-board stability of 24 hours for testing with the NeuMoDx CT/NG Assay. Results summarized in *Table 19* below.

Table 19. On-board Sample Stability Data Summary in Endocervical Swab

On-Board Specimen Stability, Cytology Specimens		T_0	4 hr	8 hr	24 hr
		% Agreement	% Agreement	% Agreement	% Agreement
NG positive	15 cells/mL	100%	100%	100%	100%
CT positive	45 EB/mL	100%	100%	100%	100%
Negative		100%	100%	100%	100%

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TRADEMARKS

SYMBOL KEY

℞ only	Prescription use only		Temperature limit
	Manufacturer		Do not re-use
IVD	In vitro diagnostic medical device		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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