December 22, 2023

NeuMoDx Molecular, Inc.
% Eveline Arnold
Director, Regulatory Affairs
Qiagen
19300 Germantown Road
Germantown, Maryland 20874

Re: K230267
Trade/Device Name: NeuMoDx CT/NG Assay 2.0
Regulation Number: 21 CFR 866.3393
Regulation Name: Device To Detect Nucleic Acids From Non-Viral Microorganism(S) Causing Sexually Transmitted Infections And Associated Resistance Marker(S)
Regulatory Class: Class II
Product Code: QEP
Dated: January 30, 2023
Received: January 31, 2023

Dear Eveline Arnold:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Himani Bisht -S

Himani Bisht, Ph.D.
Assistant Director
Viral Respiratory and HPV Branch

Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
k230267

Device Name
NeuMoDx CT/NG Assay 2.0

Indications for Use *(Describe)*
The NeuMoDx CT/NG Assay 2.0, as implemented on the NeuMoDx 96 Molecular System and NeuMoDx 288 Molecular System, is an automated, qualitative test for the direct detection and differentiation of Chlamydia trachomatis (CT) and/or Neisseria gonorrhoeae (NG) DNA as an aid in the diagnosis of chlamydial and gonococcal urogenital disease in symptomatic and asymptomatic individuals. The Assay utilizes real-time Polymerase Chain Reaction (PCR) and may be used to test male and female urine, and self-collected vaginal swab specimens (collected in a clinical setting).

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) SUMMARY

General Information

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

Contact Person: Eveline Arnold, PhD.
Director, Regulatory Affairs
NeuMoDx Molecular, Inc.
19300 Germantown Rd
Germantown, MD 20874

Phone: (240) 461-9489
Email: eveline.arnold@qiagen.com

Date Prepared: December 21, 2023

Device Name: NeuMoDx™ CT/NG Assay 2.0
Trade Name: NeuMoDx™ CT/NG Assay 2.0
Common Name: NeuMoDx CT/NG Assay 2.0

Classification Name: Nucleic Acid Detection System For Non-viral Microorganism(s) Causing Sexually Transmitted Infections(21 C.F.R. §866.3393),

Product code: QEP

Predicate Device

Manufacturer	Product Name	510(k) No.
Hologic, Inc.	Aptima Combo 2 Assay (Panther System)	k190515

Device Description

The NeuMoDx CT/NG Assay 2.0 is an automated in vitro diagnostic test for the direct detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (CT/NG) DNA from asymptomatic and symptomatic patient specimens. The assay utilizes real-time polymerase chain reaction (PCR) for the amplification of CT and/or NG DNA and fluorogenic target-specific TaqMan probes for the detection of the amplified DNA. At the end of the test, a determination of the presence/absence of CT and/or NG DNA in the specimen is automatically made based on the amplification status of the CT and/or NG DNA and/or Sample Process Control sequences using pre-established decision criteria. The NeuMoDx CT/NG Assay 2.0 is intended as an aid to diagnose CT and NG infections in symptomatic.

or asymptomatic individuals, but not to guide or monitor treatment for CT and NG infections. Concomitant cultures may be necessary to recover organisms for epidemiological typing or for further susceptibility testing.

Intended Use

The NeuMoDx CT/NG Assay 2.0, as implemented on the NeuMoDx 96 Molecular System and NeuMoDx 288 Molecular System, is an automated, qualitative test for the direct detection and differentiation of Chlamydia trachomatis (CT) and/or Neisseria gonorrhoeae (NG) DNA as an aid in the diagnosis of chlamydial and gonococcal urogenital disease in symptomatic and asymptomatic individuals. The Assay utilizes real-time Polymerase Chain Reaction (PCR) and may be used to test male and female urine, and self-collected vaginal swab specimens (collected in a clinical setting).

Special Conditions For Use Statements

For prescription use only.
For in vitro diagnostic use.

Ancillary Reagents

The NeuMoDx System consists of assay specific test strip, consumables, reagents, accessories, analyzers, and software. The following sections describe the system components.

Assay-specific components consist of the following (included with the NeuMoDx CT/NG Assay 2.0):

NeuMoDx CT/NG Test Strip 2.0 [REF 200301]
The NeuMoDx CT/NG Test Strip 2.0 is a consumable, real-time PCR reagent compatible with the NeuMoDx 288 Molecular System and NeuMoDx 96 Molecular System (NeuMoDx System(s)) used to implement the NeuMoDx CT/NG Assay 2.0.

The NeuMoDx CT/NG Test Strip 2.0 contains ambient condition stable PCR reagents at the bottom of each well (unitized reagent format). The reagents enable amplification and detection of the desired CT/NG specific targets as well as the sample process control (SPC1). It is the main component of the assay and can only be used in conjunction with the other NeuMoDx CT/NG Assay 2.0 components.

The main ancillary Consumables and Reagents that are required to perform are listed below (not included with the NeuMoDx CT/NG Assay 2.0):

NeuMoDx Extraction Plate [REF 100200]
The NeuMoDx Extraction Plate is a custom 24-well plate with ambient condition stable, dried reagents at the bottom of each well. The reagents are effective for cell lysis, protein degradation, nucleic acid binding, and process monitoring. The extraction plate is a universal consumable for all specimen types and nucleic acid targets validated for use with the system.

NeuMoDx Cartridge [REF 100100]
The NeuMoDx Cartridge incorporates a proprietary microfluidic design and allows for independent nucleic acid extraction and purification, as well as PCR amplification and detection in individual lanes for up to 12 samples when used in the NeuMoDx Systems.

NeuMoDx Lysis Buffer 2 [REF 400500]
NeuMoDx Lysis Buffer is a custom pre-filled container sealed with a pierceable septum and removable top foil. It contains a proprietary formulation of salts and chaotropic agents to provide efficient lysis of bacterial targets in human urogenital specimens. The trough itself is a flat-bottomed, free-standing, reservoir contains at least 80 mL of usable buffer.

NeuMoDx Wash Reagent [REF 400100]
The NeuMoDx Wash Reagent is supplied in a flat-bottomed, free-standing 2L bottle. It is supplied separately to the customer.

NeuMoDx Release Reagent [REF 400200]
The NeuMoDx Release Reagent is supplied in a plastic-lined, aluminum pouch inside a flat-bottomed, freestanding box. It is supplied separately to the customer.

Comparison of the NeuMoDx CT/NG Assay 2.0 and the Predicate Device

The NeuMoDx CT/NG Assay 2.0 is substantially equivalent to the predicate device:

- k190515: Aptima Combo 2 Assay (Panther System)

Similarities and differences between NeuMoDx CT/NG Assay 2.0 and the predicate device are shown in Table 1.

Table 1: Comparison of the NeuMoDx CT/NG Assay 2.0 with the predicate device

Characteristic	**Device**	**Predicate**
Name	**NeuMoDx CT/NG Assay 2.0 on the NeuMoDx 288 and 96 Molecular System**	**Aptima Combo 2 Assay (Panther)**
510(k) No.	k230267	k190515
Regulatory Number	21 CFR 866.3390	21 CFR 866.3390
Product Code	QEP	QEP
Device Class	II	II

Characteristic	**Device**	**Predicate**
	Similarities	
Intended Use	The NeuMoDx CT/NG Assay 2.0, as implemented on the NeuMoDx 96 Molecular System and NeuMoDx 288 Molecular System, is an automated, qualitative test for the direct detection and differentiation of Chlamydia trachomatis (CT) and/or Neisseria gonorrhoeae (NG) DNA as an aid in the diagnosis of chlamydial and gonococcal urogenital disease in symptomatic and asymptomatic individuals. The Assay utilizes real-time Polymerase Chain Reaction (PCR) and may be used to test male and female urine, and self-collected vaginal swab specimens (collected in a clinical setting).	The Aptima Combo 2 Assay is a target amplification nucleic acid probe test that utilizes target capture for the in vitro qualitative detection and differentiation of ribosomal RNA (rRNA) from *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (GC) to aid in the diagnosis of chlamydial and/or gonococcal disease using the Panther System as specified. On the Panther System, the assay may be used to test the following specimens from symptomatic and asymptomatic individuals: clinician-collected endocervical, vaginal, throat, rectal and male urethral swab specimens, clinician-collected gynecological specimens collected in the PreservCyt Solution, patient-collected vaginal swab specimens, and female and male urine specimens. Patient-collected vaginal swab specimens are an option for screening women when a pelvic exam is not otherwise indicated. The Aptima Multitest Swab Specimen Collection Kit has not been evaluated for home use.
Specimen Type	**Female specimens:** • Self-collected vaginal swab (collected in a clinical setting)	**Female specimens:** • Vaginal swab • Endocervical swab • Gynecological specimens in PreservCyt solution • Urine

Characteristic	**Device**	**Predicate**
	• Urine **Male Specimens:** • Urine	• Throat swab • Rectal swab **Male Specimens:** • Urethral swab • Urine • Throat swab • Rectal swab
Assay Targets	*Chlamydia trachomatis* (CT) cryptic plasmid DNA CT OMP gene *Neisseria gonorrhoeae* (NG) opacity (OPC) gene	*Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (GC) rRNA
Nucleic Acid Extraction	Extraction of nucleic acids using paramagnetic particles	Extraction of nucleic acids using Magnetic microparticles
Assay Controls	N/A	N/A
Differences		
Amplification and Detection Technology	Real-time PCR, TaqMan Chemistry	Target Capture (TC), Transcription-Mediated Amplification (TMA), Hybridization Protection Assay (HPA)
Amplification and Detection Instrument System	NeuMoDx 288 and N96 System	Panther System

Performance Characteristics - Clinical Study

The clinical performance characteristics of the NeuMoDx CT/NG Assay 2.0 as implemented on the NeuMoDx 96 and 288 Molecular Systems were established in a multi-center, pivotal, prospective urogenital specimen collection study comparing the results of the NeuMoDx CT/NG Assay 2.0 on the NeuMoDx 96 and 288 Molecular Systems (collectively named the NeuMoDx CT/NG 2.0 test system) to a patient infected status (PIS) algorithm based on results from FDA-cleared, legally marketed Nucleic Acid Amplification Tests (NAATs). A summary of the study design is provided below.

Samples Collected

A single neat urine sample was collected from each male and female subject. Additionally, a single self-collected vaginal swab (collected in a clinical setting) and two clinician-collected vaginal swabs (CCVS) were also collected from each female subject. Male and female urine, as well as the self-collected vaginal swabs (SCVS) underwent testing using the NeuMoDx CT/NG Assay 2.0. Urine and the clinician-collected vaginal swab specimens were utilized for comparator method testing to establish the patient-infected status.

Sites and Comparator Testing

All evaluable samples were tested at three external laboratories using the NeuMoDx CT/NG Assay 2.0 following the package insert instructions for use. All comparison testing was conducted at a separate reference laboratory using FDA-cleared NAAT assays in accordance with the manufacturers' package insert instructions for use. For female subjects, urine and clinician-collected vaginal swab specimens were tested using two FDA-cleared NAATs to establish the patient infected status (PIS). The infected status for female subjects was determined by combining results from two specimen types and two comparator NAATs. The male PIS was established using urine results from two FDA-cleared comparator NAATs.

Patient Infected Status Determination

The infection status of each participant was determined using a prespecified patient infected status (PIS) algorithm. For female participants, the PIS was established from the results of female urine (FU) and clinician-collected vaginal swab (CCVS) specimens tested by two FDA-cleared NAAT comparator assays. Females were classified as infected if at least one positive result was obtained by each assay. Any other combination of results was considered a non-infected status. Male subjects were classified as infected if both comparator urine NAAT results were positive. If the male urine results were conflicting (i.e. one positive and one negative), a third FDA-cleared NAAT method was performed as a tie-breaker to adjudicate male infection status.

See Tables 2 to 3 for CT/NG Assay 2.0 results vs patient infected status algorithms.

Subjects Enrolled and Exclusions

Specimens were collected from symptomatic and asymptomatic females and males enrolled from 14 geographically and demographically diverse U.S. sites, including family practice clinics, obstetrics/gynecology practices, public health centers, sexual transmitted disease (STD) clinics, family planning clinics, college campus and adolescent clinics, and hospital emergency departments/urgent care centers. Each subject was classified as symptomatic if the subject reported symptoms and asymptomatic if the subject did not report symptoms. The average age of all study subjects in the eligible population was 31 ± 10 years and ranged from 15 to 77.

A total of 4017 participants with a mean age of 31±10 years (median: 29; range:15–77 years) were enrolled in the study, including 1825 males and 2192 females. Among the 1825 males enrolled in the study, five participants were excluded after consent as a result of not meeting eligibility criteria (n=3), previous enrollment (n=1), or withdrawal owing to insufficient urine collection (n=1), which resulted in 1820 valid male participants. Among the 2192 females enrolled, 36 were excluded after consent, with reasons including ineligibility or unconfirmed eligibility (n=13), withdrawal from the study (n=4), and samples evaluable by the NeuMoDx CT/NG Assay 2.0 not collected (n=19), leaving 2156 females included in the study.

Of the remaining 1820 male participants, a urine specimen was collected from each, but 119 specimens were excluded from analysis owing to protocol deviation, collection or instrument device events, and other specimen testing issues which prevented a final result from being obtained by NeuMoDx CT/NG Assay 2.0 or comparator method, which left data from a total of 1701 male subjects included in the data analysis.

Of the 1701 male urine specimens, for CT, there were 10 cases where either a valid NeuMoDx CT/NG Assay 2.0 result could not be determined (n=2) or where the PIS result could not be determined (n=8), therefore there were 1691 male urine specimens included in the primary analysis. Similarly, for NG, there were 3 cases where a valid NeuMoDx CT/NG Assay 2.0 result could not be determined, therefore there were 1698 male urine specimens included in the primary analysis.

Among the evaluable specimens collected from the 2156 female participants, 138 urine specimens and 140 self-collected vaginal swab specimens were excluded due to protocol deviations, collection device events, instrument device events, and other events which prevented a final result from being obtained by NeuMoDx CT/NG Assay 2.0 or comparator, allowing for 2018 and 2016 female subject specimens included in the study for urine and SCVS respectively.

Of the 2018 female urine specimens, there were 11 cases where a valid NeuMoDx CT/NG Assay 2.0 result could not be determined for the CT target and 12 cases where a valid result was not obtained for the NG target; therefore, there were 2007 female urine specimens included in the primary analysis for CT and 2006 for NG.

Of the 2016 self-collected vaginal swab specimens, all produced valid results for CT and NG with the NeuMoDx CT/NG Assay 2.0 and comparator test; these subjects are included in the primary data analysis.

Chlamydia trachomatis Performance Results

The summary table of the performance data for the NeuMoDx CT/NG Assay 2.0 for CT detection is shown below in Table 2. Invalid results (IND and UNR) are not included in the sensitivity and specificity calculations shown below. Sensitivity and specificity were calculated by comparing NeuMoDx CT/NG Assay 2.0 results to the patient infected status algorithm.

Table 2: NeuMoDx CT/NG Assay vs Patient Infected Status for CT Detection (N96 and N288 Combined)

Specimen	Symptom Status	n	TP	FP	TN	FN	Prev.	Sensitivity	Specificity
MU	**Asymp.**	1227	102	0	1123	2	8.5%	98.1% (93.3%, 99.5%)	100.0% (99.7%, 100.0%)
MU	**Symp.**	464	82	1	377	4	18.5%	95.3% (88.6%, 98.2%)	99.7% (98.5%, 100.0%)
MU	**All**	1691	184	1	1500	6	11.2%	96.8% (93.3%, 98.5%)	99.9% (99.6%, 100.0%)
FU	**Asymp.**	1054	40	1	1010	3	4.1%	93.0% (81.4%, 97.6%)	99.9% (99.4%, 100.0%)
FU	**Symp.**	953	56	3	889	5	6.4%	91.8% (82.2%, 96.4%)	99.7% (99.0%, 99.9%)
FU	**All**	2007	96	4	1899	8	5.2%	92.3% (85.6%, 96.1%)	99.8% (99.5%, 99.9%)
SCVS	**Asymp.**	1052	43	2	1007	0	4.1%	100.0% (91.8%, 100.0%)	99.8% (99.3%, 99.9%)
SCVS	**Symp.**	964	58	7	896	3	6.3%	95.1% (86.5%, 98.3%)	99.2% (98.4%, 99.6%)
SCVS	**All**	2016	101	9	1903	3	5.2%	97.1% (91.9%, 99.0%)	99.5% (99.1%, 99.8%)

MU = Male Urine, FU = Female Urine, SCVS = Self-Collected Vaginal Swab
Symp. = Symptomatic, Asymp. = Asymptomatic
Prev. = Prevalence, TP = True Positive, FP = False Positive, TN = True Negative, FN = False Negative

Neisseria Gonorrhoeae Performance Results

The summary table of the performance data for the NeuMoDx CT/NG Assay 2.0 for NG detection is shown below in Table 3. Invalid results (IND and UNR) are not included in the sensitivity and specificity calculations shown below. Sensitivity and specificity were calculated by comparing NeuMoDx CT/NG Assay 2.0 results to the patient infected status algorithm.

Table 3: NeuMoDx CT/NG Assay vs Patient Infected Status for NG Detection (N96 and N288 Combined)

Specimen	Symptom Status	n	TP	FP	TN	FN	Prev.	Sensitivity	Specificity
MU	**Asymp.**	1231	11	1	1219	0	0.9%	100.0% (74.1%, 100.0%)	99.9% (99.5%, 100.0%)
MU	**Symp.**	467	81	1	384	1	17.6%	98.8% (93.4%, 99.8%)	99.7% (98.5%, 100.0%)
MU	**All**	1698	92	2	1603	1	5.5%	98.9% (94.2%, 99.8%)	99.9% (99.5%, 100.0%)
FU	**Asymp.**	1053	22	0	1029	2	2.3%	91.7% (74.2%, 97.7%)	100.0% (99.6%, 100.0%)
FU	**Symp.**	953	20	1	931	1	2.2%	95.2% (77.3%, 99.2%)	99.9% (99.4%, 100.0%)
FU	**All**	2006	42	1	1960	3	2.2%	93.3% (82.1%, 97.7%)	99.9% (99.7%, 100.0%)
SCVS	**Asymp.**	1052	24	0	1028	0	2.3%	100.0% (86.2%, 100.0%)	100.0% (99.6%, 100.0%)
SCVS	**Symp.**	964	20	2	941	1	2.2%	95.2% (77.3%, 99.2%)	99.8% (99.2%, 99.9%)
SCVS	**All**	2016	44	2	1969	1	2.2%	97.8% (88.4%, 99.6%)	99.9% (99.6%, 100.0%)

MU = Male Urine, FU = Female Urine, SCVS = Self-Collected Vaginal Swab
Symp. = Symptomatic, Asymp. = Asymptomatic
Prev. = Prevalence, TP = True Positive, FP = False Positive, TN = True Negative, FN = False Negative

Chlamydia trachomatis Patient Infected Status Tables

The frequency of test outcomes from the cleared comparator NAATs and investigational NeuMoDx System testing is summarized in Tables 4 and 5 for CT.

Table 4: Patient Infected Status – Male Urine, CT

PIS	NAAT 1	NAAT 2	NAAT 3	NeuMoDx	Count		
Overall	**Urine**	**Urine**	**Urine**	**Urine**	**Symp.**	**Asymp.**	**Total**
NI	-	-	NA	IND	0	1	1
NI	-	-	NA	-	377	1123	1500
NI	-	-	NA	+	1	0	1
NI	-	-	NA	UNR	0	1	1
Total					*378*	*1125*	*1503*
I	-	+	+	-	1	0	1
I	+	+	NA	-	3	2	5
I	+	+	NA	+	82	102	184
Total					*86*	*104*	*190*

IND = Indeterminate, UNR = Unresolved, EQ = Equivocal
NI = Non-infected, I = Infected, NA=Not Available

Table 5: Patient Infected Status – Female CT

PIS	NAAT 1		NAAT 2		NeuMoDx		Count		
Overall	**CCVS**	**Urine**	**CCVS**	**Urine**	**SCVS**	**Urine**	**Symp.**	**Asymp.**	**Total**
NI	NA	NA	-	-	-	NA	1	0	1
NI	-	NA	-	NA	-	-	0	2	2
NI	-	NA	-	-	-	NA	1	0	1
NI	-	NA	-	-	-	-	5	3	8
NI	-	NA	-	-	-	UNR	4	0	4
NI	NA	-	NA	-	-	-	1	2	3
NI	NA	-	-	-	-	-	7	3	10
NI	NA	-	+	+	+	-	1	0	1
NI	-	-	-	NA	-	-	0	1	1
NI	-	-	EQ	-	-	-	0	1	1
NI	-	-	-	-	-	NA	5	0	5
NI	-	-	-	-		-	6	4	10
NI	-	-	-	-	-	-	856	990	1846
NI	-	-	-	-	+	-	1	2	3
NI	-	-	-	-	-	+	2	0	2
NI	-	-	-	-	+	+	1	0	1
NI	-	-	-	-	-	UNR	5	2	7
NI	-	-	+	-	-	-	6	2	8
NI	-	-	+	-	+	-	2	0	2
NI	-	-	-	+	-	-	1	0	1
NI	-	-	-	+	-	+	0	1	1
NI	-	-	+	+	+	-	1	0	1
NI	+	-	-	-	+	-	1	0	1
NI	NA	-	NA	-	-	-	1	0	1
Total							*908*	*1013*	*1921*
I	+	NA	+	+	+	NA	1	0	1
I	+	-	+	-	-	-	1	0	1
I	+	-	+	-	+	-	1	1	2
I	+	-	+	+	-	-	1	0	1
I	+	-	+	+	+	-	0	2	2
I	+	-	+	+	+	+	0	3	3
I	NA	+	+	+	+	+	2	1	3
I*	-	+	-	+	-	+	1	0	1
I	-	+	-	+	+	+	1	0	1
I	-	+	+	+	+	+	2	2	4
I	+	+	+	EQ	+	+	1	0	1
I	+	+	+	-	+	+	1	0	1
I	+	+	-	+	+	+	1	1	2
I	+	+	+	+	-	-	1	0	1

PIS	NAAT 1		NAAT 2		NeuMoDx		Count		
Overall	**CCVS**	**Urine**	**CCVS**	**Urine**	**SCVS**	**Urine**	**Symp.**	**Asymp.**	**Total**
I	+	+	+	+	+	-	1	0	1
I	+	+	+	+	+	+	46	33	79
I	+	+	NA	+	+	+	1	0	1
Total							62	43	105

SCVS = Self-Collected Vaginal Swab
IND = Indeterminate, UNR = Unresolved, EQ = Equivocal
NI = Non-infected, I = Infected, NA=Not Available

*One female subject tested negative for CT in the swab specimens by both comparator NAATs and positive in urine by both comparator NAATs. For calculations of performance, the swab sample from this subject was considered a "True Negative", while the urine sample was considered a "True Positive."

Neisseria Gonorrhoeae Infected Status Tables

The frequency of test outcomes from the cleared comparator NAATs and investigational NeuMoDx System testing is summarized in Tables 6 and 7 for NG.

Table 6: Patient Infected Status – Male Urine, NG

PIS	NAAT 1	NAAT 2	NAAT 3	NeuMoDx	Count		
Overall	**Urine**	**Urine**	**Urine**	**Urine**	**Symp.**	**Asymp.**	**Total**
NI	-	-	NA	IND	0	2	2
NI	-	-	NA	-	384	1219	1603
NI	-	-	NA	+	1	1	2
NI	-	-	NA	UNR	0	1	1
Total					*385*	*1223*	*1608*
I	+	+	NA	-	1	0	1
I	+	+	NA	+	81	11	92
Total					*82*	*11*	*93*

IND = Indeterminate, UNR = Unresolved, EQ = Equivocal
NI = Non-infected, I = Infected, NA = Not Available

Table 7: Patient Infected Status – Female NG

PIS	NAAT 1		NAAT 2		NeuMoDx		Count		
Overall	**CCVS**	**Urine**	**CCVS**	**Urine**	**SCVS**	**Urine**	**Symp.**	**Asymp.**	**Total**
NI	NA	NA	-	-	-	NA	1	0	1
NI	-	NA	-	NA	-	-	0	2	2
NI	-	NA	-	-	-	NA	2	0	2
NI	-	NA	-	-	-	-	6	3	9
NI	-	NA	-	-	-	UNR	4	0	4
NI	NA	-	NA	-	-	-	2	2	4
NI	NA	-	-	-	-	-	10	4	14
NI	-	-	-	NA	-	-	0	1	1
NI	-	-	NA	-	-	-	1	0	1

PIS	NAAT 1		NAAT 2		NeuMoDx		Count		
Overall	**CCVS**	**Urine**	**CCVS**	**Urine**	**SCVS**	**Urine**	**Symp.**	**Asymp.**	**Total**
NI	-	-	-	-	-	NA	5	0	5
NI	-	-	-	-	-	IND	0	1	1
NI	-	-	-	-	NA	-	6	4	10
NI	-	-	-	-	-	-	904	1011	1915
NI	-	-	-	-	+	-	1	0	1
NI	-	-	-	-	+	+	1	0	1
NI	-	-	-	-	-	UNR	5	2	7
NI	-	-	+	-	-	-	0	1	1
NI	-	+	-	-	-	-	1	1	2
Total							*949*	*1032*	*1981*
I	+	-	+	-	+	-	0	1	1
I	+	+	+	EQ	+	+	0	1	1
I	+	+	+	-	+	+	0	1	1
I	+	+	+	+	-	-	1	0	1
I	+	+	+	+	+	-	0	1	1
I	+	+	+	+	+	+	20	20	40
Total							*21*	*24*	*45*

SCVS = Self-Collected Vaginal Swab
IND = Indeterminate, UNR = Unresolved, EQ = Equivocal
NI = Non-infected, I = Infected, NA = Not Available

Indeterminate or Unresolved Result Rates
Invalid (indeterminate or unresolved) rates were calculated for each of the NeuMoDx Molecular Systems (N96 and N288), and for the instruments combined. Among all specimen types, instrument models, targets and symptom status, 1/5738 (0.0%; 95% CI 0.0%-0.1%) were Indeterminate (IND) and 12/5738 (0.2%; 95% CI: 0.1% to 0.4%) were Unresolved (UNR).

Performance Characteristics - Non-Clinical Studies

Precision/Reproducibility

Within-Laboratory Precision
The within-laboratory precision study was conducted using three (3) NeuMoDx 288 Molecular Systems and three (3) NeuMoDx 96 Molecular Systems over 12 days. The precision panel was prepared using two (2) specimen matrices, urine and universal transport medium, and included negative, low positive, moderate positive, and high negative samples for both *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG). Low and moderate positive panel members were subjected to nested ANOVA analysis to establish repeatability (between replicates), between run, between day, within system, between system, and within laboratory (total) precision, the results of which are presented Tables 8 – 11 below

Table 8: Within Lab Precision of NeuMoDx CT/NG Assay 2.0 for CT target, N96 System

Matrix	CT Level	Ct Avg	n	% Agreement† (95% CI)	Repeatability		Between Run		Between Day		Within System		Between System		Within Lab	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Swab	MC	29.7	216	100 (98.2-100)	0.614	2.1	0.191	0.6	0.000	0.0	0.643	2.2	0.104	0.3	0.651	2.2
	LC*	31.7	432	100 (99.1-100)	0.771	2.4	0.196	0.6	0.000	0.0	0.795	2.5	0.115	0.4	0.803	2.5
	HN	N/A	218	16.5 (12.7-22.0)	N/A											
	N	N/A	216	99.5 (97.4-99.9)	N/A											
Urine	MC	30.1	216	100 (98.2-100)	0.545	1.8	0.300	1.0	0.101	0.3	0.630	2.1	0.094	0.3	0.637	2.1
	LC*	32.0	432	100 (99.1-100)	0.648	2.0	0.294	0.9	0.108	0.3	0.720	2.2	0.056	0.2	0.722	2.3
	HN	N/A	215	14.4 (10.2-20.0)	N/A											
	N	N/A	215	99.5 (97.4-99.9)	N/A											

MC = Moderate CT, LC= Low CT, HN = High Neg, N = Negative

*Low level data pooled from two panel members

†% Agreement with expected results

Table 9: Within Lab Precision of NeuMoDx CT/NG Assay 2.0 for CT target, N288 System

Matrix	CT Level	Ct Avg	n	% Agreement† (95% CI)	Repeatability		Between Run		Between Day		Within System		Between System		Within Lab	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Swab	MC	29.6	216	100 (98.2-100)	0.733	2.5	0.000	0.0	0.303	1.0	0.793	2.7	0.099	0.3	0.799	2.7
	LC*	31.7	431	100 (99.1-100)	0.771	2.4	0.082	0.3	0.193	0.6	0.799	2.5	0.133	0.4	0.810	2.6
	HN	N/A	216	14.4 (10.3-19.6)	N/A											
	N	N/A	217	100 (98.3-100)	N/A											
Urine	MC	29.9	215	100 (98.2-100)	0.702	2.3	0.079	0.3	0.117	0.4	0.716	2.4	0.158	0.5	0.734	2.5
	LC*	31.9	432	100 (99.1-100)	0.646	2.0	0.096	0.3	0.128	0.4	0.666	2.1	0.000	0.0	0.666	2.1
	HN	N/A	216	16.2 (11.7-22.0)	N/A											
	N	N/A	215	100 (98.2-100)	N/A											

MC = Moderate CT, LC= Low CT, HN = High Neg, N = Negative

*Low level data pooled from two panel members

†% Agreement with expected results

Table 10: **Within Lab Precision of NeuMoDx CT/NG Assay 2.0 for NG target, N96 System**

Matrix	CT Level	Ct Avg	n	% Agreement† (95% CI)	Repeatability		Between Run		Between Day		Within System		Between System		Within Lab	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Swab	MN	27.3	216	100 (98.2-100)	0.528	1.9	0.166	0.6	0.000	0.0	0.553	2.0	0.014	0.1	0.554	2.0
	LN*	29.1	432	100 (99.1-100)	0.538	1.8	0.155	0.5	0.101	0.3	0.569	2.0	0.116	0.4	0.581	2.0
	HN	N/A	215	57.7 (51.0-64.1)	N/A											
	N	N/A	216	100% (98.2-100)	N/A											
Urine	MN	28.4	216	100 (98.2-100)	0.503	1.8	0.337	1.2	0.000	0.0	0.605	2.1	0.095	0.3	0.612	2.2
	LN*	30.0	432	100 (99.1-100)	0.549	1.8	0.273	0.9	0.000	0.0	0.614	2.0	0.073	0.2	0.618	2.1
	HN	N/A	215	32.1 (26.2-38.8)	N/A											
	N	N/A	215	100 (98.2-100)	N/A											

MN = Moderate NG, LN= Low NG, HN = High Neg, N = Negative
*Low level data pooled from two panel members
†% Agreement with expected results

Table 11: **Within Lab Precision of NeuMoDx CT/NG Assay 2.0 for NG target , N288 System**

Matrix	CT Level	Ct Avg	n	% Agreement† (95% CI)	Repeatability		Between Run		Between Day		Within System		Between System		Within Lab	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Swab	MN	27.2	216	100 (98.2-100)	0.541	2.0	0.217	0.8	0.000	0.0	0.583	2.1	0.093	0.3	0.590	2.2
	LN*	29.1	432	100 (99.1-100)	0.643	2.2	0.128	0.4	0.108	0.4	0.664	2.3	0.049	0.2	0.666	2.3
	HN	N/A	216	56.0 (49.3-62.5)	N/A											
	N	N/A	216	100 (98.2-100)	N/A											
Urine	MN	28.3	216	100 (98.2-100)	0.491	1.7	0.191	0.7	0.000	0.0	0.527	1.9	0.026	0.1	0.528	1.9
	LN*	30.0	431	99.8 (98.7-99.96)	0.680	2.3	0.000	0.0	0.210	0.7	0.712	2.4	0.134	0.4	0.725	2.4
	HN	N/A	216	32.4 (26.5-38.9)	N/A											
	N	N/A	215	100 (98.2-100)	N/A											

MN = Moderate NG, LN= Low NG, HN = High Neg, N = Negative
*Low level data pooled from two panel members
†% Agreement with expected results

An additional precision study was conducted internally in order to evaluate performance of the system at concentrations near the LoD, utilizing urine as a representative matrix. Low positive CT samples at 1X and 2X of the confirmed LoD (7.08 EB/mL and 14.16 EB/mL,

respectively, and low positive NG samples at 1X and 2X LoD (0.68 cells/mL and 1.36 cells/mL, respectively) were processed on each of 5 days, along with a negative control sample, on one NeuMoDx 96 Molecular System and one NeuMoDx 288 Molecular System, using three lots of the NeuMoDx CT/NG Test Strip 2.0. Each panel consisted of two replicates of CT at 1X LoD, two replicates of NG at 1X LoD, two replicates of 2x LoD of CT and NG together, and two negative control samples, resulting in 60 data points per target level per system across five days, for a total of 120 replicates per level across both systems. The summary of results for each sample type is shown below in Tables 12 and 13, demonstrating acceptable reproducibility performance across both NeuMoDx CT/NG systems with samples near the LoD.

Table 12: Precision of NeuMoDx CT/NG Assay 2.0 with Low-Positive Samples in Urine for CT and NG Targets (N96 and N288 Systems)

System	Level	CT (Serovar D)					NG (B5025)				
		n	% Agreement† (95% CI)	Avg Ct	SD	%CV	n	% Agreement† (95% CI)	Avg Ct	SD	%CV
N96	1x LoD CT	59*	96.6 (88.5-99.1)	33.37	1.01	3.04	N/A				
	1x LoD NG	N/A					60	100.0 (94.0-100.0)	32.68	0.66	2.01
	2x LoD CT/NG	60	100.0 (94.0-100.0)	33.05	0.84	2.55	60	100.0 (94.0-100.0)	31.89	0.54	1.71
N288	1x LoD CT	60	100.0 (94.0-100.0)	33.70	0.62	1.85	N/A				
	1x LoD NG	N/A					59*	100.0 (93.9-100.0)	32.66	0.48	1.46
	2x LoD CT/NG	60	100.0 (94.0-100.0)	33.05	0.66	2.00	60	100.0 (94.0-100.0)	32.01	0.50	1.57

***1 replicate invalid**
†% Agreement with expected results

Table 13: Precision of NeuMoDx CT/NG Assay 2.0 with Low-Positive Samples in Urine for CT and NG Targets (N96 and N288 Systems) by Reagent Lot

System	Level	Lot	CT (Serovar D)					NG (B5025)				
			n	% Agreement† (95% CI)	Avg Ct	SD	%CV	n	% Agreement† (95% CI)	Avg Ct	SD	%CV
N96	1x LoD	1	20	90.0 (69.9–97.2)	33.36	1.28	3.83	20	100 (83.9–100.0)	32.86	0.48	1.45
		2	20	100 (83.9–100.0)	33.21	1.03	3.11	20	100 (83.9–100.0)	32.53	0.60	1.84
		3	19*	100 (83.2–100.0)	33.54	0.68	2.04	20	100 (83.9–100.0)	32.75	0.79	2.40
	2x LoD	1	20	100 (83.9–100.0)	33.17	0.86	2.59	20	100 (83.9–100.0)	31.87	0.52	1.62
		2	20	100 (83.9–100.0)	32.68	0.73	2.22	20	100 (83.9–100.0)	31.69	0.58	1.83
		3	20	100 (83.9–100.0)	33.32	0.84	2.51	20	100 (83.9–100.0)	32.09	0.48	1.51

N288	1x LoD	1	20	100 (83.9–100.0)	33.79	0.7	2.07	20	100 (83.9–100.0)	32.79	0.37	1.13
		2	20	100 (83.9–100.0)	33.51	0.47	1.41	19*	100 (83.2–100.0)	32.42	0.48	1.49
		3	20	100 (83.9–100.0)	33.81	0.66	1.56	20	100 (83.9–100.0)	32.75	0.50	1.53
	2x LoD	1	20	100 (83.9–100.0)	32.97	0.66	1.99	20	100 (83.9–100.0)	31.84	0.54	1.68
		2	20	100 (83.9–100.0)	33.00	0.45	1.36	20	100 (83.9–100.0)	32.00	0.36	1.13
		3	20	100 (83.9–100.0)	33.86	0.75	2.20	20	100 (83.9-100.0)	32.18	0.55	1.71

*1 replicate invalid
†% Agreement with expected results

Reproducibility

The NeuMoDx CT/NG Assay 2.0 reproducibility was evaluated at three (3) U.S. sites by two (2) operators at each site. Testing was done on six (6) NeuMoDx Molecular Systems, with each site hosting a NeuMoDx 288 Molecular System and NeuMoDx 96 Molecular System. Each operator performed one (1) run per day over five (5) days. Each run tested three (3) replicates of each reproducibility panel member. All testing was performed using one (1) lot of the NeuMoDx CT/NG Test Strip 2.0. Each testing site was provided with a six-member reproducibility panel that included one (1) panel member negative for both *Chlamydia trachomatis* and *Neisseria gonorrhoeae* and five (5) panel members positive for one (1) or both targets. Positive panel members were created by spiking target organisms into two (2) different specimen matrices: urine and universal transport medium. The variability within run, between run, between day, between site and total was calculated separately for each low and moderate positive panel member and is presented in Tables 14–17 below for the N96 and N288 instruments.

Table 14: Reproducibility for *Chlamydia trachomatis* (CT) Results, N96 System

Matrix	CT Level	Ct Avg	n	% Agreement† (95% CI)	Within Run		Between Run		Between Day		Between Site		Total	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Swab	MC	29.4	90	100 (95.9-100)	0.63	2.1	0.15	0.5	0.22	0.7	0.23	0.8	0.69	2.4
	LC*	31.2	180	100 (97.9-100)	1.06	3.4	0.00	0.0	0.00	0.0	0.16	0.5	1.07	3.4
	HN	N/A	90	85.6 (76.9-91.4)	N/A									
	N	N/A	180	100 (95.9-100)	N/A									
Urine	MC	29.5	90	100 (95.9-100)	0.51	1.7	0.00	0.0	0.00	0.0	0.24	0.8	0.54	1.8
	LC*	31.0	180	100 (97.9-100)	0.71	2.3	0.12	0.4	0.02	0.1	0.37	1.2	0.78	2.5
	HN	N/A	90	97.8 (92.3-99.4)	N/A									
	N	N/A	180	99.4 (96.9-99.9)	N/A									

MC = Moderate CT, LC= Low CT, HN = High Neg, N = Negative
*Low level data pooled from two panel members
†% Agreement with expected results

Table 15: Reproducibility for *Chlamydia trachomatis* (CT) Results, N288 System

Matrix	CT Level	Ct Avg	n	% Agreement† (95% CI)	Within Run		Between Run		Between Day		Between Site		Total	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Swab	MC	29.4	90	100 (95.9-100)	0.56	1.9	0.00	0.0	0.00	0.0	0.38	1.3	0.64	2.2
	LC*	31.4	180	100 (97.9-100)	0.60	1.9	0.00	0.0	0.11	0.4	0.35	1.1	0.67	2.1
	HN	N/A	90	77.8 (68.2-85.1)	N/A									
	N	N/A	180	100 (97.9-100)	N/A									
Urine	MC	29.8	90	100 (95.9-100)	0.39	1.3	0.00	0.0	0.07	0.2	0.29	1.0	0.46	1.5
	LC*	31.4	180	100 (97.9-100)	0.62	2.0	0.00	0.0	0.00	0.0	0.21	0.7	0.64	2.0
	HN	N/A	90	94.4 (87.6-97.6)	N/A									
	N	N/A	180	100 (97.9-100)	N/A									

MC = Moderate CT, LC= Low CT, HN = High Neg, N = Negative

*Low level data pooled from two panel members

†% Agreement with expected results

Table 16: Reproducibility for *Neisseria gonorrhoeae* (NG) Results, N96 System

Matrix	NG Level	Ct Avg	n	% Agreement† (95% CI)	Within Run		Between Run		Between Day		Between Site		Total	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Swab	MN	27.6	90	100 (95.9-100)	0.64	2.3	0.00	0.0	0.22	0.8	0.11	0.4	0.67	2.4
	LN*	29.4	180	100 (97.9-100)	0.61	2.1	0.00	0.0	0.00	0.0	0.29	1.0	0.66	2.2
	HN	N/A	90	46.7 (36.7-56.9)	N/A									
	N	N/A	180	100 (97.9-100)	N/A									
Urine	MN	28.3	90	98.9 (94.0-99.8)	0.50	1.8	0.00	0.0	0.15	0.5	0.28	1.0	0.56	2.0
	LN*	30.0	180	100 (97.9-100)	0.51	1.7	0.04	0.1	0.00	0.0	0.37	1.3	0.60	2.0
	HN	N/A	90	70.0 (59.9-78.5)	N/A									
	N	N/A	180	98.9 (96.0-99.7)	N/A									

MN = Moderate NG, LN= Low NG, HN = High Neg, N = Negative

*Low level data pooled from two panel members

†% Agreement with expected results

Table 17: Reproducibility for *Neisseria gonorrhoeae* (NG) Results, N288 System

Matrix	NG Level	Ct Avg	n	% Agreement† (95% CI)	Within Run		Between Run		Between Day		Between Site		Total	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Swab	MN	27.7	90	100 (95.9-100)	0.50	1.8	0.00	0.0	0.19	0.7	0.35	1.3	0.60	2.2
	LN*	29.7	180	100 (97.9-100)	0.54	1.8	0.00	0.0	0.04	0.1	0.40	1.4	0.63	2.1
	HN	N/A	90	27.8 (19.6-37.8)	N/A									
	N	N/A	180	100 (97.9-100)	N/A									
Urine	MN	28.4	90	100 (95.9-100)	0.45	1.6	0.00	0.0	0.00	0.0	0.40	1.4	0.56	2.0
	LN*	30.3	180	100 (97.9-100)	0.48	1.6	0.08	0.2	0.09	0.3	0.41	1.4	0.60	2.0
	HN	N/A	90	54.4 (44.2-64.3)	N/A									
	N	N/A	180	100 (97.9-100)	N/A									

MN = Moderate NG, LN= Low NG, HN = High Neg, N = Negative
*****Low level data pooled from two panel members
†% Agreement with expected results

Linearity/Assay Reportable Range:
Not Applicable

Traceability, Stability, Expected Values (Controls, Calibrators or Methods)

Internal Control
The full process internal control, Sample Process Control (SPC1), serves as both an extraction and PCR internal control. It is provided with the test kit. The primers and probe specific for SPC1 are included in each well of the NeuMoDx CT/NG Test Strip 2.0. The SPC1 target itself is encapsulated bacteriophage T7 that is incorporated into each well of the NeuMoDx Extraction Plate. The SPC1 is co-extracted with the DNA from each sample processed and enables monitoring of the efficacy of the entire test process (nucleic acid isolation and real-time PCR amplification/detection) and key process steps not monitored actively by the NeuMoDx Software. The NeuMoDx CT/NG Assay 2.0 incorporates the results of SPC1 amplification as part of the results processing algorithm.

External Controls
External controls are not provided with the NeuMoDx CT/NG Assay 2.0; however, testing of user-defined positive and negative controls are recommended in the assay labeling. User-defined controls should be tested in conformance with local, state, and/or federal regulations or accreditation requirements and each laboratory's standard quality control procedures.

Specimen Stability
The specimen stability study indicated that it is acceptable to store swab and urine specimens at the following conditions prior to testing:

Swab specimens

- After collection, swab specimens in transport tubes must be refrigerated within two hours of collection. Specimens may be stored at 2 to 8 °C for up to seven days

Urine specimens

- After collection, urine specimens in the primary collection container must be refrigerated within two hours of collection. Specimens may be stored at 2 to 8 °C for up to seven days

The specimen stability study evaluated negative urine samples collected in sterile specimen cup without any preservatives and using vaginal swab samples collected in universal viral transport medium (BD Universal Viral Transport System, BD Diagnostics, Sparks, MD, USA, BD UVT). Testing was performed using a combined panel of positive clinical specimens and contrived positive samples in addition to negatives. Contrived positive CT/NG samples consisted of pooled clinical negative vaginal swab matrix and negative donor urine spiked with CT and NG at ≤3X LoD. Samples were stored onboard for up to 24 hours, or at 2– 8°C for up to 7 days or a combination of both, before being processed separately on the N96 and N288. Results showed 100% concordance with the expected results through 8 hours onboard for every day of testing. Therefore, urine and vaginal swab specimens may be stored up to 7 days at 2–8°C and up to 8 hours when stored onboard the N96 and N288.

Detection Limit

The limit of detection (LoD) of the NeuMoDx CT/NG Assay 2.0 was initially evaluated by testing separate dilutions of CT (serovar D) elementary bodies (EB) and NG (strain B5025) cells at five levels surrounding the anticipated LoD in both pooled clinical negative urine and vaginal swab matrix. Testing was performed on 20 replicates per level across multiple instruments, days, and three key reagent lots. Probit analyses were performed to generate a preliminary LoD of 3.54 EB/mL CT and 0.34 cells/mL NG in urine and 6.27 EB/mL CT and 0.87 cells/mL NG in vaginal swab.

The limit of detection was confirmed for each instrument system individually, across two CT Serovars (D, J) and two NG strains (B5025, NHI 1) in a hit-rate style analysis. Targets were dosed together at low levels, with Serovar D paired with NG strain B5025, and Serovar J paired with NG strain NHI 1. Evaluation began at target levels equal to the preliminary Probit LoD, with concentrations increasing in proportions of LoD in the event that 95% positivity was not reached. The LoD claim for the assay was finalized by accepting the target level reaching ≥95% positivity across all system types and serovars/strains (see Table 18).

Table 18: Limit of Detection, NeuMoDx CT/NG Assay 2.0

Specimen Type	***Chlamydia trachomatis*** **LoD (EB/mL)**	***Neisseria gonorrhoeae*** **LoD (cells/mL)**
Urine	7.08	0.68
Vaginal Swab	12.54	0.87

Competitive Inhibition

A competitive inhibition was performed for each specimen type, where one target was at a low concentration level while the other was at very high concentration. The results of the study demonstrated no impact to sensitivity for either target in combinations of high and low levels, respectively.

Analytical Reactivity (Inclusivity)

Inclusivity of the NeuMoDx CT/NG Assay 2.0 was verified with an additional 13 serovars of CT and 20 isolates of NG shown in Table 19. Panels were prepared in clinical negative urine and transport medium and initially dosed to levels equal to preliminary Probit LoD. For each serovar/isolate, a minimum of 3 replicates per matrix were tested across both NeuMoDx CT/NG systems, and a minimum detection rate of 100% of each isolate was required to verify equivalent sensitivity across variants tested. For each serovar/isolate, a minimum of 3 replicates per matrix were tested across both NeuMoDx CT/NG systems. If the positivity rate was less than 100%, additional testing was performed with higher target concentrations until 100% positivity was achieved. All variants tested were detected at a rate of 100% within 2X the claimed limit of detection.

Table 19: CT Serovars and NG Strains Evaluated for Inclusivity

CT Serovar	NG Strain	NG Strain
A	Strain 2686	Strain NCTC 8375 [B 5025]
B	MHD 446 [NCTC 10933]	Strain BDMS T4145 [CDC 83015965, Difco A0726]
Ba	ATCC 9793	Strain ODH 2915 [NCTC 10928]
C	Strain CDC Ng-98	Strain 40836 (Uri)
E*	BDMS 8658 [CIP 104217]	Strain B-1094
F	83F0120 [DC-83-82]	Strain MHD 340 [NCTC 10929]
G	83F0091 [HWD 4345]	Strain GC/CB/001 [CDC M-2]
H	WHO V [CDC 78-63856]	
I	76.061782	
K	F-18 [89-018314, CDC 10,001, P935]	
LGV I	Strain FA1090	
LGV II	Strain C-58 [D-10]	
LGV III	Strain CDC Ng-116	

Antibiotic resistant strains: Strain FA1090 – Streptomycin resistant; Strain BDMS T4145 [CDC 83015965, Difco A0726] – Spectinomycin resistant

*E_{nv} (Swedish variant) was also evaluated and tested positive

Analytical Specificity

Cross-Reactivity

A panel of 143 non-target bacteria, fungi, and viruses, including those commonly found in the urogenital tract and those phylogenetically related to CT or NG, were tested with the NeuMoDx CT/NG Assay 2.0 to assess the potential for cross-reactivity. Cross-reactivity was assessed by processing samples containing high titers (0.02-0.03 McFarland standard or 6-9×10^6 CFU/mL) of these organisms in the absence of CT or NG targets. Three (3) replicates of samples containing each pool of organisms were processed. The NeuMoDx CT/NG Assay 2.0 did not generate a false positive test result with any microorganisms listed in Table 20.

Table 20: Microorganisms Evaluated for Cross-Reactivity

Bacteria/Fungi	Bacteria/Fungi	Bacteria/Fungi
Achromobacter xerosis	*Helicobacter pylori*	*Peptostreptococcus magnus*
Acinetobacter baumannii	*Kingella denitrificans*	*Peptostreptococcus productus*
Acinetobacter calcoaceticus	*Kingella kingae*	*Plesiomonas shigelloides*
Acinetobacter lwoffii	*Klebsiella oxytoca*	*Propionibacterium acnes*
Actinomyces israelii	*Klebsiella pneumoniae*	*Proteus mirabilis*
Actinomyces pyogenes	*Lactobacillus acidophilus*	*Proteus vulgaris*
Aerococcus viridans	*Lactobacillus brevis*	*Providencia stuartii*
Aeromonas hydrophila	*Lactobacillus crispatus*	*Pseudomonas aeruginosa*
Alcaligenes faecalis	*Lactobacillus jensenii*	*Pseudomonas fluorescens*
Bacillus subtilis	*Lactobacillus lactis*	*Pseudomonas putida*
Bacteriodes fragilis	*Lactobacillus oris*	*Rahnella aquatilis*
Bacteroides caccae	*Lactobacillus parabuchnerri*	*Rhizobium radiobacter*
Bacteroides ureolyticus	*Lactobacillus vaginalis*	*Rhodospirillum rubrum*
Bergeriella denitrificans	*Lactococcus lactis cremoris*	*Saccharomyces cerevisiae*
Bifidobacterium adolescentis	*Legionella pneumophila*	*Salmonella minnesota*
Bifidobacterium breve	*Listeria monocytogenes*	*Salmonella typhimurium*
Bifidobacterium longum	*Micrococcus luteus*	*Serratia marcescens*
Brevibacterium linens	*Mobiluncus mulieris*††	*Staphylococcus aureus*
Campylobacter jejuni	*Moraxella catarrhalis*	*Staphylococcus epidermidis*
Candida albicans	*Moraxella lacunata*	*Staphylococcus saprophyticus*
Candida glabrata	*Moraxella osloensis*	*Streptococcus agalactiae*
Candida krusei	*Morganella morganii*	*Streptococcus anginosus* (group C)
Candida parapsilosis	*Mycobacterium smegmatis*	*Streptococcus bovis*
Candida tropicalis	*Mycoplasma genitalium*†	*Streptococcus dysgalactiae*
Chlamydia pneumoniae††	*Mycoplasma hominis*†	*Streptococcus equinus*
Chromobacterium violaceum	*Neisseria cinerea* (strain 194)	*Streptococcus mitis* (F0392)
Citrobacter freundii	*Neisseria elongata*	*Streptococcus mutans* (serogroup A)
Clostridium perfringens	*Neisseria flavescens*	*Streptococcus pneumoniae*
Corynebacterium genitalium	*Neisseria lactamica*	*Streptococcus pyogenes*
Corynebacterium xerosis	*Neisseria meningitidis* (serogroup A)	*Streptococcus salivarius*
Cryptococcus neoformans	*Neisseria meningitidis* (serogroup B)	*Streptococcus sanguinis*
Deinococcus radiodurans	*Neisseria meningitidis* (serogroup C)	*Streptomyces griseus*
Derxia gummosa	*Neisseria meningitidis* (serogroup D)	*Trichomonas vaginalis*

Eikenella corrodens	*Neisseria meningitidis* (serogroup WL35)	*Ureaplasma urealyticum**
Elizabethkingia miricola††	*Neisseria meningitidis* (serogroup Y)	*Veillonella parvula*
Enterobacter aerogenes	*Neisseria mucosa*	*Vibrio parahaemolyticus*
Enterobacter cloacae	*Neisseria perflava* (strain 28)	*Weissella paramesenteroides*
Enterococcus avium	*Neisseria perflava* (strain 7078)	*Yersinia enterocolitica*
Enterococcus faecalis	*Neisseria perflava* (strain 831F)	**Viruses**
Enterococcus faecium	*Neisseria polysaccharea*	Cytomegalovirus**
Erysipelothrix rhusiopathiae	*Neisseria sicca*	Epstein-Barr virus**
Escherichia coli	*Neisseria subflava*	Hepatitis B virus***
Flavobacterium meningosepticum	*Neisseria subflava* (strain CDN-1.7)	Hepatitis C virus***
Fusobacterium nucleatum	*Neisseria weaveri*	Herpes simplex virus I**
Gardnerella vaginalis	*Pantoea agglomerans*	Herpes simplex virus ll**
Gemella haemolysans	*Paracoccus denitrificans*	Human immunodeficiency virus 1***
Haemophilus ducreyi	*Peptoniphilus asaccharolyticus*	Human papillomavirus 16††
Haemophilus influenzae	*Peptostreptococcus anaerobius*	Human papillomavirus 18††

* tested at 1 x 10^6 CCU/mL (CCU=Color-Changing Unit)
** tested at 1 x 10^6 copies/mL
*** tested at 1 x 10^6 IU/mL
† tested at 1 x 10^6 CFU/mL
†† tested at 10 ng/mL purified DNA or RNA

Microbial Interference

The same organisms that were tested in the cross-reactivity study were evaluated in the microbial interference study. Each sample tested contained CT and NG at a concentration of 3X LoD and high titer microorganism concentration. Once prepared, each sample was tested in triplicate. No microbial interference with the NeuMoDx CT/NG Assay 2.0 was observed.

Interfering Substances

A potentially interfering substances evaluation study was performed for the NeuMoDx CT/NG Assay 2.0 in urine and vaginal swab matrix. The study was performed using endogenous and exogenous substances that could be present in clinical urogenital specimens. The interfering effects were characterized with samples prepared by adding potentially interfering substances to pooled negative clinical urine and pooled negative vaginal swab specimens in BD UVT. For swab matrices, mucin and exogenous substances were dosed using saturated swab from solutions of each prepared in molecular grade water via weight per volume or volume per volume. The saturated swab containing the interfering substance was added to an aliquot of a bulk preparation of pooled clinical negative vaginal swabs. CT and NG were then spiked at a concentration ≤ 3X LoD. In addition, a control group was included that contained spiked targets at the same concentration of pooled clinical negative urine and swab specimens without any potentially interfering substances.

Lists of interfering substances and their respective levels that were tested are presented in Table 21 and Table 22.

Table 21: Endogenous/Exogenous Interfering Substances Tested – Urine Specimens

Target Concentration	Endogenous	Concentration
3X LoD CT/NG	Blood	7% v/v
	PBMCs	10^5 Cells/mL
	Acidic Urine	pH 4.0
	Alkaline Urine	pH 9.05
	Glucose	10.0 mg/mL
	Bilirubin*	1.4 mg/dL
	Albumin	10.5 mg/mL
	Exogenous	**Concentration**
	Progesterone	7.5 mg/mL
	Vagisil Feminine Powder	3.6 mg/mL
	Norforms Deodorant Suppositories	2.8 mg/mL
	Talcum Powder	12.0 mg/mL
	Aspirin (Salicylic Acid)	40.0 mg/mL
	Azithromycin	2.2 mg/mL
	Doxycycline	4.5 mg/mL
	Acetaminophen	3.8 mg/mL
	Seminal Fluid	5.0% v/v

**Clinical positive specimen*

Table 22: Endogenous/Exogenous Interfering Substances Tested, Including Dosing Method – Swab Specimens

Target Concentration	Interference Samples- Endogenous	Concentration/Dosing Plan - Endogenous
≤ 3x LoD CT/NG	Blood	7% v/v
	Mucin*	37.4 mg/mL
	PBMCs	10^5 cells/mL
	Interference Samples- Exogenous	**Concentration/Dosing Plan - Exogenous**
	Seminal Fluid	25.1 mg/mL
	Progesterone Cream	3.9 mg/mL
	Vagisil Anti Itch Cream	3.9 mg/mL
	Clotrimazole Vaginal Cream	6.3 mg/mL
	Preparation H Hemorrhoidal Cream	6.9 mg/mL
	Miconazole 3	7.1 mg/mL
	Monistat 1	7.8 mg/mL
	Abreva Cold Sore Cream	4.1 mg/mL
	Vagisil Moisturizer	2.6 mg/mL
	Replens Moisturizer	3.1 mg/mL
	KY Jelly Personal Lubricant	9.4 mg/mL
	Yeast Gard Douche	34.9 mg/mL
	VCF Contraceptive Gel	0.25 mg/mL

	Summer's Eve Medicated Douche	42.2 mg/mL

*Mucin dosed by swab from a 0.8% stock

No interference was observed with substances evaluated at the concentrations listed above (i.e., all positive replicates tested produced positive results and all negative replicates tested produced negative results).

Carryover Contamination

Carryover was assessed in a two-part study and on the NeuMoDx 288 Molecular System and NeuMoDx 96 Molecular System separately. The first part of the study involved the processing of high positive CT/NG samples (pooled negative urine and neat transport medium at 106 EB/mL CT and 106 cells/mL NG) alternated with negative samples in a checkerboard arrangement. Next, full runs of high positive samples were immediately proceeded with full runs of negative samples to evaluate contamination between runs. Across both the NeuMoDx 288 Molecular System and NeuMoDx 96 Molecular Systems, a total of 72 high positive and 72 negative samples were tested for each matrix in the second part of the study. The study included 4 runs (2 alternating checkerboard, 1 positive followed by a negative run). No contamination was observed.

Assay Cut-Off

Assay results from each test are determined by a cut-off based on the end point ratio (EPR), end point signal (EP), and cycle threshold (Ct). A test may be negative, positive, indeterminate, no result, or unresolved as determined by these measurements for the detection of CT and NG DNA. Table 23 and Table 24 below describe the criteria associated with each possible assay result for swab and urine specimens, respectively.

Table 23: Processing and Cutoff Criteria (ADF CTNG 2.11.0) for Swab Workflow

Result	Target (CT)	Target (NG)	Process Control (SPC1)	Interpretation
Positive	**AMPLIFIED** [25< Ct ≤42 AND EP ≥500] **OR** [Ct ≤25 AND EP ≥500 AND EPR >1.3]	N/A	N/A	CT/NG DNA detected**
	N/A	**AMPLIFIED** [25< Ct ≤42 AND EP ≥900] **OR** [Ct ≤25 AND EP ≥900 AND EPR >1.3]	N/A	CT/NG DNA detected**

Result	Target (CT)	Target (NG)	Process Control (SPC1)	Interpretation
Negative	**NOT AMPLIFIED** N/A **OR** [25< Ct ≤42 AND EP <500] **OR** [Ct ≤25 AND EPR ≤1.3] **OR** [Ct >42]	**NOT AMPLIFIED** N/A **OR** [25< Ct ≤42 AND EP <900] **OR** [Ct ≤25 AND EPR ≤1.3] **OR** [Ct >42]	**AMPLIFIED** [30≤ Ct ≤37 AND EP ≥1000	CT/NG DNA not detected
IND/NR	NOT AMPLIFIED/System Errors Noted			All target results were invalid; 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/UNR result is automatically reprocessed to minimize delays in result reporting.
**A re-test may be performed if desired in the event of only CT or NG target being amplified.

Table 24: Processing and Cutoff Criteria (ADF CTNG 2.11.0) for Urine Workflow

Result	Target (CT)	Target (NG)	Process Control (SPC1)	Interpretation
Positive	**AMPLIFIED** [25< Ct ≤42 AND EP ≥700] **OR** [Ct ≤25 AND EP ≥700 AND EPR >1.3]	N/A	N/A	CT/NG DNA detected**
	N/A	**AMPLIFIED** [25< Ct ≤42 AND EP ≥1000] **OR** [Ct ≤25 AND EP ≥1000 AND EPR >1.3]	N/A	CT/NG DNA detected**
Negative	**NOT AMPLIFIED** N/A **OR** [25< Ct ≤42 AND EP <700] **OR** [Ct ≤25 AND EPR ≤1.3] **OR** [Ct >42]	**NOT AMPLIFIED** N/A **OR** [25< Ct ≤42 AND EP <1000] **OR** [Ct ≤25 AND EPR ≤1.3] **OR** [Ct >42]	**AMPLIFIED** [29≤ Ct ≤35 AND EP ≥2000]	CT/NG DNA not detected

IND/NR	NOT AMPLIFIED/System Errors Noted	All target results were invalid; 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/UNR result is automatically reprocessed to minimize delays in result reporting.
**A re-test may be performed if desired in the event of only CT or NG target being amplified.

Method Comparison with Predicate Device
The comparison studies were conducted in the clinical study with prospectively collected samples (see the Clinical Study section above).

Matrix Comparison
A matrix equivalency study was conducted to demonstrate the equivalence of samples collected in neat BD UVT used in analytical testing to samples collected in clinical negative vaginal swab matrix (collected in BD UVT) for use with the NeuMoDx CT/NG Assay 2.0. Performance of each matrix was assessed in parallel using moderate positive, low positive, and negative samples. Moderate positive samples were contrived to 5x Limit of Detection (LoD) and low positive samples were contrived to 2x LoD, as determined by selecting the target level that reached ≥95% positivity during Probit analysis portion of the Limit of Detection testing.

Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) targets were dosed together in positive samples. Negative samples consisted of only the matrix being evaluated. Each matrix was tested in replicates of n=10 at 5x LoD and n=30 at 2x LoD. Ten replicates were tested in each matrix lacking both analytes, in neat BD UVT and clinical negative vaginal swab matrix (collected in BD UVT).

The 5x LoD and 2x LoD level produced rates of 100% positivity in both matrices for the CT and NG targets. Negative samples in both matrices resulted in no amplification of the CT or NG targets , with valid negative results achieved at a rate of 100%. These results demonstrated matrix equivalency for the purposes of the analytical studies conducted within this submission.

Clinical Cutoff
Not Applicable.

Expected Values/Reference Range
A summary of the positivity rates of CT and NG, by specimen collection site and specimen type, as determined by the CT/NG Assay 2.0 on the NeuMoDx 96 Molecular System and NeuMoDx 288 Molecular System from a multi-center clinical study is shown in Table 25. The positivity rates shown below include all cases.

Table 25: Positivity Rates for CT and NG Infections by the NeuMoDx CT/NG Assay 2.0, by Specimen Collection Site

Specimen Collection Site Number	Specimen Type	N (CT) Number of Results	Positivity CT (%)	N (NG) Number of Results	Positivity NG (%)
1	SCVS	216	9.72%	216	4.17%
	Female Urine	213	8.92%	213	4.23%
	Male Urine	175	15.43%	174	17.24%
	Total	604	11.09%	603	7.96%
2	SCVS	9	11.11%	9	0.00%
	Female Urine	9	0.00%	9	0.00%
	Male Urine	38	5.26%	38	10.53%
	Total	56	5.36%	56	7.14%
3	SCVS	85	3.53%	85	2.35%
	Female Urine	85	3.53%	85	2.35%
	Male Urine	N/E	N/E	N/E	N/E
	Total	170	3.53%	170	2.35%
4	SCVS	487	7.19%	487	3.70%
	Female Urine	485	5.98%	485	3.30%
	Male Urine	233	11.59%	233	7.30%
	Total	1205	7.55%	1205	4.23%
5	SCVS	118	2.54%	118	1.69%
	Female Urine	117	2.56%	117	1.71%
	Male Urine	76	11.84%	76	9.21%
	Total	311	4.82%	311	3.54%
6	SCVS	36	2.78%	36	0.00%
	Female Urine	35	2.86%	35	0.00%
	Male Urine	259	10.04%	259	7.34%
	Total	330	8.48%	330	5.76%
7	SCVS	229	8.73%	229	3.06%
	Female Urine	228	9.21%	227	3.08%
	Male Urine	220	19.55%	220	4.55%
	Total	677	12.41%	676	3.55%

Specimen Collection Site Number	Specimen Type	N (CT) Number of Results	Positivity CT (%)	N (NG) Number of Results	Positivity NG (%)
8	SCVS	167	5.99%	167	1.80%
	Female Urine	165	6.06%	165	1.82%
	Male Urine	120	16.67%	120	1.67%
	Total	452	8.85%	452	1.77%
9	SCVS	340	0.88%	340	1.18%
	Female Urine	340	0.59%	340	0.88%
	Male Urine	236	2.12%	236	0.42%
	Total	916	1.09%	916	0.87%
10	SCVS	217	4.61%	217	0.46%
	Female Urine	219	4.57%	219	0.46%
	Male Urine	131	13.74%	131	1.53%
	Total	567	6.70%	567	0.71%
11	SCVS	9	0.00%	9	0.00%
	Female Urine	9	0.00%	9	0.00%
	Male Urine	87	3.45%	87	0.00%
	Total	105	2.86%	105	0.00%
12	SCVS	13	7.69%	13	0.00%
	Female Urine	13	7.69%	13	0.00%
	Male Urine	22	13.64%	22	4.55%
	Total	48	10.42%	48	2.08%
13	SCVS	1	0.00%	1	0.00%
	Female Urine	1	0.00%	1	0.00%
	Male Urine	35	5.71%	35	0.00%
	Total	37	5.41%	37	0.00%
14	SCVS	89	2.25%	89	0.00%
	Female Urine	88	1.14%	88	0.00%
	Male Urine	67	2.99%	67	1.49%
	Total	244	2.05%	244	0.41%
All Sites	SCVS	2016	5.46%	2016	2.28%
	Female Urine	2007	4.98%	2006	2.14%

Specimen Collection Site Number	Specimen Type	N (CT) Number of Results	Positivity CT (%)	N (NG) Number of Results	Positivity NG (%)
	Male Urine	1699	11.01%	1698	5.54%
	Total	5722	6.94%	5720	3.20%

N based on valid NeuMoDx CT/NG Assay 2.0 results
SCVS = Self-Collected Vaginal Swab
N/E = Site did not enroll any subjects in this category as the site was a gynecology clinic.

Specimen Identification
By hand-held barcode reader or automated barcode reader and positional checks.

Specimen Sampling and Handling
Fully automated.

Calibration
Not applicable.

Quality Control
In addition to user-defined controls chosen and validated by the user, the NeuMoDx Molecular System contains process controls that employ both hardware and software components. The process controls include, but are not limited to:

- Verification that the sequence of assay processing steps is correct for each reaction.
- Verification that the reaction incubation times and temperatures are correct.
- Verification that reagents and fluids are appropriately dispensed.

Conclusions

The NeuMoDx CT/NG Assay 2.0 is substantially equivalent to the legally marketed Hologic, Inc. Aptima Combo 2 Assay.