REF **617007 NeuMoDx™ HPV Test Strip** ℞ only

CAUTION: For US Export Only

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

Electronic version is available at www.qiagen.com/neumodx-ifu
For detailed instructions, refer to the NeuMoDx 288 Molecular System Operator's Manual; P/N 40600108
For detailed instructions, refer to the NeuMoDx 96 Molecular System Operator's Manual; P/N 40600317

INTENDED USE

The NeuMoDx HPV Assay, as performed on the NeuMoDx 96 Molecular System and NeuMoDx 288 Molecular System (NeuMoDx System(s)), is a rapid, automated, *in vitro* diagnostic, real-time PCR-based nucleic acid amplification assay for the qualitative detection of high-risk types of human papillomavirus (HPV) DNA in cervical specimens. The test specifically identifies HPV16 and HPV18 while concurrently detecting the other high risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, and 68) at clinically relevant infection levels. Cervical specimens that may be tested with the NeuMoDx HPV Assay include clinician-collected cervical specimens using a brush/broom-type collection device preserved in PreservCyt® (HOLOGIC) and SurePath™ (BD) liquid based cytology. The assay is intended to be used as a primary test in screening of women of 21 years and older for the risk of cervical (pre)cancer to determine the need for referral to colposcopy or other follow-up procedures and as a follow-up test for women with Pap test results with atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous intra-epithelial legion (LSIL) to determine the need for referral to colposcopy or other follow-up procedures. This information, together with the physician's assessment of cytology, history, other risk factors, and professional guidelines, may be used to guide patient management.

This product is intended to be used by professional users, such as technicians and laboratorians who are trained in *in vitro* diagnostics procedures, and molecular biological techniques.

SUMMARY AND EXPLANATION

Cervical cancer and its precursor lesions (cervical intraepithelial neoplasia, CIN) are caused by a persistent infection with a high-risk type of human papillomavirus (HPV).[1-3] HPV belong to the family of Papillomaviridae and are small double-stranded DNA viruses. The circular genome is approximately 7.9 kilo bases in size. More than 100 types of HPV have been identified, of which certain HPV types, known as high-risk HPV (hrHPV) like HPV 16 and 18, are associated with the induction of mucosal lesions that can progress to malignancy. The viral genome contains early (E) and late (L) genes, which encode proteins necessary for early and late stages of the HPV life cycle, respectively. The E6 and E7 gene products of hrHPV types have carcinogenic properties and are necessary for malignant transformation of the host cell.[4] Malignant progression is often associated with viral integration into the genome of the host cell.[5] Integration results in interruption of the viral genome in a region that may extend from the E1 to the L1 open reading frame.[6] This may have consequences for PCR-mediated amplification of viral DNA in these regions. As not only the initiation but also the maintenance of the transformed phenotype depends on continuous expression of the viral oncoproteins, the viral E6/E7 region is invariably retained in integrated viral genomes in cervical cancers.[6,7,8]

Cervical cancer is a rare complication of an HPV infection; the lifetime risk of an hrHPV infection is estimated to be around 80% and the large majority of infections are cleared by the host immune system and do not give rise to lesions.[9] After the clearing of the HPV infection the CIN lesions usually regresse.[10]

Testing for HPV DNA provides better protection against cervical cancer and its CIN precursor lesions compared to cytomorphological analysis (i.e. Pap smear) in cervical samples in primary screening in women aged 30 years and older and in the triage of women aged 21 and older with ASC-US or LSIL cervical cytology (ASC-US).[11-15] Primary HPV-based cervical screening is implemented in several countries globally and international guidelines for HPV DNA test requirements for primary cervical cancer screening have been published.[16] The NeuMoDx HPV Assay targets a conserved region within the E7 gene of the HPV genome, thereby overcoming potential false-negative results upon viral integration into the host genome.

PRINCIPLES OF THE PROCEDURE

The NeuMoDx HPV Assay combines automated DNA extraction and amplification/detection by real-time PCR. Cervical specimens are collected in liquid cytology solution and then transferred to a compatible secondary specimen tube, barcoded, and placed on the NeuMoDx System. The NeuMoDx System automatically aspirates an aliquot of the specimen to mix with NeuMoDx Lysis Buffer 2 and the agents 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/detection of the target sequences using real-time PCR. β-globin (βG) DNA which is present in every properly collected specimen serves as an endogenous sample process control and helps monitor for the presence of inhibitory substances and for system, process, or reagent failures. No operator intervention is necessary once the specimen and necessary consumables are loaded onto the NeuMoDx System.

The NeuMoDx System automatically performs lysis, DNA extraction, and removal of inhibitors. The released nucleic acids are captured by paramagnetic particles. The particles, with bound nucleic acid, are loaded into the NeuMoDx Cartridge where the unbound elements are washed away with NeuMoDx Wash Reagent. The bound DNA is then eluted using NeuMoDx Release Reagent. The NeuMoDx System uses the eluted DNA to rehydrate proprietary NeuDry™ amplification reagents containing all the components necessary for 40 cycles of amplification of the 15 HPV targets (if present), as well as the β-globin target. This enables simultaneous amplification and detection of target and control DNA sequences. Upon 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 sequences (if present) occur in the PCR chamber. The NeuMoDx Cartridge is designed to contain the amplicon following PCR, virtually eliminating the risk of post-amplification contamination.

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

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

A TaqMan probe labeled with a fluorophore at the 5' end and a dark quencher at the 3' end is used to detect HPV 16 (470/510 nm), HPV 18 (625/660 nm), and the remaining clinically significant high risk (HR) types ('HPV Other'; 530/555 nm). For detection of the β-globin, the TaqMan probe is labeled with an alternate fluorescent dye (585/610 nm) at the 5' end and a dark quencher at the 3' end. The NeuMoDx System software monitors the fluorescent signal emitted by the TaqMan probes at the end of each amplification cycle. When amplification is complete, the NeuMoDx System software analyzes the data and reports a result (POSITIVE/NEGATIVE/INDETERMINATE/UNRESOLVED/NO RESULT).

REAGENTS / CONSUMABLES

Material Provided

REF	Contents	Units per package	Tests per unit	Tests per package
617007	**NeuMoDx HPV Test Strip** *Dried PCR reagents containing HPV and βG 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 β-globin control*
400500	**NeuMoDx Lysis Buffer 2**
401600	**NeuMoDx Viral Lysis Buffer***
400100	**NeuMoDx Wash Reagent**
400200	**NeuMoDx Release Reagent**
100100	**NeuMoDx Cartridge**
235903	**Hamilton® CO-RE / CO-RE II Tips (300 µL) with Filters**
235905	**Hamilton CO-RE / CO-RE II Tips (1000 µL) with Filters**

**Required for processing of Pre-treated SurePath samples*

Instrumentation Required

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

WARNINGS AND PRECAUTIONS

- The NeuMoDx HPV Test Strip is for *in vitro* diagnostic use with NeuMoDx Systems only.
- Do not use the reagents or consumables past the listed expiration date.
- Do not use any reagents if the safety seal is broken or if the packaging is damaged upon arrival.
- Do not use consumables or reagents if the protective pouch is open or broken upon arrival.
- Minimum specimen volume of secondary aliquots is dependent on the tube size/specimen tube carrier as defined below. Volume below the specified minimum may result in a "Quantity Not Sufficient" error.
- The use of specimens stored at improper temperatures or beyond the specified storage times may produce invalid or erroneous results.
- Only SurePath specimens pre-treated with Viral Lysis Buffer may be used on the NeuMoDx Molecular Systems. Neat specimens may produce invalid or sub-optimal results.

- Up to 20% evaporation of the specimen was observed in validation studies performed to evaluate the on-system specimen stability due to high volatility of the PreservCyt collection medium. This is not expected to affect sample results negatively but should be taken into consideration when preparing samples for delayed processing. No significant evaporation was observed with pre-treated SurePath specimens.
- Avoid microbial and deoxyribonuclease (DNase) contamination of all reagents and consumables. The use of sterile DNase-free, disposable transferring pipettes is recommended when using secondary tubes. Use a new pipette for each specimen.
- To avoid contamination, do not handle or break apart any NeuMoDx Cartridge post-amplification. Do not retrieve NeuMoDx Cartridges from the Biohazard Waste Container (NeuMoDx 288 Molecular System) or Biohazard Waste Bin (NeuMoDx 96 Molecular System) under any circumstances. The NeuMoDx Cartridge is designed to prevent contamination.
- In cases where open-tube PCR tests are also conducted by the laboratory, care must be taken to ensure that the NeuMoDx HPV Test Strip, the additional consumables and reagents required for testing, personal protective equipment such as gloves and lab coats, and the NeuMoDx System are not contaminated.
- Clean, powder-free, nitrile gloves should be worn when handling NeuMoDx reagents and consumables. Care should be taken not to touch the top surface of the NeuMoDx Cartridge, the foil seal surface of the NeuMoDx HPV Test Strip and 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.qiagen.com/neumodx-ifu.
- Wash hands thoroughly after performing the test.
- Do not pipette by mouth. Do not smoke, drink, or eat in areas where specimens or reagents are being handled.
- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in *Biosafety in Microbiological and Biomedical Laboratories*[17] and in CLSI Document M29-A4.[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 HPV Test Strips are stable in the primary packaging through the stated expiration date on the immediate product label when stored at 15 to 23 °C.
- Do not reload any test product that has previously been loaded onto *another* NeuMoDx System.
- Once loaded, the NeuMoDx HPV 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, HANDLING, STORAGE AND TRANSPORT

1. The NeuMoDx HPV Assay is intended for use with samples obtained from cervical specimens. The validated collection media for cervical specimens is PreservCyt and SurePath. Follow the specimen collection device manufacturer instructions for preparation and storage.
2. SurePath Specimens must be pre-treated prior to use following specific instructions below.
3. **Refrigerated samples should be equilibrated to room temperature for at least 30 minutes before processing for proper system performance.**
4. Prepared cervical specimens may be stored on the NeuMoDx System for up to 24 hours prior to processing. If additional storage time is required, it is recommended that the specimens be stored according to the following:

   Cervical specimens in **PreservCyt**:

   a. Up to 6 weeks after sampling when stored at 15–25 °C
   b. Up to 3 months after sampling when stored at 2–8 °C
   c. Up to 8 years when stored at -80 °C. If specimens are frozen, allow to completely thaw at room temperature (15–30 °C) and vortex to generate a uniformly distributed sample.

   Cervical specimens in **SurePath**:

   a. Up to 30 days after sampling when stored at 2-30 °C
   b. Up to 180 days after sampling when stored at 2–8 °C
   c. Up to 180 days when stored at -20 °C. If specimens are frozen, allow to completely thaw at room temperature (15–30 °C) and vortex to generate a uniformly distributed sample.
5. If specimens are shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations.
6. Label specimens clearly and indicate specimens that are for HPV testing.

INSTRUCTIONS FOR USE

Test Preparation- PRESERVCYT

1. Apply specimen barcode label to a specimen tube compatible with the NeuMoDx System.
2. Place the barcoded tube into a Specimen Tube Carrier and ensure the cap is removed prior to loading onto the NeuMoDx System.
3. Aliquot the specimen according to the volumes defined below for **PreservCyt** Samples:

    - Specimen Tuber Carrier (32-tube): 11 – 14 mm in diameter and 60 – 120 mm in height; minimum fill volume = 400 µL
    - Specimen Tube Carrier (24-tube): 14.5 – 18 mm in diameter and 60 – 120 mm in height; minimum fill volume = 850 µL
    - Low Volume Specimen Tube Carrier (32-tube): 1.5 mL conical bottom microcentrifuge tube; minimum fill volume = 250 µL

Test Preparation- SUREPATH

1. Pre-treat SurePath specimen with a 1:1 volume of NeuMoDx Viral Lysis Buffer and mix thoroughly. Use appropriate volume to meet minimum specimen volume as defined below.
2. Incubate at 90°C for 20 minutes followed by equilibration to room temperature before proceeding.
3. Apply specimen barcode label to a specimen tube compatible with the NeuMoDx System.
4. Place the barcoded tube into a Specimen Tube Carrier and ensure the cap is removed prior to loading onto the NeuMoDx System.
5. Aliquot the specimen according to the volumes defined below for **SurePath** samples:

    - Specimen Tuber Carrier (32-tube): 11 – 14 mm in diameter and 60 – 120 mm in height; minimum fill volume = 450 µL
    - Specimen Tube Carrier (24-tube): 14.5 – 18 mm in diameter and 60 – 120 mm in height; minimum fill volume = 800 µL
    - Low Volume Specimen Tube Carrier (32-tube): 1.5 mL conical bottom microcentrifuge tube; minimum fill volume = 300 µL

NeuMoDx System Operation

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

1. Load the Test Order onto the NeuMoDx System according to the desired specimen type.

    - PreservCyt samples are tested by defining the specimen as "Cytology".
    - Pre-treated SurePath samples are tested by defining the specimen as "UserSpecified1".

    If not defined in the Test order, the PreservCyt specimen type will be used as default.
2. Populate one or more NeuMoDx System Test Strip Carrier(s) with NeuMoDx HPV 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 Molecular System only), Tip Waste Bin (NeuMoDx 96 Molecular System only), or Biohazard Waste Bin (NeuMoDx 96 Molecular System only), as appropriate.
5. Load the specimen tube(s) into a Specimen Tube Carrier and ensure caps are removed from all tubes.
6. Place the Specimen Tube Carrier(s) on the autoloader shelf and use the touchscreen to load the carrier(s) into the NeuMoDx System. This will initiate processing of the loaded specimens for the tests identified, given a valid test order is present in the system.

LIMITATIONS

1. The NeuMoDx HPV Test Strip can only be used on NeuMoDx Systems.
2. The performance of the NeuMoDx HPV Test Strip has been established for use with samples obtained from cervical specimens (scrapes) in PreservCyt, SurePath or equivalent cytology media. Use of the NeuMoDx HPV Test Strip with other sources has not been assessed, and performance characteristics are unknown for other specimen types or collection media.
3. Only SurePath specimens pre-treated with Viral Lysis Buffer may be used on the NeuMoDx Molecular Systems. Neat specimens may produce invalid or sub-optimal results.
4. Because detection of HPV is dependent on the amount of tissue present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
5. Erroneous results could occur from improper specimen collection, handling, storage, technical error, or specimen tube confusion. In addition, false negative results could occur because the number of viral particles in the sample is below the limit of detection of the NeuMoDx HPV Assay.
6. Operation of the NeuMoDx System is limited to use by personnel trained on the use of the NeuMoDx System.
7. If both the HPV targets and the β-globin target do not amplify, an invalid result (Indeterminate, No Result, or Unresolved) will be reported and the test should be repeated.
8. A positive result does not necessarily indicate the presence of viable HPV. However, a positive result is presumptive for the presence of HPV DNA.

9. Deletions or mutations in the conserved regions targeted by the NeuMoDx HPV Assay may affect detection and could lead to an erroneous result.
10. Results from NeuMoDx HPV Assay should be used as an adjunct to clinical observations and other information available to the physician.
11. Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination.

RESULTS PROCESSING

Available results may be viewed or printed from the 'Results' tab in the Results window on the NeuMoDx System touchscreen. NeuMoDx HPV Assay results are automatically generated by the NeuMoDx System software using the decision algorithm and results processing parameters specified in the NeuMoDx HPV Assay Definition File (HPV ADF). A NeuMoDx HPV Assay result may be reported as Negative, Positive, Indeterminate (IND), No Result (NR), or Unresolved (UNR), based on the amplification status of the targets and sample process control. Results are reported based on the ADF decision algorithm, summarized below in *Table 1*.

Ct thresholds for each of the targets were established and are presented in *Table 2* below to align with clinical relevance of the assay. There may be scenarios where a target amplification curve is observed but a Negative result is reported. This reporting is consistent with the result processing and cutoff criteria validated by NeuMoDx.

The results reported by the NeuMoDx HPV Test must be evaluated by physician in context of other findings.

Table 1. Summary of HPV Assay Decision Algorithm

RESULT	HPV16	HPV18	HPV Other	PROCESS CONTROL (βG)
POSITIVE	AMPLIFIED	N/A^	N/A^	N/A^
POSITIVE	N/A^	AMPLIFIED	N/A^	N/A^
POSITIVE	N/A^	N/A^	AMPLIFIED	N/A^
NEGATIVE	NOT AMPLIFIED	NOT AMPLIFIED	NOT AMPLIFIED	AMPLIFIED
IND	NOT AMPLIFIED, System Error Detected, Sample Processing Completed			
IND/NR*	NOT AMPLIFIED, System Error Detected, Sample Processing Aborted			
UNR	NOT AMPLIFIED, No System Errors Noted			

** No Result flag is only reported on NeuMoDx System software versions 1.8 and higher.*
^ N/A = Not Applicable

Table 2. Ct Cut Off Values for Positive Calls

RESULT	HPV16	HPV18	HPV Other	PROCESS CONTROL (βG)
POSITIVE	33	33	30	N/A*

** N/A = Not Applicable*

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.

User-Defined (External) Controls

1. Appropriate user-defined controls must be chosen and validated by the laboratory in accordance with local guidelines. Note that the user-defined controls must meet the same minimum volume specifications as clinical samples specified above based on the Specimen Tube Carrier size.
2. When processing user-defined controls, place the labeled controls in a Specimen Tube Carrier and use the touchscreen to load the carrier into NeuMoDx System from the autoloader shelf. Once defined, the NeuMoDx System will recognize the barcodes and start processing controls.
3. It is recommended that users process one set of positive and negative user-defined controls every 24 hours.
4. A positive test result reported for a negative user-defined control sample may indicate 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 user-defined 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.

Sample Process (Internal) Control

β-Globin (βG) serves as an endogenous internal control as it is present in properly collected cervical scrapes. The βG target undergoes the entire process of nucleic acid extraction and real-time PCR amplification with each sample, and also functions as a sample quality check. Primers and probe specific to βG are included in each NeuMoDx HPV Test Strip along with the primers and probes for the multiple HPV targets, enabling detection of βG with the target HPV DNA (if present) via multiplex PCR. Detection of βG amplification allows the NeuMoDx System software to monitor the efficacy of the specimen collection, DNA extraction and PCR amplification processes.

NeuMoDx System(s) Controls

The NeuMoDx System(s) perform various instrument internal controls as follows:

1. Prior to PCR, the NeuMoDx System automatically performs a 'FILL CHECK' to ensure that the PCR chamber is filled with solution and contains an adequate amount of fluorescent probe.
2. The NeuMoDx System software continuously monitors on-board sensors and actuators to ensure a safe and effective operation of the System.
3. Multiple fluidic error recovery modes are implemented by active monitoring of aspiration and dispense operations to ensure that the System can either complete processing of all samples in a safe and effective manner or provide an appropriate error code.
4. The NeuMoDx System is equipped with automatic Rerun/Repeat capability that the end user can choose to use to ensure that an INVALID result is automatically reprocessed to minimize delays in result reporting.

Invalid Results

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

An IND 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.

A UNR result will be reported if no valid amplification of HPV DNA or βG is detected, which indicates possible reagent failure or the presence of inhibitors. If a UNR result is reported, a retest is recommended as a first step. If a retest fails, a specimen dilution may be used to mitigate the effects of any sample inhibition.

A NR result will be reported if sample processing is aborted due to a system error. If a NR result is reported, a retest is recommended. This flag is only reported on NeuMoDx Software versions 1.8 and higher. In lower versions of the software, this error is reported as IND.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The limit of detection (LoD) was determined using a serial three-fold gBlock (double-stranded blocks of genomic DNA) dilution series containing the amplicon region from each of the targeted HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, 68) and β-globin. Each six-member dilution series was prepared in a background of 2000 ng/mL human DNA (except for β-globin) and concentration tested in 45-fold. Results of the study where the LoD was determined using 95% hit-rate analysis is presented in *Table 3* below.

Table 3. Limit of Detection (LoD) of the NeuMoDx HPV Assay of 15 hrHPV types and β-globin gene

Target	Limit of Detection (copies/mL)
HPV 16	8,230
HPV 18	2,743
HPV 31	24,691
HPV 33, 35, 39, 45, 51, 56, 66, 67	74,074
HPV 52, 58, 59	222,222
HPV 68	666,667
β-globin	74,074

Analytical Specificity

Analytical specificity of the NeuMoDx HPV Assay was determined against DNA of non-targeted HPV genomes (*Table 4*) at 1 x 10^6 copies/mL and against the potentially pathogenic vaginal microorganisms shown in *Table 5* at 1 x 10^6 CFU/mL or 1 x 10^5 PFU/mL. The assay did not show any cross-reactivity with the non-targeted HPV types 6, 11, 26, 30, 34, 53, 69, 73, 82, 85, or the microorganisms. Positive 'HPV Other' results were observed with HPV 70—likely due to high sequence homology between types 39, 68, and 70—and a subsequent titration study indicated this type could be detected at ≥ 4.12 x 10^6 copies/mL. HPV 70 is considered probably carcinogenic on the basis of epidemiological, phylogenetic, and functional studies.

Table 4. Non-target HPV types evaluated for cross-reactivity

HPV Non-Targeted Genotypes	
HPV 6	HPV 69
HPV 11	HPV 70
HPV 26	HPV 73
HPV 30	HPV 82
HPV 34	HPV 85
HPV 53	

Table 5. Microorganisms evaluated for cross-reactivity

Microorganism		
Adenovirus*	*Enterococcus spp.*	*Proteus vulgaris*
Bacteroides fragilis	Epstein Barr virus	*Pseudomonas aeruginosa*
Bifidobacterium adolescentis	*Escherichia coli*	*Staphylococcus agalactiae*
Candida albicans	*Fusobacterium spp.*	*Staphylococcus aureus*
Chlamydia trachomatis	Herpes simplex virus 1	*Staphylococcus epidermidis*
Clostridium perfringens	Herpes simplex virus 2	*Staphylococcus faecalis*
Corynebacterium spp.	*Klebsiella oxytoca*	*Staphylococcus pyogenes*
Cytomegalovirus	*Lactobacillus acidophilus*	*Trichomonas vaginalis*
Enterobacter aerogenes	*Neisseria gonorrhoeae*	
*Treponema pallidum***	*Peptostreptococcus anaerobius*	

** tested at 1 x 10^5 ($TCID_{50}$)/mL*
***performed by in silico analysis*

Analytical Reproducibility

The analytical reproducibility of the NeuMoDx HPV Assay was assessed using the same data set as used for the limit of detection study. The samples were tested at 3X LoD across 3 different NeuMoDx Molecular Systems, 1 N288, and 2 N96 systems using 3 different lots of NeuMoDx HPV Test Strips. Data showed excellent reproducibility overall, with maximum CV of 3.0% for each of the genotypes tested as shown in *Table 6*. In addition, this data set was used to demonstrate the reproducibility between lots of reagents and systems as shown in *Table 7*.

Table 6. hrHPV genotypes tested

Target	Target concentration	copies/ mL	Hitrate	Overall CV
B-globin	3X LoD	222222	100% (45/45)	1.8%
HPV 16		24691	100% (44/44)	1.3%
HPV 18		8230	100% (45/45)	1.3%
HPV 31		74074	100% (45/45)	1.3%
HPV 33		222222	100% (45/45)	1.6%
HPV 35		222222	100% (45/45)	0.8%
HPV 39		222222	100% (45/45)	1.4%
HPV 45		222222	100% (45/45)	1.5%
HPV 51		222222	100% (45/45)	1.8%
HPV 52		666667	97.8% (44/45)	3.0%
HPV 56		222222	100% (45/45)	1.3%
HPV 58		666667	100% (44/44)	2.4%
HPV 59		666667	100% (45/45)	2.5%
HPV 66		222222	100% (45/45)	1.8%
HPV 67		222222	100% (45/45)	1.4%
HPV 68		2000000	100% (45/45)	2.9%

Table 7. Between lot and between system reproducibility

Target	Lot Variability CV			System Variation CV		
	Lot 1	Lot 2	Lot 3	System 1 (N96)	System 2 (N288)	System 3 (N96)
B-globin	1.5%	2.4%	1.0%	1.7%	2.4%	1.0%
HPV 16	0.9%	1.1%	1.6%	1.8%	1.0%	0.9%
HPV 18	1.2%	1.6%	0.9%	1.1%	1.0%	1.5%
HPV 31	1.3%	1.5%	1.1%	1.1%	1.2%	1.1%
HPV 33	2.1%	1.4%	1.2%	0.9%	2.5%	0.9%
HPV 35	0.7%	0.7%	0.9%	0.9%	0.7%	0.8%
HPV 39	1.6%	1.6%	0.8%	1.1%	1.9%	0.9%
HPV 45	1.5%	1.4%	1.7%	1.4%	1.6%	1.1%
HPV 51	2.1%	1.2%	1.9%	1.1%	2.3%	1.4%
HPV 52	2.2%	4.0%	2.5%	1.5%	3.9%	1.6%
HPV 56	1.4%	1.5%	1.1%	0.6%	1.5%	1.3%
HPV 58	1.3%	3.2%	2.2%	2.1%	1.8%	3.0%
HPV 59	2.3%	2.4%	2.7%	1.1%	2.3%	0.9%
HPV 66	2.5%	1.5%	0.8%	1.3%	2.3%	1.3%
HPV 67	1.1%	1.2%	1.8%	0.6%	2.1%	1.1%
HPV 68	1.4%	3.1%	3.8%	1.5%	3.9%	1.9%

Interfering Substances

Contrived samples of PreservCyt were spiked with a recombinant baculovirus incorporating amplicon regions of HPV 16, 18, 51 and β-globin at 1000 copies/mL and substances listed in *Table 8.* None of the agents had a significant inhibitory effect on the performance of the assay.

Table 8. Potentially interfering substances tested

	Substance	Concentration
Endogenous	Whole blood (human)	1% (v/v)
	Leukocytes	10^6 cells/mL
	Mucin	1% (v/v)
Exogenous	Douche	1% (v/v)
	Anti-fungal cream	1% (w/v)
	Spermicide	1% (w/v)
	Vaginal lubricant	1% (w/v)
	Feminine spray	1% (v/v)
	Contraceptive foam	1% (w/v)

On-board Sample Stability

Recombinant baculovirus control containing the targets for HPV 16, 18, 51 and β-globin was spiked at ~3x LOD cp/mL in either SurePath or PreservCyt Collection Medium and processed using the NeuMoDx HPV Assay. At the end of processing, all the positive and negative specimen tubes were left on the system worktable for 4, 8, and 24 hours and then tested again. The expected result at all time points was POSITIVE for all the cytology specimens spiked with target and NEGATIVE (for all targets) in the cytology specimens that were not spiked with target. Complete concordance with expected result was observed at the 24-hour time point, demonstrating an on-board stability of 24 hours for testing with the NeuMoDx HPV Assay. Results summarized in *Table 9* below. The PreservCyt samples experienced up to 20% evaporation while stored onboard the system for 24 hours but it did not impact the detection of targets at level tested.

Table 9. On-board Sample Stability Data Summary

On-Board Specimen Stability	Target	PreservCyt		SurePath	
		T_0	24 hr	T_0	24 hr
		% Agreement	% Agreement	% Agreement	% Agreement
Positive Set	HPV 16	100%	100%	100%	100%
	HPV 18	100%	100%	100%	100%
	HPV Other	100%	100%	100%	100%
	β-Globin	100%	100%	100%	100%
Negative Set	Negative (β-Globin only)	100%	100%	100%	100%

Clinical Performance-PreservCyt Collection Medium

The clinical sensitivity and specificity of the NeuMoDx HPV Assay for cervical intraepithelial neoplasia grade 2 or higher (CIN2+) in cervical specimens collected in PreservCyt were evaluated by a non-inferiority analysis relative to the reference assay (i.e. high-risk HPV GP5+/6+-PCR-EIA) following international guidelines for HPV test requirements for cervical cancer screening.[16] Using a case-control study format, 67 samples were tested from women at and over the age of 30 with histologically confirmed CIN2+ (i.e. cases; *Table 10*). For the clinical specificity, 823 consecutively collected liquid-based cytology samples from the screening population of women with normal cytology and without evidence of CIN2+ within 2 years of follow-up were tested (i.e. controls). Overall success rate with the NeuMoDx HPV Assay was 99.4% (818/823), as shown in *Table 11*. The clinical sensitivity of the NeuMoDx HPV assay for CIN2+ was 92.5% (62/67; 95%CI 83.3–96.9) and the clinical specificity for CIN2+ was 95.6% (782/818; 95%CI 92.2–97.6), both of which were non-inferior to that of the reference assay GP5+/6+-PCR-EIA (P=0.02 and P<0.0001, respectively).

Table 10. Clinical sensitivity results of samples from women 30+ years with confirmed CIN2+

	NeuMoDx HPV Assay		
Reference Test	POS	NEG	TOTAL
POS	61	2	63
NEG	1	3	4
TOTAL	62	5	67
Clinical Sensitivity NeuMoDx HPV Assay: 92.5% (95%CI 83.3–96.9)			

Table 11. Clinical specificity results of samples from women with normal cytology and no confirmed CIN2+

	NeuMoDx HPV Assay		
Reference Test	POS	NEG	TOTAL
POS	28	19	47
NEG	8	763	771
TOTAL	36	782	818
Clinical Specificity NeuMoDx HPV Assay: 95.6% (95%CI 92.2–97.6)			

For women under the age of 30, 173 liquid-based cytology samples were tested from women attending an outpatient clinic. The success rate of the NeuMoDx HPV Assay was 98.3% (170/173) (*Table 12*). The CIN3+ sensitivity of the NeuMoDx HPV Assay was 91.1% (41/45; 95% CI 78.6-96.6) and CIN3+ specificity was 51.2% (64/125; 95%CI 42.5-60.0). Relative sensitivity and specificity values compared to the QIAScreen HPV PCR Test were 1.03 and 1.10, respectively.

Table 12. Performance NeuMoDx HPV assay in women aged <30 stratified by histology and QIAScreen HPV PCR Test

Histology	QIAScreen HPV PCR Test	NeuMoDx HPV Assay		
		NEG	POS	TOTAL
<=CIN1	NEG	53	1	54
	POS	6	43	49
	TOTAL	59	44	103
CIN2	NEG	4	-	4
	POS	1	17	18
	TOTAL	5	17	22
CIN3+	NEG	4	1	5
	POS	-	40	40
	TOTAL	4	41	45
OVERALL	NEG	61	2	63
	POS	7	100	107
	TOTAL	68	102	170

For women with ASC-US or LSIL, the clinical sensitivity for CIN2+ was 91.7% (11/12; 95%CI 58.7–98.8) and the clinical specificity for CIN2+ was 75.0% (15/20; 95%CI 52.2–89.2) (*Table 13*).

Table 13. Performance NeuMoDx HPV assay in women with ASC-US/LSIL cytology stratified by histology and reference test result

Histology	Reference Assay	NeuMoDx HPV assay		
		NEG	POS	TOTAL
<=CIN1	NEG	13	-	13
	POS	2	5	7
	TOTAL	15	5	20
CIN2	NEG	-	-	-
	POS	-	6	6
	TOTAL	-	6	6
CIN3+	NEG	1	-	1
	POS	-	5	5
	TOTAL	1	5	6
OVERALL	NEG	14	-	14
	POS	2	16	18
	TOTAL	16	16	32

Clinical Performance-SurePath Collection Medium

The clinical sensitivity and specificity of the NeuMoDx HPV Assay for detection of CIN2+ was determined using 948 cervical scrape specimens collected in SurePath collection medium using a case-control study design. Relative sensitivity and specificity for CIN2+ of the NeuMoDx HPV Assay compared to a clinically validated reference assay (i.e. HPV-Risk Assay) was determined based on the statistical method of a "non-inferiority score test".

Clinical sensitivity was determined using 106 samples from women diagnosed with histologically confirmed CIN2+ status (i.e. cases). Average age of the women was 38 (range 30-58) years. The sensitivity of the NeuMoDx HPV Assay was determined to be 92.5% (98/106; 95% CI: 85.6-96.2) and equal to that of the reference assay HPV-Risk (*Table 14*). The relative sensitivity of the NeuMoDx HPV Assay compared to the HPV-Risk assay was 1.00 with non-inferiority score test value of P=0.0009.

Clinical specificity was determined based on 842 collected LBC samples (SurePath) from the screening population of women with normal cytology and without evidence of CIN2+ within 2 years of follow-up. Average age of the women was 43 (range 30-59) years and 98.6% (935/948) of the samples tested valid. The specificity of the NeuMoDx HPV Assay was 93.5% (775/829; 95% CI:91.6-95.0) and that of the reference assay HPV-Risk was 91.9% (762/829; 95% CI: 89.9-93.6) (*Table 15*). The relative specificity of the NeuMoDx HPV Assay compared to the HPV-Risk assay was 1.02 with non-inferiority score test value of P<0.0001.

Table 14. Clinical sensitivity results of samples from women with confirmed CIN2+ in SurePath collection medium

Reference Test	NeuMoDx HPV Assay		
	POS	NEG	TOTAL
POS	97	1	98
NEG	1	7	8
TOTAL	98	8	106
Clinical Sensitivity NeuMoDx HPV Assay: 92.5% (95%CI 85.6–96.2)			

Table 15. Clinical specificity results of samples from women with normal cytology and no confirmed CIN2+ in SurePath collection medium

	NeuMoDx HPV Assay		
Reference Test	POS	NEG	TOTAL
POS	48	6	54
NEG	19	756	775
TOTAL	67	775	842
Clinical Specificity NeuMoDx HPV Assay: 93.5% (95%CI 91.6–95.0)			

Clinical Reproducibility

The intra-laboratory reproducibility and inter-laboratory agreement of the test on clinical specimens collected in PreservCyt were evaluated according to the international guidelines for HPV test requirements for cervical cancer screening.[16] The intra-laboratory reproducibility on cervical specimens over the length of the study was 96.0% (484/504; 95%CI 94.3–97.4) with a kappa value (κ) of 0.90 (*Table 16*). Results of these test moments were then assessed for agreement with those of another testing site, giving inter-laboratory agreements of 96.4% (486/504; 95%CI 94.8–97.7) with κ=0.91 and 94.4% (476/504; 95%CI 92.5-96.1) with κ=0.86 for the first and second test moments, respectively (*Table 17*).

Table 16. Intra-lab reproducibility over time of the NeuMoDx HPV assay

NeuMoDx HPV Assay Test Result 1	**NeuMoDx HPV Assay Test Result 2**		
	NEG	POS	TOTAL
NEG	347	13	360
POS	7	137	144
TOTAL	354	150	504
Reproducibility = 96.0% (95%CI 94.3–97.4); κ=0.90			

Table 17. Inter-lab agreement of the NeuMoDx HPV assay

NeuMoDx HPV Assay External Test	**NeuMoDx HPV Assay – Internal Test Result 1**			**NeuMoDx HPV Assay – Internal Test Result 2**		
	NEG	POS	TOTAL	NEG	POS	TOTAL
NEG	355	13	368	347	21	368
POS	5	131	136	7	129	136
TOTAL	360	144	504	354	150	504
	96.4% Agreement (95%CI 94.8–97.7); κ=0.91			94.4% Agreement (95%CI 92.5-96.1); κ=0.86		

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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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