AutoGenomics

INFINITI® UroGen QUAD

Directional Package Insert (DPI)

For *In Vitro* Diagnostic Use

CE

FOR EXPORT ONLY

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Doc EM-34039E (English) Rev. J (CO 3922) February 2017
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INTENDED USE

The INFINITI UroGen QUAD is an *in vitro* diagnostic test for the detection and identification of three pathogens in genomic deoxyribonucleic acid (DNA) obtained from human endocervical and cervical specimens. The INFINITI UroGen QUAD is designed to identify the following pathogens: *Ureaplasma urealyticum (UU), Mycoplasma genitalium (MG), and Mycoplasma hominis (MH)*, The INFINITI UroGen QUAD is a qualitative assay for use in clinical laboratories upon prescription by the attending physician.

BACKGROUND INFORMATION

The family *Mycoplasmataceae* contains two genera that infect humans: *Mycoplasma* and *Ureaplasma*, which are usually referred to collectively as mycoplasmas. The mycoplasmas are the smallest free-living bacteria. Although there are many species of mycoplasmas, only four are recognized as human pathogens; *Mycoplasma pneumoniae, Mycoplasma hominis, Mycoplasma genitalium,* and *Ureaplasma urealyticum*. *Mycoplasma hominis* and *Ureaplasma* species, known collectively as the genital mycoplasmal organisms, cause invasive infections in susceptible populations. *Mycoplasma hominis* and *Ureaplasma urealyticum* have been isolated from the genitourinary tract and are known to cause non-gonococcal urethritis, pelvic inflammatory disease (PID), or bacterial vaginosis. *Mycoplasma genitalium* is known to cause urethritis in men. Other than culture, there is no direct method for the detection of these pathogens.

The two *Ureaplasma* biovars, *Ureaplasma urealyticum* and *Ureaplasma parvum*, have now been designated as separate species. Separation of these species is not possible except via molecular techniques such as polymerase chain reaction (PCR). Therefore, they are considered together as *Ureaplasma* species.

Although there are other species that have been isolated from humans, their role in disease is not well established. The diseases caused by *M. pneumoniae, M. hominis, M. genitalium* and *U. urealyticum* are presented in the following below (Adapted from: Murray, *et al.*, Medical Microbiology 3rd Ed., Table 42-1).

Organism	Disease
M. pneumoniae	Upper respiratory tract disease, tracheobronchitis, atypical pneumonia
M. hominis	Pyelonephritis, pelvic inflammatory disease, postpartum fever
M. genitalium	Non-gonococcal urethritis
U. urealyticum	Non-gonococcal urethritis

TEST PRINCIPLE/ASSAY OVERVIEW

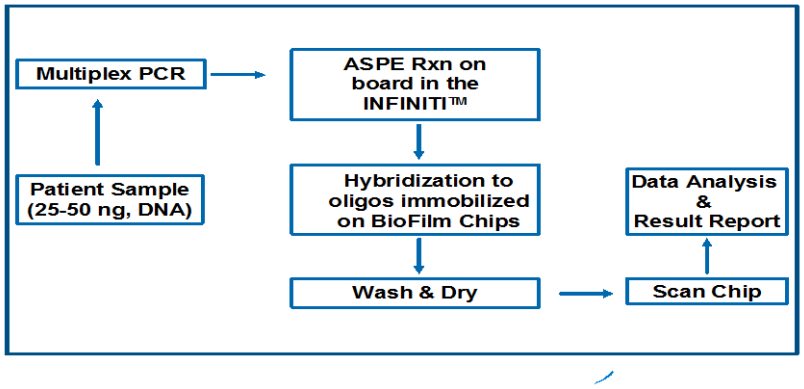
The INFINITI UroGen QUAD utilizes AutoGenomics' proprietary film-based microarray technology combined with process automation, reagent management and software technology for multiplex detection of the presence of UU, MG, and MH pathogens in deoxyribonucleic acid (DNA) obtained from cervical specimens.

The INFINITI UroGen QUAD is based on the following processes:

a) DNA extraction from endocervical and urine specimens.
b) PCR amplification of purified DNA.
c) Fluorescent label incorporation using analyte specific primer extension (ASPE).
d) Hybridization of the labeled ASPE primers to a microarray followed by washing.
e) Scanning of the microarray.
f) Signal detection and analysis.

Steps (c) through (f) are automated by the INFINITI Analyzer and INFINITI PLUS Analyzer.

A schematic overview of the assay is shown below.



DEVICE DESCRIPTION

The INFINITI UroGen QUAD is an *in vitro* diagnostic device that consists of reagents and instrumentation which includes polymerase chain reaction (PCR) primers, microarrays, a thermal cycler, an imager, and software designed to detect the presence of the following pathogens: *Ureaplasma urealyticum (UU), Mycoplasma genitalium (MG), and Mycoplasma hominis (MH)*.

The INFINITI UroGen QUAD is comprised of the BioFilmChip® Microarray, the Intellipac® Reagent Module, and the PCR Amplification Mix. The instrumentation for the INFINITI UroGen QUAD is the AutoGenomics INFINITI Analyzer or INFINITI PLUS Analyzer with the Qmatic® Operating Software.

The **BioFilmChip Microarray** consists of a polyester film coated with proprietary multi-layer components designed for DNA analysis. The layers have been designed to provide a versatile surface to enhance test performance. The INFINITI UroGen QUAD uses a microarray chip (P-Chip) which has capture probes spotted on the surface of the film. Four (4) samples can be run on one microarray. Twelve (12) microarrays are housed in a magazine.

The **Intellipac Reagent Module** which acts as a communication link contains four reservoirs that house the test reagents and has an integrated memory chip. Reagent information such as lot number, expiration date, and volume usage are stored in the memory chip. The Intellipac Reagent Module communicates with the INFINITI Analyzer and provides the reagent information which appears on the assay report and printout. The Intellipac Reagent Management Module provides test reagent for 96 samples.

The **PCR Amplification Mix** consists of the reagents needed for the PCR amplification step of the assay. Each box of the PCR Amplification Mix provides 4 x 500µl vials of PCR Amplification.

The **INFINITI Analyzer** and **INFINITI PLUS Analyzer** automates the INFINITI UroGen QUAD and integrates all the discrete processes of sample (PCR reaction product) handling, reagent management, hybridization, detection, and results analysis. The assays are processed automatically and read by the built-in confocal microscope. Results are analyzed and presented as positive or negative for the presence of UU, MG, and MH pathogens.

The Analyzers are provided with Operator's Manuals. The Operator's Manual provides detailed description and operating principle of the Analyzer and instruction for use.

The INFINITI Analyzer and INFINITI Analyzer are CE marked.

WARNINGS AND PRECAUTIONS

Handling Requirements

- **For *in vitro* diagnostic use. To be used by qualified laboratory personnel.**
- This test is to be used only with endocervical and urine specimens.
- To minimize the risk of cross contamination, sample preparation, PCR reaction set up and PCR product analysis should be performed according to approved guidelines such as CLSI (Molecular Diagnostic Methods for Genetic Diseases: Approved Guideline).
- All patient specimens are potentially hazardous and care should be taken when handling materials of human origin. No test method can offer complete assurance that HCV, HIV or other infectious agents are absent.

  Follow the CLSI Guidelines (Molecular Diagnostics Methods for Infectious Diseases; Approved Guidelines; MM3-A).
- Do not pool/mix reagents from different lots.
- Do not use a kit or reagent past its expiration date.
- Store kits and reagents according to the product label.

Laboratory Procedures

- Follow normal precautions for handling laboratory reagents. Do not mix reagents from different containers or from different lots.
- Follow safe laboratory procedures: do not pipette by mouth; wear protective clothing (e.g., disposable gloves laboratory coats) and eye protection; do not eat, drink or smoke in the laboratory work areas; wash hands thoroughly after handling samples and reagents.

Waste Handling

- Dispose of unused reagents, specimens and waste according to applicable country, federal, state and local regulations.
- Safety data Sheets (SDS) are available upon request from AutoGenomics Customer Service.

Sample Preparation

- Refer to the instructions provided with the sample processing kit.
- The PCR product cannot be stored prior to loading it onto the microarray. Use immediately**.**

INFINITI Analyzer or INFINITI PLUS Analyzer

- **Read the Operator's Manuals before operating the instruments.** Pay particular attention to "Notes".
- Follow the Caution and Safety Warning in the Operator's Manual.
- Refer to the Installation Requirements Section when installing the instrument.
- Refer to the Errors Section when errors are encountered while operating the instrument.
- Refer to the Help Section when problems are encountered.

STORAGE / STABILITY

BioFilmChip Microarray: 24 months Refrigerated (2 to 8°C)
Intellipac Reagent: 18 months Refrigerated (2 to 8°C)
Note: Remove the Intellipac from the Analyzer and store refrigerated as soon as possible. Do not use after Intellipac has been opened for four weeks.
Amplification Mix: 18 months Frozen (-30 to -15°C)

Note: Specific product expiration date is printed on the product label.

REAGENTS REQUIRED AND PROVIDED BY AUTOGENOMICS

ASSAY REAGENTS (SUFFICIENT FOR 192 TESTS)

- Catalog Number 04-1100-02 INFINITI UroGen QUAD Magazine – BioFilmChip® Microarray
  4 magazines per package; 48 tests per magazine
- Catalog Number 04-2100-02 INFINITI UroGen QUAD Intellipac® Reagent Management Module
  2 modules per package; 96 tests per module which contains:4 x 1.1 ml of ASPE
  - dNTPs
  - Labeled -dCTP
  - Allele Specific Primers
  - Extension reaction Buffer
- Catalog Number 04-3100-02 INFINITI UroGen QUAD Amp Mix: 4 x 500µl vials of PCR reaction master mix containing:
  - dNTPs
  - Multiplex Primer Mix
  - $MgCl_2$
  - PCR Reaction Buffer

REAGENTS REQUIRED BUT NOT PROVIDED WITH THE ASSAY REAGENTS

- AutoGenomics Catalog Number 12-0470-02 INFINITI Sample processing Kit
- Internal Control 1 Catalog Number 12-0170-02
- Catalog Number 12-0030-02 Solution
- Hybridization Buffer 2 : 6 x 30ml bottles. The hybridization buffer consists of:
  SSC
  Sodium Azide Preservative 0.08%
  EDTA
  10X Blocking Buffer
  Catalog Number 12-0130-02 UU DNA Template Control
- Catalog Number 12-0140-02 MG DNA Template Control
- Catalog Number 12-0150-02 MH DNA Template Control

REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- Molecular Grade Water (DNAse and RNAse free)
- Platinum Taq DNA Polymerase (Invitrogen Catalog Number 10966-018)

EQUIPMENT

The following equipment is required but not provided with the assay reagents

- Genesee Scientific Catalog Number 27-125 8-well PCR tube strips with hinged caps
- Pipettors
- Mini Centrifuge
- Pipette tips
- Microfuge tube Racks
- Thermocycler
- Vortex
- 0.2 ml thin wall tubes for PCR
- 1.5 ml microcentrifuge tubes
- 8-well Flat Strip Caps (Genesee Scientific, Catalog No. 22-623)
- AutoGenomics Product Number 11-0060-00: INFINITI Waste tray Stir Bars
- AutoGenomics Product Number 11-0020-00: INFINITI Waste Tray Liners
- AutoGenomics Product Number 11-0080-00: INFINITI Pipette Tips

- **FOR INFINITI Analyzer***:*
  - AutoGenomics Product Number 10-0010-99: INFINITI Analyzer
  - AutoGenomics Product Number 11-0030-00: 24-Well Plates with Lids
  - AutoGenomics Product Number 11-0050-00: INFINITI Temp Cycle Plate

- **FOR INFINITI PLUS Analyzer***:*
  - AutoGenomics Product Number 10-0020-99: INFINITI PLUS Analyzer
  - AutoGenomics Product Number 11-0100-00: 48-Well Plates and Product Number 11-0110-00: 48 Well Plate Lid

ASSAY PROCEDURE

Specimen Processing

It is recommended that the INFINITI Sample Processing Kit (Catalog Number 12-0470-00) be used for sample processing. Follow the instructions provided with the processing kit.

DNA Controls

It is required to run known positive controls and a negative control should also be included in each test run. The following positive controls, available from AutoGenomics, are recommended for use with the INFINITI UroGen QUAD (refer to section on REAGENTS REQUIRED for Catalog Number):

(a) UU DNA Template Control
(b) MG DNA Template Control
(c) MH DNA Template Control

Note: Please use proper PCR technique to prevent contamination of reagents with positive controls. Sealing the 24WP containing sample DNA and "no template control" samples with caps **before** adding the positive controls is recommended to prevent cross contamination.

Amplification Reaction

Note:

- Keep Taq DNA polymerase on ice.
- Completely thaw reagents at room temperature then immediately place on ice.
- Vortex the amplification mix for 2 to 5 seconds then centrifuge briefly to bring the contents to the bottom of the tube.
- To avoid potential contamination, a separate area is recommended for assembly of the PCR reaction. Decontaminate pipettes and all work surfaces with freshly prepared 10% bleach.
- Filter tips and gloves must be used when handling samples and controls.
- Make sure there is no abnormal evaporation of the PCR product. After PCR is complete, visually inspect for any volume change. All amplification reaction volumes should be about 15 µl. Otherwise, do not proceed with the assay.

Note:

- For the INFINTI Analyzer use the 24WP.
- For the INFINTI PLUS Analyzer use the 48WP.

1. Prepare the PCR master mix

Amplification mix	9.75 µl
Platinum Taq polymerase	0.25 µl
Internal Control 1	1.00 µL
Total volume of PCR master mix	11.00 µl

Note: Calculate the amount of each reagent needed based on the number of reactions.

2. Gently vortex the PCR master mix then dispense 11.0 µl of master mix into wells of the well plate
3. Add 4.0 µl of sample or control DNA to each well

PCR master mix	11.0 µl
Sample	4.0 µl
Total volume of amplification reaction	15.0 µl

Note: **This is a QUAD assay**. When loading samples, always load the samples in multiples of four and in consecutive order. For example, if loading 8 samples, load wells A1 to A8. **Do not** load the B wells**.**

4. Place the well plate, sealed with 8-well flat strip caps, in a thermocycler and immediately commence the amplification reaction using the following program.

Step No.	Temperature °C	Time (sec)	No. of Cycles
1	94	120	1
2	94 62-54 (-0.8/cycle) 72	5 90 30	10x
3	94 54 72	5 90 30	40x
4	4	hold	1

Note: After each cycle in step 2, the temperature is decreased by 0.8°C. When an Eppendorf Mastercycler EP was used with the ramp rate set at 75%, the total cycling time was two (2) hours and 32 minutes (± 5 min). If using other thermocycler models, we recommend adjusting the ramp rate in order to obtain an equivalent total cycling time.

Sample Loading

1) Carefully remove the 8-well flat strip caps to avoid splashing.
2) Load the well plate in the appropriate orientation (with well A1 in the back left corner) into the Analyzer
   - **INFINITI Analyzer:** Load the assembled 24WP with the associated lid (Catalog # 11-0030-00).
   - **INFINITI PLUS Analyzer:** Load the assembled 48WP with a clean 48WP lid (see instructions in the INFINITI PLUS Analyzer Operator's Manual) (Catalog # 11-0110-00, reusable).
3) Load the following: assay specific magazines, Intellipac, INFINITI Static Free Pipette tips, and buffer.
   - **FOR INFINITI Analyzer***:*
     Wash buffer and Hybridization buffer should be placed in the INFINITI bottle holders. Wash buffer goes in the left holder (near the magazine) and Hybridization buffer 2 in the right holder (near the Intellipac).
   - **FOR INFINITI PLUS Analyzer***:*
     Hybridization buffer 2 should be placed in the INFINITI bottle holders. Hybridization Buffer 2 goes in the right holder (near the Intellipac).

Operation of the Analyzers

Follow the instructions in the Operator's Manuals

INFINITI Analyzer Operator's Manual (Part Number EM-34000)
INFINITI PLUS Analyzer Operator's Manual (Part Number EM-34041)

QUALITY CONTROL

- Maintain calibration of thermocycler according to manufacturer's specifications.
- Maintain calibration of INFINITI or INFINITI PLUS Analyzer according to AutoGenomics' specifications.
- Maintain calibration of pipettes according to manufacturer's specifications.

LIMITATIONS
The results obtained from the INFINITI UroGen QUAD should be used and interpreted only in the context of the overall clinical diagnosis. AutoGenomics is not responsible for any clinical decisions that are made.

INTERPRETATION OF RESULTS
Results from the INFINITI UroGen QUAD are reported to the user as "Positive" or "Negative" for the presence of pathogens detected.

The Internal Control is intended to identify specimens that contain polymerase inhibitors. INFINITI UroGen QUAD results are interpreted as follows:

Analyte Result	Internal Control (IC) Result	INTERPRETATION
All Analytes Negative	Positive	Negative
All Analytes Negative	Negative	Invalid Test Result
Any Analyte Positive	Positive or Negative	Valid Test Result

If the analyzer detects a problem or an error (e.g., assay parameters not met), no results will be reported. Instead, a description of the problem or error code will be displayed. The Trouble Shooting section of the INFINITI Analyzer Operator's Manual provides an explanation of the errors. The assay needs to be repeated.

DISPOSAL
Waste materials for the INFINITI UroGen QUAD are common waste materials generated in clinical laboratories, and should be handled/disposed of in accordance with the policies/procedures in place in the clinical laboratory.

PERFORMANCE CHARACTERISTICS
Analytical Specificity
Studies related to specificity were conducted during assay development. PCR primer specificity was determined by amplicon size on a gel and sequencing the amplicon. ASP primer specificity was determined by the correct calls made by the assay using known genomic samples. Capture probe specificity was determined by hybridizing different oligos and demonstrating that correct oligo hybridizes to the known spot.

Analytical Specificity/Cross-Reactivity study demonstrated no cross-reactivity of the INFINITI UroGen QUAD with the following microbial DNA.

Escherichia coli	*Streptococcus pyogenes*	*Enterococcus cassioflavus* (ATCC 700327)
Klebsiella pneumoniae	*Staphylococcus aureus*	*Mobiluncus mulieris* (RMA 13861)
Pseudomonas aeruginosa	*Staphylococcus epidermidis*	*Mobiluncus curtisii* (RMA 13851)
Haemophilus influenzae	*Corynebacterium striatum*	*Gardenella vaginalis* (RMA 12507)

Proteus vulgaris	*Peptostreptococcus anaerobis*	*Prevotella bivia* (RMA 15003)
Enterobacter cloacae	*Clostridium sordelli*	*Atopobium vaginae* (ATCC BAA-55)
Acinetobacter baumannii	*Clostridium difficile*	*Candida parapsilosis* (ATCC 22019)
Moraxella (Branhamella) catarrhalis	*Bacteriodes fragilis*	*Candida tropicalis* (ATCC 750)
Neisseria meningitidis	*Bacteriodes uniformis* (ATCC 8492)	*Candida krusei* (ATCC 6258)
Streptococcus agalactiae	*Fusobacterium nucleatum* (ATCC 25586)	*Candida glabrata* (RMA 16114)
Enterococcus (Streptococcus) faecalis	*Candida albicans* (ATCC 14053)	*Lactobacillus jensenii* (ATCC 25258D)

Analytical Sensitivity (Level **o**f **D**etection)
Analytical sensitivity studies performed using plasmid samples established the limit of detection (copies/test) of the INFINITI UroGen QUAD. The limit of detection is the lowest concentration of DNA that gives all correct calls. The lower limit of detection for *Ureaplasma urealyticum (UU), Mycoplasma genitalium (MG), and Mycoplasma hominis (MH)* were 50, 10, and 50copies/test, respectively. The upper limit of detection was determined to be above 10^8copies/test.

The INFINITI UroGen QUAD operating sample volume range is from 5 to 9µl. The sample volume range is the upper and lower sample volume limits where the INFINITI UroGen QUAD gave all correct calls.

Sample Carry-over
There is no sample carry-over with the INFINITI UroGen QUAD.

Potential Interference from Blood and other Substances
Interference from substances which may commonly be found in the vaginal tract (e.g., whole blood, douche, anti-fungal cream and contraceptive jelly) is not expected for the INFINITI UroGen QUAD.

Precision and Reproducibility
Precision and reproducibility of the INFINITI UroGen QUAD was evaluated using plasmid DNA controls at the LOD and three instruments. The reproducibility study demonstrated reproducibility of the INFINITI UroGen QUAD for UU, MG and MH to be 100%, 100% and 99%, respectively.

Lot-to-lot reproducibility was demonstrated during the manufacturing process validation. Three lots of INFINITI UroGen QUAD tested during the validation consistently met the specifications for the three target analytes (UU, MG, MH).

Clinical Validation Studies
Clinical validation of the INFINITI UroGen QUAD was conducted at three clinical laboratories. Samples tested were comprised of clinical patient samples, lyphocult (cultures) resuspended in OSB buffer and known DNA controls added to cell re-suspension buffer. Validation involved comparing the results from the INFINITI UroGen QUAD to the results obtained using the current laboratory method (e.g., culture), or to the expected results (known DNA Control). Table 1 is a summary of the combined results of validation studies (three sites).

Table 1 Summary Clinical Validation - 3 Sites

	UU	MH	MG
# of Samples Tested	173	176	189
Sensitivity	77%	97%	93%
Specificity	97%	92%	96%
Positive Predictive Value	85%	76%	81%

Negative Predictive Value	95%	99%	99%
% Correct Call (vs. Current Method/Control)	94%	96%	93%

During the validation study at Site 3, five (5) patient samples Positive for Ureaplasma genus tested Negative for *Ureaplasma urealyticum* with the INFINITI UroGen QUAD. Current direct methods (e.g., culture) are not able to distinguish between the two *Ureaplasma* biovars (*Ureaplasma urealyticum* and *Ureaplasma parvum)* and will give a Positive result when either biovar is detected. The INFINITI UroGen QUAD is specific and detects only UU; it will not detect *Ureaplasma parvum* and will therefore give a Negative result if only *Ureaplasma parvum* is present. The five samples were most likely *Ureaplasma parvum*, and therefore Negative for *Ureaplasma urealyticum*.

The inability of the current direct methods to distinguish between the two *ureaplasma* species and the separation of these species via molecular techniques have been documented.

REFERENCES

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7. Safedin H et al Evaluation of STD6 Multiplex PCR Assay for the Detection of *U. urealyticum, M. genitalium, M. hominis and T. vaginalis* on the AutoGenomics INFINITI System.