AutoGenomics

INFINITI® H.pylori QUAD Assay

Directional Package Insert (DPI)

For *In Vitro* Diagnostic Use

CE

FOR EXPORT ONLY

Manufactured by AutoGenomics, Inc., 1600 Faraday Avenue, Carlsbad, CA USA 92008

Authorized EU Agent: Medical Device Safety Service GmbH (MDSS)
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INTENDED USE

The INFINITI H.pylori QUAD assay is an *in vitro* diagnostic test for the detection of *Helicobacter pylori* in human biopsy and stool/fecal specimens, and to determine the resistance to clarithromycin. The INFINITI H.pylori QUAD Assay is a qualitative Assay for use in clinical laboratories upon prescription by the attending physician. Together with the patient's cytology history, other risk factors and relevant clinical information, the information from the INFINITI H.pylori QUAD Assay may be used to guide patient management.

BACKGROUND INFORMATION

Helicobacter pylori (*H.pylori)* is the microorganism responsible for the most frequent and persistent bacterial infection worldwide. H.pylori infection affects nearly half of the world's population. H.*pylori* is associated with peptic ulcer, gastric ulcers, mucosa-associated lymphoid tissue lymphoma and gastric cancer. (Elvira et al). In developing countries, the prevalence of infection is as high as 90%, whereas in developed countries, excluding Japan, the prevalence is below 40 % (Tonkic et al).

The patients infected by *H. pylori* are treated with antibiotics such as amoxicillin or clarithromycin. Resistance to clarithromycin, the first line of treatment for *H. pylori,* is on the rise. Two mutations in the 23S Ribosomal RNA (rRNA) gene, A2142G and A2143G are related to a patient's resistance to clarithromycin. When one of these mutations is present, clarithromycin is unable to bind to the large subunit of H .pylori ribosome to inhibits the protein synthesis. Detection of these mutations aid in the management of patients infected with *H. pylori.*

Several methods are currently available to detect the presence of H.pylori each with its own advantages, disadvantages and limitations. In Histological sections, H.pylori appears as a curved or spiral bacillus on the epithelial surface, in mucous layer and within gastric glands.(Morgner et al). At present, there is no test commercially available to determine resistance to clarithromycin.

The INFINITI H.*pylori* QUAD Assay is a PCR based molecular diagnostic assay designed to detect the presence of *H. pylori* and its two mutations in the 23S Ribosomal RNA (rRNA) gene, A2142G and A2143G, in human biopsy and stool/fecal specimens.

TEST PRINCIPLE/ASSAY OVERVIEW

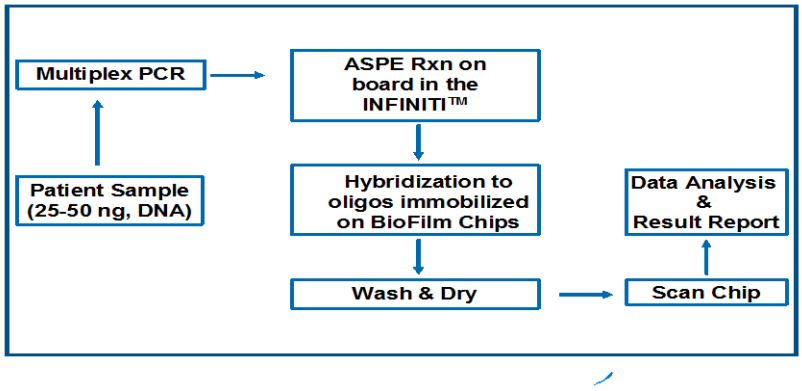
The INFINTI H.pylori QUAD Assay utilizes AutoGenomics' proprietary film-based microarray technology combined with process automation, reagent management and software technology for multiplex detection of *H. pylori* and its two mutations in the 23S Ribosomal RNA (rRNA) gene, A2142G and A2143G, in human biopsy and stool/fecal specimen.

The INFINTI H.pylori QUAD Assay is based on the following processes:

a) DNA extraction from human biopsy or stool/fecal 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 PLUS Analyzer.

A schematic overview of the assay is shown below.



DEVICE DESCRIPTION

The INFINITI H.pylori QUAD Assay utilizes the AutoGenomics proprietary film-based microarray technology for detection of *Helicobacter pylori* bacterial DNA and its two mutations in the 23S Ribosomal RNA (rRNA) gene, A2142G and A2143G, in deoxyribonucleic acid (DNA) obtained from biopsy and stool/fecal specimens.

The INFINITI H.pylori QUAD Assay is based on the principle of isolation of DNA from human biopsy specimen and stool/fecal sample , PCR amplification of the purified DNA, fluorescent labeling of ASPE primers, hybridization of the labeled ASPE primers to a microarray, removal of unbound fluorescent products, and detection of fluorescence.

The INFINITI H.pylori QUAD Assay is comprised of the BioFilmChip® Microarray, the Intellipac® Reagent Module and the PCR Amplification Mix. The INFINITI H.pylori QUAD Assay should be run using the AutoGenomics INFINITI PLUS Analyzer. The INFINITI PLUS Analyzer is CE-marked.

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. 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 PLUS 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. Two vials each containing 500µl vials of PCR Amplification are packaged in a pouch.

The **INFINITI PLUS Analyzer** automates the INFINTI H.pylori QUAD Assay and integrates all the discrete processes of sample (PCR reaction product) handling, reagent management, hybridization, detection, and results analysis. The tests are processed automatically and read by the built-in confocal microscope. Results are analyzed and presented as positive or negative for the presence of H.*pylori* and the A2143G and A2142G mutations. The INFINITI PLUS is provided with Operator's Manuals. The Operator's Manual provides instructions for use, and description and operating principle of the INFINITI PLUS.

WARNINGS AND PRECAUTIONS

Handling Requirements

- **For *in vitro* diagnostic use. To be used by qualified laboratory personnel.**
- This test is to be used with human biopsy specimens and stool/fecal sample.
- 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.
- Material Safety data Sheets (MSDS) are available upon request from AutoGenomics Customer Service.

SPECIMEN

- The human biopsy specimen for the INFINTI H.pylori QUAD Assay is collected using standard endoscopic biopsy procedures.
- Stool specimen collected using commercially available stool collection kit will be suitable for use with the INFINITI® H.pylori QUAD Assay.

INFINITI PLUS

- **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 INFINITI PLUS 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 04-1270-02 INFINTI H.pylori QUAD Assay Magazine – BioFilmChip® Microarray
  4 magazines per package; 48 tests per magazine
- Catalog Number 04-2270-02 INFINTI H.pylori QUAD Assay Intellipac® Reagent Management Module
  2 modules per package; 96 tests per module which contains:
  dNTPs
  Labeled -dCTP
  Analyte Specific Primer Mix
  Extension Reaction Buffer
- Catalog Number 04-3270-02 INFINTI H.pylori QUAD Assay Amp Mix: 2 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

- Catalog Number 12-0170-00 Internal Control 1
- Catalog Number 12-0030-02 Hybridization Buffer 2: 6 x 30ml bottles. The hybridization buffer consists of:
  - SSC
  - Sodium Azide Preservative 0.08%
  - EDTA
  - Tween 20
  - Sorbitol
  - Polyvinylpyrrolidone (PVP)
  - Poly (ethylene glycol) (PEG)
- Catalog Number 12-0330-00 Wash Buffer
- Catalog # 12-0900-00 H. pylori Mutant Combo Pack Positive Control

REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- *Helicobacter pylori* Positive Control (ATCC® 43504D-5™)
- Molecular Grade Water (DNAse and RNAse free)
- Titanium Taq DNA polymerase (Clontech, a Takara Company (Catalog # 639209)
- QIAamp DNA Stool Mini kit (Catalog # 51504)

EQUIPMENT

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

- INFINITI® PLUS Analyzer (Catalog # 10-0020-99)
- INFINITI® PipetteTips (Catalog # 11-0080-00)
- INFINITI® Waste Tray Liners (Catalog # 11-0020-00)
- INFINITI® Waste Tray Stir Bars (Catalog # 11-0060-00)
- INFINITI® 48 Well Plates (Catalog # 11-0100-00)
- INFINITI® 48 Well Lid (Catalog
- 8-well flat strip caps (Axygen Catalog # PCR-2CP-RT-C)
- Pipettors
- Mini Centrifuge
- Microfuge Tube Racks
- Thermocycler
- Vortex
- 1.5 ml Microcentrifuge Tubes

REAGENTS REQUIRED FOR DNA EXTRACTION

- Ethanol
- Chloroform
- Distilled water

ASSAY PROCEDURE

Specimen Processing

- **Biopsy Specimen**

  The following chloroform extraction method is recommended for extracting DNA from the biopsy specimen.

  Note: Incubating is on the bench. Other steps are performed in the Biosafety Cabinet.

  1. Turn on the incubator on the bench and set the temperature at 60°C.
  2. Shake all the samples tubes for at least 30 seconds.
  3. Put all sample tubes into the shaker/incubator.
  4. Incubate specimens at 60°C in the incubator for 1.5 to 2 hours . Shake the tubes every one hour to make sure that there is no solid tissue left and the liquid is clean, and then go to the next step.
  5. Transfer 0.9ml sample to a labeled 1.5ml tube.
  6. Add 0.5ml chloroform, mix well and centrifuge at 14,000rpm for 7 min.
  7. Transfer 0.5ml supernate to a labeled 1.5ml tube for DNA precipitation.
  8. Precipitate: Add 0.9ml ethanol to the supernate and gently mix the samples by inverting the tube up and down a few times.
  9. Centrifuge 5min at 14000 rpm.
  10. Discard the supernate.
  11. Decant the ethanol and air dry the pellet about 1 hour.
  12. Re-suspend the pellet and air dry the pellet for about 1 hour.
  13. Re-suspend the pellet (DNA) in 50µl in distilled H_20, vertex the tubes.
  14. After completing this step, the specimen DNA is transferred to 4°C refrigerator and is ready for PCR detection.

- **Stool/Fecal Samples**
  1. QIAamp DNA stool Mini kit (Catalog # 51504) is **required** for extracting DNA from stool/fecal specimen.

Required Controls

Positive controls and a no template control (i.e., molecular grade water) are required in each test run. The no template control serves as a contamination control. If this control is positive, then samples should be tested again taking appropriate measures to prevent contamination.

The following positive controls, available from AutoGenomics, are required for use with the INFINTI H.pylori QUAD Assay (refer to section on REAGENTS REQUIRED for Catalog Number):

- Catalog # 12-0900-00 H. pylori Mutant Combo Pack Positive Control
- *Helicobacter pylori* Positive Control (ATCC® 43504D-5™)

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 in deionized or distilled water.
- 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.

1. Prepare the PCR master mix

Amplification mix	9.7 µl
Titanium Taq polymerase	0.3 µl
Internal Control 1	1.0 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 48-well plate.

3. Add 4.0 µl of extracted DNA sample or control DNA to each well

PCR master mix	11.0 µl
Sample (or control) DNA	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	60	1
2	94 60 72	30 30 30	35
3	72	60	1
4	4	hold	1

Note: When an Eppendorf Mastercycler EP/Pro was used with the ramp rate set at 75% [Step 2], the total cycling time was one hour and 23 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.
   - Load the assembled 48WP with a clean 48WP lid (Catalog # 11-0110-00, reusable). See instructions in the INFINITI PLUS Analyzer Operator's Manual
3) Load the following: assay specific magazines, Intellipac, INFINITI Static Free Pipette tips, and buffers.
   - Hybridization Buffer 2 should be placed in the INFINITI PLUS bottle holders. Hybridization Buffer 2 goes in the right holder (near the Intellipac).

Operation of the Analyzer

Follow the instructions in the **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 PLUS Analyzer according to AutoGenomics' specifications.
- Maintain calibration of pipettes according to manufacturer's specifications.

LIMITATIONS

The results obtained from the INFINTI H.pylori QUAD Assay 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 INFINTI H.pylori QUAD Assay are reported to the user as "Positive" or "Negative" for the detected. The Internal Control is intended to identify specimens that contain polymerase inhibitors. The INFINTI H.pylori QUAD Assay results are interpreted as follows:

Results Analytes				Interpretation
H. pylori	**A2142G**	**A2143G**	**IC**	
Positive	Negative	Negative	Positive	H. pylori positive; Clarithromycin sensitive
Positive	Positive	Negative	Positive	H. pylori positive Clarithromycin resistant
Positive	Negative	Positive	Positive	H. pylori positive Clarithromycin resistant
Positive	Positive	Positive	Positive	H. pylori positive Clarithromycin resistant
Negative	Negative	Negative	Positive	H. pylori negative
Negative	Negative	Negative	Negative	Invalid Test Result – Repeat the test

If the INFINITI PLUS 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 PLUS Analyzer Operator's Manual provides an explanation of the errors. The assay needs to be repeated.

DISPOSAL

Waste materials for the INFINTI H.pylori QUAD Assay 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/Cross-Reactivity
Analytical Specificity/Cross-Reactivity study was performed to evaluate the effect of the presence of known bacteria on the INFINITI H.pylori QUAD Assay. The study demonstrated no cross-reactivity of the INFINITI H.pylori QUAD Assay with the bacteria species listed below.

- *Staphylococcus aureus* subsp. *aureus*
- *Escherichia coli*
- *Corynebacterium glutamicum*
- Fusobacterium nucleatum subsp. nucleatum
- *Staphylococcus epidermidis*
- *Clostridium perfringens*

Analytical Sensitivity (Limit **o**f **D**etection)
The Analytical sensitivity studies evaluated the INFINITI H.pylori QUAD at six levels: 10^6, 10^5, 10^4, 10^3, 10^2, 10^1 copies per reaction. Eight replicates were tested at each level. The limit of detection (LOD) is the lowest concentration of DNA that gives all correct calls.

The Analytical sensitivity studies established the limit of detection for INFINITI H.pylori QUAD to be 100 copies per reaction for H.pylori, 1,000 copies per reaction for A2142G and A2143G. The limit of detection was confirmed by testing an additional 30 replicates.

At 10^6 copies per reaction, the highest concentration tested, all samples gave the correct call.

Potential interference from drugs used to manage patients infected with H.pylori
An interference study was performed to determine if INFINITI H.pylori QUAD Assay will be affected by the presence of drugs commonly used to manage/treat H.pylori patients.

The interference study demonstrated that the presence of the following drugs at the standard dosage taken by a patient did not interfere with the INFINITI H.pylori QUAD Assay.

- Clarithromycin
- Amoxicillin
- Metronidazole
- Levofloxacin
  Tetracycline

Reproducibility
Reproducibility of the INFINITI H.pylori QUAD Assay was evaluated using twelve (12) known samples and three reagent lots. The 12 samples consisted of six negative samples, three positive for H.pylori, one positive for A2142G, and two positive for A2143G.

Three operators participated in the study and received 12 identical samples. The operators were blinded to the sample genotype. Each operator tested the samples following the INFINITI H.pylori Application Notes (AN-22604 Rev A), in duplicate on three non-consecutive days, each day with a different lot of reagents. A total of 216 tests were completed during the reproducibility study. All Genotype calls from the reproducibility study were 100% correct.

Clinical Studies

One hundred (100) clinical biopsy samples, 50 negative and 50 positive for H.pylori were used to evaluate the clinical performance of the INFINITI H.pylori QUAD Assay. These samples were initially tested by the reference laboratory using multiplex PCR and agarose gel band analysis to identify the positive and negative samples. DNA extracted from these samples were provided to AutoGenomics to be tested using the INFINITI H.pylori QUAD Assay.

The following summarizes the results of this clinical evaluation

- All negative samples tested negative by the INFINITI H.pylori QUAD Assay (100% concordance)
- 48 of the 50 positive samples tested positive by the INFINITI H.pylori QUAD Assay (96% concordance)
- Two (2) of the 50 positive samples tested negative by the INFINITI H.pylori QUAD Assay
- Overall concordance for H.pylori was 98% (98/100)
- Eight (8) H.pylori positive samples tested positive for A2142G
- Nine (9) H.pylori positive samples tested positive for A2143G
- One (1) H.pylori positive sample tested positive for A2142G and A2143G

Validation of stool specimen

A total of twenty five (25) stool / fecal samples were used in the validation studies. The samples were extracted using the Qiagen QIAamp DNA stool Mini Kit. Samples were tested using the commercially available ELISA test (Enzyme linked Immunosorbent Assay) and the INFINITI® H. pylori QUAD Assay. Samples for which the ELISA and the INFINITI® H. pylori QUAD Assay did not agree where sequenced (Bidirectional sequencing using the Sanger method). Correct calls (Truth), reported as positive or negative for H pylori, were established when two (2) or more methods agreed. The validation demonstrated the specificity and sensitivity of the INFINITI H.pylori QUAD Assay using stool specimens to be 100%.

Note: The reference laboratory did not test for A2142G and A2143G

REFERENCES

- González, E. G. (2014). A review of Helicobacter pylori diagnosis, treatment, and methods to detect eradication. *World J Gastroenterol*, *20*(6), 1438-1449. doi:10.1107/s0108768104025947/bm5015sup1.cif

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