HC Hybrid Capture® 2 GC-ID DNA Test

Instructions For Use

digene® HC2 GC-ID DNA Test

An *In Vitro* Nucleic Acid Hybridization Microplate Assay with Signal Amplification using Microplate Chemiluminescence for the Qualitative Detection of *Neisseria gonorrhoeae* (GC) DNA in Cervical Specimens

For use with:

digene® HC2 DNA Collection Device
digene® Female Swab Specimen Collection Kit
Hologic PreservCyt® Solution

KEY CHANGES FROM PREVIOUS PACKAGE INSERT REVISION
1. Updated product branding
2. Removed reflex test references and data.

For Professional Use Only, by trained and validated laboratory personnel. Read these instructions carefully before using the test.

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EC REP
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IVD

Σ 96

REF 5140-1330

L2172EN Rev. 3

CE The CE mark indicates that the *digene* HC2 GC-ID DNA Test is in compliance with the requirements of the *In Vitro* Diagnostic Medical Device Directive 98/79/EC.

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NAME AND INTENDED USE

The *digene*® Hybrid Capture® 2 (HC2) GC-ID DNA Test is an *In Vitro* nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of *Neisseria gonorrhoeae* DNA in cervical specimens collected with the *digene* HC2 DNA Collection Device [consisting of a cervical brush and *digene* Specimen Transport Medium (STM)] and in cervical specimens collected using the *digene* Female Swab Specimen Collection Kit (swab and STM) or specimens collected using a broom-type collection device and placed in Hologic PreservCyt® Solution. The *digene* HC2 GC-ID DNA Test is indicated for use with symptomatic or asymptomatic women as evidence of infection with *Neisseria gonorrhoeae*.

For high volume sample-throughput testing, the *digene* HC2 GC-ID DNA Test may be performed using the Rapid Capture® System (RCS) Instrument Application.

For *In Vitro* Diagnostic Use IVD

SUMMARY AND EXPLANATION

Neisseria gonorrhoeae are non-motile, Gram-negative diplococci with fairly complex growth requirements. They are aerobic, producing optimal growth at temperatures in the range of 35-37°C in the presence of 3-7% CO_2 and $\geq$70% relative humidity. Presumptive diagnosis for *Neisseria gonorrhoeae* is traditionally obtained by isolating organisms from cultures of clinical specimens and using a Gram stain for morphological examination. Definitive diagnoses can be obtained with a positive oxidase and/or catalase test of the culture. Additional confirmation of results includes carbohydrate degradation, agglutination, and sugar fermentation tests. More definitive, direct tests for *Neisseria gonorrhoeae* include antigen detection and nucleic acid probe tests. An enzyme-linked immunosorbent assay has been shown to be as sensitive and as specific as the Gram stain for detecting gonococci in male urethral and first void urine specimens, but it has decreased sensitivity when applied to endocervical specimens.[1,2] Because the antigen detection test may cross-react with commensal Neisseria and related species[3], this test can only be used for presumptive diagnosis.[3]

More recently, nucleic acid hybridization tests have been used to evaluate clinical specimens for the detection of *Neisseria gonorrhoeae* in high-risk populations using both endocervical and male urethral specimens.

PRINCIPLE OF THE PROCEDURE

The *digene* HC2 GC-ID DNA Test using *digene* Hybrid Capture 2 technology is a nucleic acid hybridization assay with signal amplification that utilizes microplate chemiluminescent detection. Specimens containing the target DNA hybridize with a specific GC RNA probe. The resultant RNA:DNA hybrids are captured onto the surface of a microplate well coated with antibodies specific for RNA:DNA hybrids. Immobilized hybrids are then reacted with alkaline phosphatase conjugated antibodies specific for RNA:DNA hybrids and detected with a chemiluminescent substrate. Several alkaline phosphatase molecules are conjugated to each antibody. Multiple conjugated antibodies bind to each captured hybrid resulting in substantial signal amplification. As the substrate is cleaved by the bound alkaline phosphatase, light is emitted, which is measured as relative light units (RLUs) on a luminometer. The intensity of the light emitted denotes the presence or absence of target DNA in the specimen.

An RLU measurement equal to or greater than a specified ratio to the positive Cutoff (CO) Value indicates the presence of GC DNA in the specimen. An RLU measurement less than a specified ratio to the positive Cutoff Value indicates the absence of GC DNA or GC DNA levels below the assay's detection limit.

The GC Probe contains a probe mixture specifically chosen to eliminate or minimize cross-reactivity with DNA sequences from human cells, other bacterial species, or *Neisseria* species other than *Neisseria gonorrhoeae*. The GC Probe supplied with the *digene* HC2 GC-ID DNA Test is complementary to approximately 9,700 bp or 0.5% of the *Neisseria gonorrhoeae* genomic DNA (1.9×10^6 bp).[4] One probe is complementary to 100% of the cryptic plasmid of 4200 bp.

High-volume sample-throughput testing with the *digene* HC2 GC-ID DNA Test can be performed utilizing a general use automated pipetting and dilution system referred to as the Rapid Capture System (RCS). This instrument, using an application specific to the *digene* HC2 GC-ID DNA Test, processes up to 352 specimens in eight hours. To enable high-volume sample-throughput testing, all the procedural steps of the assay are performed by the RCS, with the exception of specimen denaturation, chemiluminescent signal detection, and result reporting.

REAGENTS AND MATERIALS PROVIDED

There are 96 tests in one *digene* HC2 GC-ID DNA Test kit (REF 5140-1330). The number of patient results will vary, depending on the number of uses per kit:

1 use = 88 patient results
2 uses = 80 patient results
3 uses = 72 patient results
4 uses = 64 patient results

Indicator Dye INDIC Contains 0.05% w/v sodium azide.	1 x 0.35 ml
Denaturation Reagent* REAG | DENAT Dilute sodium hydroxide (NaOH) solution.	1 x 50 ml
Probe Diluent* DIL | PROBE Buffered solution with 0.05% w/v sodium azide.	1 x 5 ml
GC Probe PROBE | GC GC RNA probe in buffered solution.	1 x 200 µl
Negative Calibrator CAL | - Carrier DNA in Specimen Transport Medium (STM) with 0.05% w/v sodium azide.	1 x 2 ml
GC Positive Calibrator (PC) CAL | GC | + 1.0 pg/ml cloned GC DNA and carrier DNA in STM with 0.05% w/v sodium azide.	1 x 1 ml
Quality Control CT (QC CT) QC | CT 5.0 pg/ml cloned CT DNA and carrier DNA in STM with 0.05% w/v sodium azide.	1 x 1 ml
Quality Control GC (QC GC) QC | GC 5.0 pg/ml cloned GC DNA and carrier DNA in STM with 0.05% w/v sodium azide.	1 x 1 ml
Capture Microplate PLATE | CAPTURE Coated with Goat polyclonal anti-RNA:DNA hybrid antibodies.	1 each
Detection Reagent 1 REAG | DET | 1 Alkaline phosphatase-conjugated antibodies to RNA:DNA hybrids in buffered solution with 0.05% w/v sodium azide.	1 x 12 ml
Detection Reagent 2 REAG | DET | 2 CDP-Star® with Emerald II (chemiluminescent substrate).	1 x 12 ml
Wash Buffer Concentrate* BUF | WASH | X 30 Contains 1.5% w/v sodium azide.	1 x 100 ml

*See the *Warnings and Precautions* section of this insert for health and safety information.

MATERIALS REQUIRED BUT NOT SUPPLIED

Hybrid Capture System *In Vitro* Diagnostic Equipment and Accessories[A]

digene Hybrid Capture 2 System ("*digene* HC2 System"), consisting of a QIAGEN-approved luminometer ("luminometer"), QIAGEN-approved personal computer and computer peripherals (monitor, keyboard, mouse, printer, and printer cable), *digene* HC2 System Software ("*digene* assay analysis software"), *digene* HC2 System Assay Protocols for CT/GC, LumiCheck Plate Software, and *digene HC2 System Software User Manual*
Hybrid Capture System Rotary Shaker I
Hybrid Capture System Microplate Heater I
Hybrid Capture System Automated Plate Washer
Hybrid Capture System Multi-Specimen Tube (MST) Vortexer 2 (Optional)[B]
Conversion Rack and Rack lid (optional for manual use; required when using the Rapid Capture System with the *digene* HC2 GC-ID DNA Test and PreservCyt specimens)
digene Specimen Rack and Rack lid (optional for manual use; (required when using the Rapid Capture System with the *digene* HC2 GC-ID DNA Test and *digene* HC2 specimens collected with the *digene* HC2 DNA Collection Device)
EXPAND-4 Pipettor and Stand (optional)[C]
digene HC2 DNA Collection Device[D]
digene Female Swab Specimen Collection Kit (consists of 2 swabs and *digene* Specimen Transport Medium)[D]
Tube Sealer Dispenser and cutting device (optional, used with the MST Vortexer 2)
Rapid Capture System (optional for high-volume sample-throughput testing)[E]
Wash Apparatus
Hybridization Microplates
Microplate Lids
Empty Microplate Strips (available from Costar, Model #2581); optional for use with the Automated Plate WasherI
Extra-Long Pipette Tips for removal of specimen
Specimen Collection Tubes
Specimen Collection Tube Rack
Specimen Collection Tube Screw Caps
Disposable Reagent Reservoirs
DuraSeal® Tube Sealer Film

General Laboratory Use Equipment and Accessories

65 ± 2°C water bath of sufficient size to hold either 1 Conversion Rack (36 x 21 x 9 cm) or two *digene* Specimen Racks (each 31.7 x 15.2 x 6.4 cm)
Microcentrifuge (optional for centrifuging probe vials to obtain maximum probe volume)
Vortex mixer with cup attachment
Single-channel Micropipettor; variable settings for 20-200 µl and 200-1000 µl volumes
Repeating positive displacement Pipettor, such as Eppendorf Repeater® Pipette or equivalent
8-channel Pipettor: variable settings for 25-200 µl volumes
Timer
Sodium hypochlorite solution, 0.5% final concentration (of household bleach)
Parafilm® or equivalent
Disposable aerosol-barrier Pipette Tips for single-channel pipettor (20 to 200 µl and 200-1000 µl)
Disposable Tips for Eppendorf Repeater® Pipette (25 and 500 µl)
Disposable Tips for 8-channel pipettor (25 to 200 µl)
Kimtowels® Wipers or equivalent low-lint paper towels
Disposable bench cover
Powder-free gloves
5-ml and/or 15-ml snap-cap, round-bottom Polypropylene Tubes (for Probe dilution)
2.0-ml polypropylene microcentrifuge tubes with caps

Additional Equipment and Accessories for PreservCyt Solution Specimen Processing

Swinging Bucket Centrifuge capable of reaching 2900 ± 150 x *g* and holding 10-ml or 15-ml conical polypropylene centrifuge tubes

5-ml serological pipettes or transfer pipettes
digene HC2 Sample Conversion Kit[A]
Disposable tips for Eppendorf Repeater Pipette (50 and 100 µl)

<u>For Manual Vortex Procedure:</u>

digene HC2 Sample Conversion Tubes (15-ml conical)[F], Sarstedt® 10-ml Conical tubes with Caps or VWR® or Corning® brand 15-ml conical-bottom polypropylene centrifuge tubes with caps
Tube rack to hold 10-ml or 15-ml conical tubes

<u>For Multi-Specimen Tube Vortexer 2 Procedure</u>

digene HC2 Sample Conversion Tubes (15-ml conical)[F]
Multi-Specimen Tube (MST) Vortexer 2
Conversion Rack and Lid (specific for 15-ml conical tubes)
Tube Sealer dispenser and cutting device
DuraSeal Tube Sealer Film (used with the MST Vortexer 2)

[A] Only equipment and accessories validated with *digene* HC2 CT/GC DNA Tests are available from QIAGEN.
[B] Also required for use when performing the Semi-automated RCS Application.
[C] Custom item. Other custom expandable multi-channel pipettes can be used, provided tip spacing of 3.2 cm is achievable when expanded. Alternatively, a single-channel pipette capable of pipetting 75 µl may be used.
[D] The performance characteristics of the *digene* HC2 GC-ID DNA Test were established only with the collection kits indicated.
[E] Refer to the *Rapid Capture System User Manual* for instructions specific to the use of that system for high volume sample-throughput testing with this assay.

[F] The *digene* HC2 Sample Conversion Tubes (VWR or Corning® brand) available from QIAGEN must be used to assure proper assay performance when using the Multi-Specimen Tube Vortexer 2 procedure.

WARNINGS AND PRECAUTIONS

READ ALL INSTRUCTIONS CAREFULLY BEFORE USING THE TEST.

SAFETY PRECAUTIONS

ALL SPECIMENS should be considered potentially infectious. No known test method can offer complete assurance that specimens will not transmit infection. It is recommended that human specimens be handled in accordance with the appropriate national/local biosafety practices.[5,6,7,8] Use these biosafety practices with materials that contain or are suspected of containing infectious agents. These precautions include, but are not limited to, the following:

1. Do not pipette by mouth.
2. Do not smoke, eat, or drink in areas where reagents or specimens are handled.
3. Wear disposable powder-free gloves while handling reagents or specimens. Wash hands thoroughly after performing the test.
4. Clean and disinfect all spills of specimens using a tuberculocidal disinfectant such as 0.5% v/v sodium hypochlorite, or other suitable disinfectant.[9,10]
5. Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with national and local regulations.[11,12]

Some reagents contain sodium azide. Sodium azide has been reported to form lead or copper azide in laboratory plumbing. These azides may explode upon percussion, such as hammering. To prevent formation of lead or copper azide, flush drains thoroughly with water after disposing of solutions containing sodium azide. To remove contamination from old drains suspected of azide accumulation, the National Institute for Occupational Safety and Health recommends the following: (1) siphon liquid from trap using a rubber or plastic hose, (2) fill with 10% v/v sodium hydroxide solution, (3) allow to stand for 16 hours, and (4) flush well with water.

SAFETY AND HEALTH RISK INFORMATION

The materials below have been assessed according to the requirements of EC Directives 2001/59/EC and 99/45/EC.


T

Wash Buffer Concentrate. Contains sodium azide: Toxic (T)
R25: Toxic if swallowed.
R52/53: Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
S45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).


C

Denaturation Reagent. Contains sodium hydroxide: Corrosive (C)
R35: Causes severe burns.
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
S45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).


Xi

Probe Diluent. Contains BES and Acetic acid: Irritant (Xi)
R36/38: Irritating to eyes and skin.
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.

24-HOUR EMERGENCY INFORMATION

EMERGENCY MEDICAL INFORMATION IN ENGLISH, FRENCH, AND GERMAN CAN BE OBTAINED 24 HOURS A DAY FROM:

POISON INFORMATION CENTER MAINZ, GERMANY

TEL: +49-6131-19240

Refer to the *Rapid Capture System User Manual* for additional Warnings and Precautions specific to the use of that system for high-volume sample-throughput testing with this assay.

HANDLING PRECAUTIONS

1. For *In Vitro* Diagnostic Use only.
2. Cervical Brush for use with non-pregnant women only.
3. Do not use the reagents beyond the expiration date indicated next to the symbol  on the outer box label.
4. Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges are invalid and must be repeated.
5. The *digene* HC2 GC-ID DNA Test Procedure, Assay Calibration Verification Criteria, Quality Control, and the Interpretation of Specimen Results must be followed closely to obtain reliable test results.
6. It is important to pipette the exact reagent volume indicated and to mix well after each reagent addition. Failure to do so could result in erroneous test results. Ensuring that the noted color changes occur will confirm that these conditions have been met.
7. These components have been tested as a unit. **Do not** interchange components from other sources or from different lots.
8. Nucleic acids are very sensitive to environmental nuclease degradation. Nucleases are present on human skin and on surfaces or materials handled by humans. Clean and cover work surfaces with a disposable bench cover **and wear powder-free gloves when performing all assay steps.**
9. Take care to prevent contamination of the Capture Microplate and Detection Reagent 2 with exogenous alkaline phosphatase during performance of the assay. Substances that may contain alkaline phosphatase include Detection Reagent 1, bacteria, saliva, hair and oils from skin. **Covering the Capture Microplate after the wash step and during the Detection Reagent 2 incubation step is especially important because exogenous alkaline phosphatase may react with Detection Reagent 2 producing false-positive results.**
10. Protect Detection Reagent 2 from prolonged exposure to direct light. Use the reagent within the time frame indicated immediately after aliquoting, and avoid direct sunlight.
11. The repeating pipettor should be primed in advance of reagent delivery and checked for large air bubbles periodically. Excessive amounts of large air bubbles in the repeating pipettor tip may cause inaccurate delivery and can be avoided by filling the pipettor, dispensing all of the liquid, and refilling. See pipettor instruction manuals for specific directions for use.
12. Multichannel pipetting should be performed using the reverse pipetting technique (see *Hybrid Detection*) for dispensing Detection Reagents 1 and 2. Check each pipette tip on the multichannel pipettor for proper fit and filling.
13. Take care during washing to ensure that each microwell is washed thoroughly as indicated in the Manual Washing instructions. Inadequate washing will result in increased background and may cause false-positive results. Residual Wash Buffer in wells may result in reduced signal or poor reproducibility.
14. Allow at least 60 minutes for the Microplate Heater I to equilibrate to 65°C ± 2°C from a cold start. Not allowing for this warm-up period could result in melting of the Hybridization Microplate. See the Microplate Heater I User Manual for details.

REAGENT PREPARATION AND STORAGE

1. Upon receipt, store the kit at 2-8°C. The Wash Buffer Concentrate, Denaturation Reagent and Indicator Dye may be stored at 2-30°C, as desired.
2. Do not use after the expiration date indicated next to the symbol on the outer box label or the expiration date of the prepared reagents (see below).
3. All reagents provided are ready-to-use except Denaturation Reagent, GC Probe Mix and Wash Buffer.

Refer to the *Rapid Capture System User Manual* for the preparation of the GC Probe Mix, the Wash Buffer, Detection Reagent 1 and Detection Reagent 2 as those instructions are specific to the use of that system for high-volume sample-throughput testing.

Reagent Preparation Method

<table>
<tr><td>Denaturation Reagent</td><td>PREPARE FIRST:
Add 5 drops of Indicator Dye to the bottle of Denaturation Reagent and mix thoroughly. The Denaturation Reagent should be a uniform, dark purple color.

Once prepared, the Denaturation Reagent is stable for three months when stored at 2-8°C. Label it with the new expiration date. If the color fades, add 3 additional drops of Indicator Dye and mix thoroughly before using.

Warning: Denaturation Reagent is corrosive. Wear suitable protective clothing, gloves, eye/face protection. Use care when handling.</td></tr>
<tr><td>GC Probe Mix (Prepared from GC Probe and Probe Diluent Reagents)</td><td>PREPARE DURING SPECIMEN DENATURATION INCUBATION:

IMPORTANT: SOMETIMES PROBE GETS TRAPPED IN THE VIAL LID.

Note: Take extreme care at this step to prevent RNase contamination of Probe and Probe Mix. Use aerosol-barrier pipette tips for pipetting probe. Probe Diluent is viscous. Take care to ensure thorough mixing when preparing GC Probe Mix. A visible vortex must form in the liquid during the mixing step. Incomplete mixing may result in reduced signal.

• Centrifuge the vial of GC Probe briefly to bring liquid to the bottom of the vial. Tap tube gently to mix.

• Determine the amount of Probe Mix required (25 µl/test). It is recommended that extra Probe Mix be made to account for the volume that may be lost in pipette tips or on the side of the vial. Refer to suggested volumes listed below. The smallest number of wells recommended for each use is 24. If fewer than 24 wells per assay are desired, the total number of tests per kit may be reduced due to limited Probe and Probe Diluent volumes.

• Transfer the required amount of Probe Diluent to a new disposable container. Depending on the number of tests, either a 5-ml or 15-ml snap-cap, round-bottom, polypropylene tube is recommended. Make a 1:25 dilution of GC Probe in Probe Diluent to prepare Probe Mix.

<table>
<tr><th>No. of Tests/Strips</th><th>Volume Probe Diluent*</th><th>Volume Probe*</th></tr>
<tr><td>96/12</td><td>4.0 ml</td><td>160.0 µl</td></tr>
<tr><td>72/9</td><td>3.0 ml</td><td>120.0 µl</td></tr>
<tr><td>48/6</td><td>2.0 ml</td><td>80.0 µl</td></tr>
<tr><td>24/3</td><td>1.0 ml</td><td>40.0 µl</td></tr>
<tr><td>Per Well</td><td>0.045 ml</td><td>1.8 µl</td></tr>
</table>
*These values include the recommended extra volume.

• Pipette Probe into Probe Diluent by placing pipette tip against the inner wall of the tube just above the meniscus and expelling the contents. Do not immerse the tip into the Probe Diluent.

• Vortex for at least 5 seconds at maximum speed to mix thoroughly. A visible vortex must be produced. Label as GC Probe Mix and keep in a sealed container until ready for use. Unused Probe Mix should be discarded.</td></tr>
</table>

Wash Buffer

PREPARE DURING CAPTURE STEP:
For the Automated Plate Washer, the Wash Buffer can be prepared as described below and stored in a covered container or prepared 1 L at a time and placed in the Automated Plate Washer reservoirs. See the table below for mixing volumes.

See Automated Plate Washer User Manual for additional care and maintenance instructions.

Warning: Wash Buffer Concentrate is toxic by ingestion. Wear suitable protective clothing, gloves, eye/face protection. To minimize exposure, add water to Wash Buffer Concentrate when preparing.

Amount of Wash Buffer Concentrate	Amount of Distilled or Deionized Water	Final Volume of Wash Buffer
33.3 ml	966.7 ml	1 L
66.6 ml	1,933.4 ml	2 L
100.0 ml	2,900.0 ml	3 L

Note: It is very important to always leave the power to the Automated Plate Washer on at all times. This allows the maintenance rinse to be performed after eight hours of nonuse.

Prior to each assay, make sure the Automated Plate Washer waste reservoir is empty and the rinse reservoir is filled with distilled or deionized water.

See Automated Plate Washer User Manual for additional Care and Maintenance Instructions.

For the manual plate washing method:
- Mix Wash Buffer Concentrate well.
- Dilute 100 ml Wash Buffer Concentrate with 2.9 L of distilled or deionized water and mix well (final volume should be 3 L).
- Close the container to prevent contamination or evaporation.

Once prepared, the Wash Buffer is stable for three months at 2-30°C. Label it with the new expiration date. If Wash Buffer has been refrigerated, equilibrate to 20-25°C before using.

It is recommended that the Wash Apparatus and tubing be cleaned with 0.5% sodium hypochlorite solution and rinsed thoroughly with distilled or deionized water once every three months to prevent possible contamination from alkaline phosphatase present in bacteria and molds.

Volumes for Ready-to-Use Reagents

Detection Reagent 1 and Detection Reagent 2

IMMEDIATELY PRIOR TO USE:
Mix reagent thoroughly, then carefully measure the appropriate volume of Detection Reagent 1 or Detection Reagent 2 into a clean reagent reservoir following the guidelines shown below. To avoid contamination, these reagents **MUST NOT** be returned to the original bottles: **Discard unused material after use.** If an 8-channel pipettor is not being used, an appropriate repeating pipettor may be substituted. In this case, aliquots of the reagent should be made into a polypropylene tube of sufficient size to hold the required volume as indicated below.

No. of Tests/Strips	Volume Detection Reagent 1 or 2
96/12	contents of bottle
72/9	7.0 ml
48/6	5.0 ml
24/3	3.0 ml
1 test	0.125 ml

SPECIMEN COLLECTION AND HANDLING

Cervical specimens collected and transported using the *digene* HC2 DNA Collection Device (consisting of a cervical brush and *digene* Specimen Transport Medium) and the *digene* Female Swab Specimen Collection Kit (swab and *digene* Specimen Transport Medium) or specimens collected using a broom-type collection device and placed in Hologic PreservCyt Solution are the only specimens recommended for use with the *digene* HC2 GC-ID DNA Test. Specimens taken with other sampling devices or transported in other transport media have not been qualified for use with this assay. The performance characteristics of this kit were established only with the collection kits indicated. Cervical specimens must be collected prior to the application of acetic acid or iodine if colposcopic examination is being performed. See the *digene* HC2 DNA Collection Device instructions for use for additional specimen collection and handling procedures.

CERVICAL SPECIMENS IN STM

STM specimens may be held for up to two weeks at room temperature and shipped without refrigeration to the testing laboratory. Specimens should be shipped in an insulated container using either an overnight or 2-day delivery vendor. At the testing laboratory, specimens must be stored at 2-8°C if the assay is to be performed within one week. If the assay will be performed later than one week, store specimens at -20°C for up to 3 months. A preservative has been added to the *digene* Specimen Transport Medium to retard bacterial growth and to retain the integrity of DNA. It is **not intended** to preserve viability of organisms or cells. Specimens collected in *digene* Specimen Transport Medium cannot be used for culture of other testing methods.

STM specimen stability for 2 weeks at room temperature, plus an additional week at 2-8°C is based on in-house testing of 90 simulated clinical specimens. These 90 specimens included 40 that contained low concentrations of GC organism [at or near the assay's limit of detection (LOD)], 35 that were moderately positive specimens (approximately 2-5 times the LOD), and 5 high-positive specimens that exceeded 10 times the LOD. The remaining 10 specimens were negative for GC; however, 5 contained a high level of CT organism. Performance estimates for the assay are based on specimens stored at 2-8°C or frozen and tested within 1-2 weeks of collection.

Notes:

1. A non-denatured aliquot of each of 90 specimens was subjected to extreme temperatures intended to simulate shipping conditions (storage at -20°C for 3 days, then at 50°C for 5 days, and an additional 2 weeks at room temperature). Although a loss of signal (RLU/CO) was observed after 8 days under these conditions, the qualitative interpretation of the results was not affected. After the additional two-week incubation at room temperature, qualitative differences were observed with specimens containing low levels of organism.
2. To prevent caps from popping off specimens that are shipped or stored frozen:
   - Cover caps with Parafilm® prior to shipping specimens previously frozen. Specimens may be shipped frozen or at 20-25°C.
   - When removing specimens from the freezer for testing, replace caps immediately with specimen collection tube screw caps.
3. The *digene* HC2 DNA Collection Device must not be used for pregnant women. Collect specimens from pregnant women using the *digene* Female Swab Specimen Collection Kit only.

CERVICAL SPECIMENS IN HOLOGIC PRESERVCYT SOLUTION

Specimens collected using a broom-type collection device and placed in Hologic PreservCyt Solution for use in making Hologic ThinPrep® Pap Test slides can be used with the *digene* HC2 GC-ID DNA Test. Specimens should be collected in the routine manner, and the ThinPrep Pap Test slides should be prepared according to Hologic instructions.

PreservCyt Solution specimens may be held for up to one month at room temperature (20-25°C), following collection and prior to processing for the *digene* HC2 GC-ID DNA Test. PreservCyt Solution specimens cannot be frozen. To process these specimens, refer to the *PreservCyt Specimen Preparation Procedure*.

TEST PROCEDURE

Specimens may contain infectious agents and should be handled accordingly. The *digene* HC2 GC-ID DNA Test can be performed manually (as instructed in these instructions for use) or using the Rapid Capture System instrument for high-volume sample-throughput testing.

HIGH-VOLUME SAMPLE-THROUGHPUT TESTING USING THE RAPID CAPTURE SYSTEM

The Rapid Capture System is a general use automated pipetting and dilution system that can be used with the *digene* HC2 GC-ID DNA Test for high-volume sample-throughput testing. This system handles up to 352 specimens in eight hours, including a 3.5-hour period during which user intervention is not required; up to 704 specimen results can be generated in 13 hours. Denaturation of the specimens in preparation for testing is performed independently of the RCS, in the primary collection tube (as with the manual method of the *digene* HC2 GC-ID DNA Test described below), prior to placing on the RCS platform. In addition, chemiluminescent signal detection and result reporting are performed using the offline QIAGEN-approved luminometer system common to both the manual and RCS methods. Each of the *digene* HC2 GC-ID DNA Test's procedural steps is performed in the exact sequence as the manual test procedure. The RCS Application allows for the staggered processing of up to 4 microplates, each plate containing specimens and the required assay Calibrators and Quality Controls.

When using the Rapid Capture System, refer to the *Rapid Capture System User Manual* provided with the instrument, in addition to these instructions for use, for necessary procedural and descriptive information.

MANUAL METHOD

Setup

1. Allow at least 60 minutes for the Microplate Heater I to equilibrate to 65 ± 2°C from a cold start. See the *Microplate Heater I User Manual* for details.
2. Confirm a water bath is at 65°C and the water level is high enough to immerse the entire volume in the specimen tubes.
3. Remove the specimens and **all** required reagents from the refrigerator **prior to beginning the assay**. Allow them to reach 20-25°C for 15 to 30 minutes.
4. Create a plate layout using the *digene* assay analysis software with *digene* assay protocols for GC. See the applicable software user manual for details.
5. Negative Calibrator, Positive Calibrator and Quality Controls must be prepared **fresh** for each assay. Mix the Calibrators and Quality Controls well. If using the MST Vortexer 2, remove 500 µl of each into appropriately labeled empty specimen collection tubes. Alternatively, remove 200 µl of each into appropriately labeled 2-ml polypropylene microcentrifuge tubes.
6. **The Negative Calibrator and Positive Calibrator must be tested FIRST** in triplicate for each batch of specimens tested. The Quality Controls and specimens should be tested singly. Calibrators, Quality Controls, and specimens should be tested in an 8-microwell column configuration, such that the Negative Calibrator (NC) replicates are placed in A1, B1, C1; the Positive Calibrator (PC) in D1, E1, F1; QC CT in G1; QC GC in H1; then specimens beginning in A2. See example layout below. Consult the appropriate QIAGEN-approved luminometer user manual and the appropriate *digene* assay analysis software user manual for the proper Calibrator/Quality Control/Specimen setup in software.

EXAMPLE LAYOUT FOR A TEST USING OF 24 MICROWELLS:

	Column		
Row	1	2	3
A	NC	Spec. 1	Spec. 9
B	NC	Spec. 2	Spec. 10
C	NC	Spec. 3	Spec. 11
D	PC	Spec. 4	Spec. 12
E	PC	Spec. 5	Spec. 13
F	PC	Spec. 6	Spec. 14
G	QC CT	Spec. 7	Spec. 15
H	QC GC	Spec. 8	Spec. 16

DENATURATION

Notes:

- **Caution**: Denaturation Reagent is corrosive. Wear suitable protective clothing, gloves, eye/face protection. Use care and wear powder-free gloves when handling.
- **Important**: Some specimens may contain blood or other biological material that may mask the color changes upon addition of Denaturation Reagent. Specimens that exhibit a dark color prior to the addition of Denaturation Reagent may not give the proper color changes at these steps. In these cases, failure to exhibit the proper color change will not affect the results of the assay. Proper mixing can be verified by observing the color changes of the Calibrators and Quality Controls.
- During the denaturation step, be sure that the water level in the water bath is adequate to immerse the entire volume of specimen in the tube.
- Specimens may be prepared up through the denaturation step and stored at 2-8°C overnight, or at -20°C for up to 3 months. A maximum of 3 freeze-thaw cycles may be performed with a maximum of 2 hours at room temperature during each thaw cycle. Mix well before using.
- Calibrators and Quality Controls may be prepared up through the denaturation step and stored at 2-8°C overnight, **but they may not be frozen.** If Calibrators and Quality Controls have been frozen, they must be discarded.
- Following denaturation and incubation, the specimens are no longer considered infectious[13]; however, lab personnel should still adhere to national/local precautions.

CALIBRATORS, QUALITY CONTROLS, AND STM SPECIMEN PREPARATION PROCEDURE

Notes:

- Do not remove specimen collection device prior to denaturation.
- To avoid false-positive results, it is critical that all Calibrator, Quality Control, and STM specimen material come into contact with Denaturation Reagent. Mixing after Denaturation Reagent addition is a critical step: **Make sure the Multi-Specimen Tube Vortexer 2 is set to 100 (maximum speed) and a visible vortex of liquid is observed during mixing such that the liquid washes the entire inner surface of the tube. If performing manual vortexing, make sure that each Calibrator, Quality Control, and specimen is mixed individually by vortexing each for at least 5 seconds at full speed such that the liquid vortex washes the entire inner surface of the tube followed by inverting the tube one time.**

1. Remove and discard caps from Calibrators, Quality Controls, and STM specimen tubes.

   Note: Caps removed from the specimen tubes are considered potentially infectious. Dispose of in accordance with national/local regulations.

2. Pipette Denaturation Reagent with Indicator Dye into each Calibrator, Quality Control, or STM specimen using a repeating or adjustable pipettor. Take care not to touch the sides of the tube or cross-contamination of specimens could occur. The volume of Denaturation Reagent needed is equivalent to half the specimen volume. The exact volume for each type of Calibrator, Quality Control, and specimen is listed in the table below.

- **Dilute remaining Denaturation Reagent in bottle prior to disposing according to national/local laboratory procedures.**

Calibrator, Quality Control, or Specimen	Volume of Denaturation Reagent Required
Negative Calibrator, Positive Calibrator, and Quality Control, 200 µl	100 µl
Negative Calibrator, Positive Calibrator, and Quality Control, 500 µl	250 µl
Cervical Specimen, 1 ml	500 µl

3. Mix the specimens using one of the two methods below.

   Multi-Specimen Tube Vortexer 2 Method

   Note: QIAGEN specimens mixed using the MST Vortexer 2 **must** be hybridized using the hybridization microplate and Microplate Heater I method. See the MST Vortexer 2 User Manual for further instructions, as needed.

   a) Cover the Calibrators, Quality Controls, and STM specimen tubes with DuraSeal® Tube Sealer Film by pulling the film over the tubes in the rack.
   b) Place the rack lid over the film-covered tubes and lock into place with the two side clips. Cut the film with the cutting device.
   c) Place the rack on the Multi-Specimen Tube Vortexer 2 and secure the rack with the clamp. Verify speed setting is at 100 (maximum speed), and turn the vortexer power switch to the ON position. Vortex the tubes for 10 seconds.

   Manual/Individual Tube Vortexing Method

   a) Recap the Calibrators, Quality Controls, and STM specimen tubes with clean specimen collection tube screw caps.
   b) Mix each tube thoroughly by vortexing individually, at high speed, for 5 seconds.
   c) Invert each specimen tube one time to wash the inside of the tube, cap and rim.
   d) Return the tube to the rack.

4. Independent of the vortexing method utilized, **there must be a visible vortex of liquid inside each tube during mixing such that the liquid washes the entire inner surface of the tube.** The Calibrators, Quality Controls, and specimens should turn purple.
5. Incubate the tubes in the rack in a 65 ± 2°C water bath for 45 ± 5 minutes (denatured Calibrators, Quality Controls, and specimens may be tested immediately. Calibrators Quality Controls may be stored at 2-8°C overnight, as described in **Notes** above). For specimen storage, refer to the *Optional Stop Point*. Prepare GC Probe Mix during this incubation. See *Reagent Preparation and Storage* section.

PRESERVCYT SOLUTION SPECIMEN PREPARATION PROCEDURE

Notes:

- Consult the *digene* HC2 Sample Conversion Kit instructions for use for complete details.
- Processing a 4-ml aliquot of PreservCyt Solution produces enough for 2 tests, when tested manually. The minimum volume that can be processed is 4 ml. *Refer to the Equivalence between STM and PreservCyt Solution Specimens* section for details regarding minimum residual volume.
- Prepare PreservCyt Solution specimens in batches of 36 or fewer; otherwise, pellets may become dislodged when decanting the supernatant. This is important for maintaining the integrity of the cell pellet during the decanting step. If preparing additional PreservCyt Solution vials, do not start to prepare them until after completing the preparation of the first batch.

Use either the Denaturation Reagent (DNR) provided with the *digene* HC2 GC-ID DNA Test (see *Reagent Preparation and Storage*) or the DNR provided with the *digene* HC2 Sample Conversion Kit. To prepare the DNR provided with the *digene* HC2 Sample Conversion Kit, add 3 drops of Indicator Dye to the bottle

of DNR and mix well. The solution should be a uniform, dark purple color. To determine volume requirements, use Table 1.

Table 1. Volume Requirements: Reagent Preparation.

Number of Tests	PreservCyt Solution Volume	Conversion Buffer Volume
1-2	4 ml	0.4 ml
3	6 ml	0.6 ml
4	8 ml	0.8 ml
5	10 ml	1.0 ml
6	12 ml	1.2 ml

1. Label a *digene* HC2 Sample Conversion tube, 10-ml conical Sarstedt tube, or a 15-ml VWR or Corning brand conical tube with the appropriate specimen identification number.
2. Handling one specimen at a time:
   a. Shake the PreservCyt vial vigorously by hand until cells appear to be homogenously dispersed.
   b. Immediately, as cells settle very quickly, pipette the appropriate volume of the PreservCyt specimen into the labeled tube. Deliver the PreservCyt solution to the bottom of the conical tube to minimize cellular material adhering to the inside of the tube.
3. Add the appropriate volume of Sample Conversion Buffer to each tube (see Table 1*)*.
4. Recap and mix the contents of each tube thoroughly by using a vortex mixer with cup attachment.

   Note: The MST Vortexer 2 procedure has not been validated for vortexing PreservCyt Solution specimens with Sample Conversion Buffer prior to centrifugation, and therefore it must not be used for this step.
5. Centrifuge the tubes in a swinging bucket rotor at 2,900 ± 150 x *g* for 15 ± 2 minutes.
6. During centrifugation, prepare the *digene* Specimen Transport Medium/Denaturation Reagent mixture (STM/DNR) in a 2:1 ratio, according to Table 2.

   Note: The STM/DNR Mixture must be prepared fresh each day the test is being performed.

   a. To determine the total volume of STM/DNR mixture required, use the starting volume of the PreservCyt Solution specimen as a guide and then multiply the STM and DNR "per tube" volumes by the number of specimens to be processed (see Table 2).

      Table 2. Volume Requirements: STM/DNR.

No. of Tests	PreservCyt Solution Volume	STM Volume per tube for final STM/DNR Mixture*	DNR Volume per tube for final STM/DNR Mixture*	STM/DNR Mixture added to tube
1-2	4 ml	120 µl	60 µl	150 µl
3	6 ml	170 µl	85 µl	225 µl
4	8 ml	220 µl	110 µl	300 µl
5	10 ml	270 µl	135 µl	375 µl
6	12 ml	320 µl	160 µl	450 µl

      * The volumes listed in these columns should not be added directly to the specimen tube.

   b. Mix the solution thoroughly by vortexing.
7. Remove tubes from the centrifuge one tube at a time and place into a rack or Conversion Rack. A pink/orange pellet should be present in the bottom of each tube.

   Note: Specimens that do not have a visible pellet after centrifugation are not acceptable for testing and should be discarded.

8. Handling each tube individually:
   a. Remove the cap and set aside on a clean low-lint paper towel.
   b. Carefully decant supernatant.
   c. Maintain the inverted tube position and gently blot (approximately 6 times) on absorbent low-lint paper towels until liquid no longer drips from the tube. Use a clean area of the towel each time. **Do not** allow the cell pellet to slide down the tube during blotting.

      Notes:
      - Do not blot in the same area of the absorbent low-lint paper towel more than once.
      - It is important to remove the maximum amount of PreservCyt Solution by blotting. However, it is normal to see residual PreservCyt Solution after blotting.

   d. Place the tube in a rack or the Conversion Rack.

Vortexing and Denaturation

Manual Vortexing Procedure

1. Add the appropriate volume of STM/DNR to each pellet (see Table 2). Recap each tube and resuspend the pellets by vortexing each tube individually for at least 30 seconds at the highest speed setting. If a pellet is difficult to resuspend, vortex for an additional 10-30 seconds or until the pellet floats loose from the bottom of the tube. If a pellet remains undissolved after additional vortexing (a total of 2 minutes maximum), note the specimen identification and proceed to the next step.
2. Place the tubes in a rack.
3. Place the rack in 65 ± 2°C water bath for 15 ± 2 minutes. Ensure that the water level is sufficient to cover all liquid in the tubes.
4. Remove the rack with specimens from the water bath and vortex specimens individually for 15-30 seconds.

   Note: Make sure that all pellets are completely resuspended at this point. Specimens that still have visible pellets are not acceptable for testing and should be discarded.
5. Return the rack to the 65 ± 2°C water bath and continue denaturation for another 30 ± 3 minutes.
6. Proceed to the *Hybridization* Step below or see *Optional Stop Point* for storage and treatment of denatured specimens.

Multi-Specimen Tube (MST) Vortexer 2 Procedure

Notes:

- The Multi-Specimen Tube (MST) Vortexer 2 procedure is validated for the processing of PreservCyt Solution specimens following centrifugation and decanting of the supernatant.
- Only the MST Vortexer 2 is designed for PreservCyt Solution specimen processing.
- The Conversion Rack and Lid are specifically designed to accommodate *digene* HC2 Sample Conversion Tubes (VWR or Corning brand 15-ml conical tubes). The user should use only one tube type on the Conversion Rack at a time. Other brands are not validated for use.
- Strict adherence to the specified vortexing times of the Conversion Rack and Lid is required.
- The Conversion Rack and Lid cannot be used to vortex the *digene* HC2 DNA Test kit Calibrators or Quality Controls. The height of the STM tubes prevents adequate vortexing using the Conversion Rack and Lid.

1. After blotting each labeled 15-ml conical tube, place each in its proper position in the Conversion Rack.
2. Add the appropriate volume of STM/DNR mixture to each pellet (Table 2).

3. Cover the 15-ml conical tubes with DuraSeal tube sealer film by pulling the film over the tubes in the rack.
4. Place the rack lid over the film-covered tubes, and lock the lid into place with the two side clamps. Cut the film with the cutting device after the lid is securely fastened.
5. Move the red-handled lever up so that it is in a horizontal position.
6. Place the Conversion Rack and Lid on the MST Vortexer 2 so that the largest diagonal corner of the Conversion Rack is located in the right front corner. Position the rack and lid on the MST Vortexer 2 platform so that it fits securely within the guides. Secure the rack in place by moving the red-handled lever down to the vertical position. This will lock the rack in place.
7. Verify that the speed setting is at 100 (maximum speed) and the Pulser toggle switch is in the OFF position.
8. Turn the Vortexer power switch to the ON position. **Vortex the tubes for 30 seconds.**
9. Turn the Vortexer power switch to the OFF position.
10. Remove the Conversion Rack and Lid from the MST Vortexer 2 by lifting up on the red-handled lever.
11. Place the rack in the 65 ± 2°C water bath for 15 ± 2 minutes. Be sure the water level completely covers all liquid in all of the tubes.
12. After the 15-minute incubation, remove the rack with specimens from the water bath.
13. To prevent splashing, dry the rack of excess water prior to placing it on the MST Vortexer 2.
14. Secure the Conversion Rack and Lid on the MST Vortexer 2 as described in *Step 6*.
15. Verify that the speed setting is at 100, and turn the vortexer power switch to the ON position. **Vortex the tubes for 1 minute.**
16. Turn the Vortexer power switch to the OFF position.

    Note: The MST Vortexer 2 Procedure standardizes the mixing speed, times, and process, eliminating the need to visually check for cell pellets, as is required when using the Manual Vortexing Procedure.
17. Return the rack to the 65 ± 2°C water bath, and continue denaturation for 30 ± 3 minutes.
18. Remove the rack from the water bath, dry the rack, and secure it to the vortexer.
19. Turn the Vortexer power switch to the ON position. **Vortex for 10 seconds at the maximum setting.**
20. Turn the Vortexer power switch to the OFF position. Remove the rack.
21. Immediately remove the Rack Lid and DuraSeal tube sealer film from the specimens.
22. Proceed to the *Hybridization* Step below or see *Optional Stop Point* for storage and treatment of denatured specimens.

OPTIONAL STOP POINT

After denaturation, STM specimens and converted PreservCyt specimens may be stored at 2-8°C overnight or at -20°C for up to 3 months. For overnight refrigeration, specimens may be left in the Conversion Rack with the DuraSeal film and Rack Lid replaced. Prior to storage at -20°C, the Rack Lid and DuraSeal film must be removed, and caps placed on the tubes. In either case, the specimens must be equilibrated to 20 - 25°C and thoroughly vortexed before proceeding to the *Hybridization* step.

Note: Do not store or ship denatured specimens on dry ice.

A maximum of 3 freeze/thaw cycles may be performed with a maximum of 2 hours at room temperature during each thaw cycle.

HYBRIDIZATION

Notes:

- The GC Probe Mix is viscous. Take care to ensure thorough mixing and that the required amount is completely dispensed into each Hybridization Microplate well. See *Reagent Preparation and Storage* section.
- If the denatured specimen has been stored at -20°C then allow the specimen to thaw at 20-25°C, and thoroughly vortex the specimen before proceeding with hybridization.
- Preheat the Microplate Heater I to 65 ± 2°C for at least 60 minutes prior to use. See the *Microplate Heater I User Manual* for further instructions, as needed.

1. Obtain and label a Hybridization Microplate.
2. Remove Calibrators, Quality Controls, and specimens from the water bath after the incubation. If the Multi-Specimen Tube Vortexer 2 is being used, vortex the entire rack of STM specimens for a minimum of 5 seconds on the maximum speed setting. For PreservCyt Solution specimens, vortex the entire Conversion Rack for a minimum of 10 seconds on the maximum speed setting. Alternatively, vortex each tube individually for at least 5 seconds.
3. Pipette 75 µl of each Calibrator, Quality Control, or specimen into the **bottom** of an empty Hybridization Microplate well following the plate layout created under *Setup*. Avoid touching the sides of the wells and limit formation of air bubbles. Use a clean extra-long pipette tip for each transfer to avoid cross-contamination of Calibrators, Quality Controls, or specimens. For STM specimens, do not remove the specimen collection device from the specimen transport tube. Denatured specimens may be capped with specimen collection tube screw caps and stored with specimen collection devices remaining in the tubes. Denatured PreservCyt specimens may be recapped with their original caps.

   Notes:

   - **False-positive results can occur if specimen aliquots are not carefully transferred. During transfer of specimen, do not touch the pipette tip to the inside of the tube when removing the 75-µl aliquot.**

4. After transferring the last specimen, cover the plate with a plate lid, and **incubate the hybridization microplate for 10 minutes at 20-25°C.**
5. Aliquot the prepared and thoroughly vortexed Probe Mix into a Disposable Reagent Reservoir. Carefully pipette 25 µl of the Probe Mix into each well containing Calibrators, Quality Controls, and specimens using an 8-channel pipettor and fresh tips for each row. Dispense the volume of Probe Mix into each hybridization well, avoiding back splashing. Avoid touching the sides of the wells.

   Note: For the above step, use an 8-channel pipettor that is equipped with 25-200 µl tips and that is capable of delivering 25-75 µl. For a small number of wells, use a single-channel pipettor (equipped with 25-200 µl tips) in place of an 8-channel pipettor.
6. Cover the Hybridization Microplate with a plate lid. Shake the Hybridization Microplate on the Rotary Shaker I set at 1100 ± 100 rpm for 3 ± 2 minutes. *The Calibrators, Quality Controls, and specimens should turn yellow after shaking*. Wells that remain purple may not have received the proper amount of Probe Mix. Add an additional 25 µl of Probe Mix to specimens that remain purple and shake again. If wells remain purple after following this procedure, retest the specimens.
7. Incubate in a preheated and equilibrated to 65 ± 2°C Microplate Heater I for 60 ± 5 minutes.

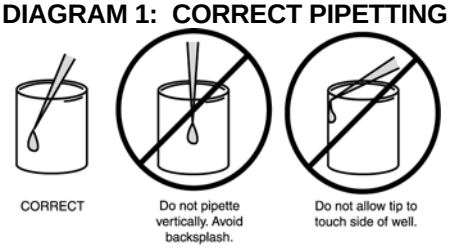
   Notes:

   - When placing the Hybridization Microplate in the Microplate Heater I, take care not to cause splashing.
   - After shaking, PreservCyt Solution specimens should turn pink instead of yellow.

HYBRID CAPTURE

1. Remove all but the required number of Capture Microplate wells from the plate frame. Return the unused microwells to the original bag and reseal. With a marker, number each column 1, 2, 3. . . and label microplate with an appropriate identifier. The specimens will be added to the wells according to the example layout previously prepared under *Setup*.
2. Carefully remove Hybridization Microplate containing Calibrators, Quality Controls and specimens from Microplate Heater I. Immediately remove the plate lid and placed on a clean surface.
3. Transfer the entire contents (approximately 100 µl) of the Calibrators, Quality Controls, and specimens from Hybridization Microplate wells to the bottom of the corresponding Capture Microwell using an 8-channel pipettor. Use new pipette tips on the 8-channel pipettor for each column transferred, and allow each pipette tip to drain well to ensure complete specimen transfer. If desired, the pipettor may be steadied by resting the **middle** of the pipette tips on the top edge of the capture microwells (see Diagram 1).

DIAGRAM 1: CORRECT PIPETTING



4. Cover microplate with the plate lid and shake on Rotary Shaker I at 1100 ±100 rpm, at 20-25°C for 60 ± 5 minutes.
5. During this incubation, prepare the Wash Buffer and, if applicable, check Automated Plate Washer rinse and waste reservoirs. See *Reagent Preparation and Storage* section.
6. When the capture step is complete, remove the Capture Microplate from the Rotary Shaker I and carefully remove the plate lid. Remove the liquid from the wells by discarding into a sink: fully invert the plate over sink and shake hard with a downward motion being careful not to cause a backsplash by decanting too closely to the bottom of the sink. **Do not reinvert plate**; blot by tapping firmly 2-3 times on clean Kimtowels® Wipers or equivalent low-lint paper towels. Ensure that all liquid is removed from the wells and the top of the plate is dry.

HYBRID DETECTION

Notes:

- Make additions across the plate in a left-to-right direction using an 8-channel pipettor.
- It is recommended that the reverse pipetting technique be utilized to improve consistency of reagent delivery. With this technique, the pipette tips are initially over-filled by using the second stop on the pipettor's aspirate/dispense control (plunger). See procedure below. Wipe tips on reagent reservoir or on a clean low-lint paper towel to remove excess reagent before delivery to plate.
- If desired, the pipettor may be steadied by resting the middle of the pipette tips on the top edge of the microwells. Take care not to touch the sides of the microwells or cross-contamination of specimens could occur. Refer to Diagram 1 shown earlier.

1. Aliquot the appropriate volume of Detection Reagent 1 into a reagent reservoir (see *Reagent Preparation and Storage section* for instructions). Carefully pipette 75 µl of Detection Reagent 1 into each well of the Capture Microplate using an 8-channel pipettor and the reverse pipetting technique, described below.

   Reverse Pipetting Technique:
   a) Attach tips to an 8-channel pipettor; ensure all tips are firmly seated.
   b) Push the plunger of the pipettor past the first stop to the second stop.
   c) Immerse tips into the Detection Reagent 1 solution.
   d) Release plunger slowly and allow solution to fill the tips.
   e) Dispense solution into microwells (75 µl) by pressing the plunger to the first stop. Do not release plunger until pipette tips have been re-immersed into the Detection Reagent 1 solution.

f) Refill tips and repeat until all wells are filled. Fill wells of microplate from left to right. *Verify that all wells have been filled accurately by observing the intensity of the pink color. All wells should have similar intensity.*

2. Cover plate with plate lid and incubate at 20-25°C for 30-45 minutes.

WASHING

Wash the capture plate using one of the two methods below.

AUTOMATED PLATE WASHER METHOD

Note: Always keep the Automated Plate Washer on. Ensure that the rinse reservoir is filled and the waste reservoir is empty. The Automated Plate Washer will routinely rinse the system for cleaning. See the *Automated Plate Washer User Manual* for further instructions, as needed.

BEFORE EACH USE:

- Verify that the Wash Reservoir is filled at least to the 1 L mark with Wash Buffer Solution. If not prepare the Wash Buffer solution. See *Reagent Preparation and Storage* section.
- Verify the rinse reservoir is filled with distilled or deionized water.
- Verify that the waste reservoir is empty and the cap is securely fastened.
- The Automated Plate Washer will automatically prime itself before each wash and rinse after each wash.

1. Remove plate lid and place plate on Automated Plate Washer platform.
2. Verify that the power is on and that the display reads “Digene Wash Ready” or “P1.”

   Note: If only a partial strip of capture wells is being used, empty microplate wells will need to be placed in capture plate to complete the column prior to washing. See *Accessories* section for ordering information.

3. Select the number of strips to be washed by pressing the “Rows” key and then “+” or “-” to adjust. Press “Rows” key to return to “Digene Wash Ready” or “P1.”
4. Press “Start/Stop” to begin.
5. The Automated Plate Washer will perform six fill-and-aspirate cycles taking approximately 10 minutes. There will be a brief pause during the program so be sure not to remove the plate prematurely. When the Automated Plate Washer is finished washing, it will read “Digene Wash Ready” or “P1.”
6. Remove the microplate from the washer when the program is finished. Plate should appear white, and no residual pink liquid should remain in the microwells.

MANUAL WASHING METHOD

Note: Inadequate washing may cause increased background and false-positive results (due to residual alkaline phosphatase). To ensure efficient washing using the Wash Apparatus, Wash Apparatus should be placed at least 61 cm and not more than 91 cm above the wash area such that the plate will be between 61 cm and 91 cm below the Wash Apparatus when being washed. The stop-cock of the Wash Apparatus should be turned to the full “open” position when in use and placed in the “off” position when not in use. During use, the Wash Apparatus must contain at least 1.0 L of Wash Buffer to ensure adequate pressure.

1. Remove Detection Reagent 1 from the wells by placing clean Kimtowels Wipers or equivalent low-lint paper towels on top of the plate and carefully inverting. Before inverting, ensure that the paper is in contact with the entire surface area of the plate. Allow the plate to drain for 1-2 minutes. Blot well on clean Kimtowels Wipers or equivalent low-lint paper towels. Carefully discard the used low-lint paper towels to avoid alkaline phosphatase contamination of later steps.
2. Using the Wash Apparatus, hand-wash the plate 6 times. Each well is washed to overflow to remove conjugate from the tops of the wells. Washing begins at well A1 and continues in a serpentine fashion to the right and downward. After all wells have been filled, decant liquid into sink with a strong

downward motion. The second wash is started at well H12 moving in a serpentine motion to the left and upward. This sequence of 2 washes is repeated 2 more times for a total of 6 washes per well.

3. After washing, blot the plate by inverting on clean Kimtowels Wipers or equivalent low-lint paper towels and tapping firmly 3-4 times. Replace the low-lint paper towels and blot again. Leave plate inverted and allow to drain for 5 minutes. Blot the plate one more time.
4. Plate should appear white, and no pink residual liquid should remain in the microwells.

SIGNAL AMPLIFICATION

Notes:

- Use a new pair of powder-free gloves for handling Detection Reagent 2.
- Aliquot **only** the amount of reagent required to perform the assay into the reagent reservoir in order to avoid contamination of Detection Reagent 2. See *Reagent Preparation and Storage* section. **DO NOT return Detection Reagent 2 to the original bottle. Discard unused material after use.**
- Detection Reagent 2 addition should be made without interruption. The incubation time of all wells must be as close as possible.
- Take care not to touch the sides of the microwell or splash reagent back onto tips because cross-contamination of specimens could occur (See Diagram 1).

1. Carefully pipette 75 µl of Detection Reagent 2 into each well of the Capture Microplate using an 8-channel pipettor and the reverse pipetting technique as previously described. *All microwells should turn a yellow color.* Verify that all wells have been filled accurately by observing the intensity of the color. All wells should have similar intensity.
2. Cover the microplate with a plate lid or clean Parafilm (or equivalent), and incubate at 20-25°C for 15 minutes. Avoid direct sunlight.
3. Read the microplate on a QIAGEN-approved luminometer after 15 minutes of incubation (and no later than 30 minutes of incubation).
4. The *digene* assay analysis software will allow the entry of pertinent assay information directly into the software.
5. If a full microplate was not used, remove used microwells from the microplate holder, rinse the holder thoroughly with deionized water, dry and reserve for next assay.

ASSAY CALIBRATION VERIFICATION CRITERIA

Assay Calibration Verification is performed to ensure that the reagents and furnished Calibrator and Quality Control material are functioning properly, permitting accurate determination of the assay cutoff value. The Verification Criteria are automatically calculated and verified valid or invalid by the *digene* assay analysis software. The *digene* HC2 GC-ID DNA Test requires calibration with each assay. Therefore, it is necessary to verify each assay using the following criteria. This verification procedure is not intended as a substitute for internal quality control testing.

1. Negative Calibrator
   The Negative Calibrator must be tested in triplicate with each assay. The mean RLU value of the Negative Calibrator must be ≥ 10 and ≤ 150 RLUs in order to proceed. The coefficient of variation (%CV) for the Negative Calibrator replicates should be ≤ 25%. If the %CV is > 25%, the software will discard the replicate with the RLU value farthest from the mean as an outlier and recalculate the mean and %CV using the remaining two replicates. The recalculated %CV should be ≤ 25%; otherwise, **the assay calibration verification is invalid and the assay must be repeated for all patient specimens. Accordingly, patient specimen results should not be reported.**

2. Positive Calibrator
   The Positive Calibrator must be tested in triplicate with each assay. The %CV for the Positive Calibrator replicates should be ≤ 20%. If the %CV is > 20%, the software will discard the replicate with the RLU value farthest from the mean as an outlier and recalculate the mean and %CV using the remaining two replicates. The recalculated %CV should be ≤ 20%; otherwise, **the assay calibration verification is invalid, and the assay must be repeated for all patient specimens. Accordingly, patient specimen results should not be reported.**

3. Mean PC/Mean NC Ratio
   The mean of the Positive Calibrator replicates (mean PC) and the mean of the Negative Calibrator replicates (mean NC) are used to calculate the mean PC/mean NC ratio. The software will calculate the mean PC/mean NC ratio. This ratio must meet the following criteria to verify the assay calibration **before the specimen results can be interpreted**. If the ratio is ≥ 2.0 and ≤ 20, the software will proceed to the cutoff calculation. If the ratio is < 2.0 or > 20, **the assay calibration verification is invalid, and the assay must be repeated for all patient specimens. Accordingly, patient specimen results should not be reported.**

Note: To determine the reproducibility of the Calibrators for the *digene* HC2 GC-ID DNA Test, the results generated with the *digene* Microplate Luminometer 2000 (DML 2000) during internal studies involving 62 assays performed using the Rapid Capture System Application and 43 assays performed using the manual method were compiled (Table 3). The results showed that the average of the %CV for the Positive Calibrator for these 105 assays was equal to or lower than 6.5% and the average of the %CV for the Negative Calibrator was equal to or lower than 14.6%. As indicated by the mean Negative Calibrator mean RLU value of 43 obtained for the manual assays, compared to the RCS Application mean of 54, the RCS application has been shown to yield NC RLU values that are shifted slightly upwards relative to the manual method. This shift has been shown to have no effect on the test results generated using either optional method. The mean RLU threshold for the Negative Calibrator has been defined as 250 RLUs based on a statistical calculation of ±3SD of the mean RLU value for the Negative Calibrator observed for the *digene* HC2 CT/GC DNA Test system observed during extensive testing that took place during the development of the RCS Application. The upper end of that ±3SD range was extended an additional 20% to ensure that the NC RLU threshold can be achieved in routine clinical practice.

The mean RLU value of the NC should routinely be observed at ≤ 150 and the CV ≤ 25%. Each laboratory should monitor quality control and calibration performance according to the National Committee for Clinical Laboratory Standards (NCCLS) document C24-2A. The mean RLU using the RCS Application may occasionally exceed 150, possibly with a corresponding decrease in the PC/NC, which, according to Table 3, has been shown to yield an average value upon calibration of 8.29. In this case, results are acceptable provided the NC RLU remains ≤ 250 and the PC/NC ratio is ≥ 2.0. Should the NC RLU exceed 250 or the PC/NC fall below 2.0 or be greater than 20, the assay is invalid.

Table 3. Statistical Summary of Negative Calibrator and Positive Calibrator Values for the RCS Application and Manual Method Assays.

Method	No. of Plates	PC/NC Calculated Means				Test Kit Quality Controls (Mean RLU/CO)	
		Mean	Median	Min	Max	QC CT	QC GC
RCS	62	8.29	8.99	3.95	12.72	0.22	4.73
Manual	43	8.22	8.83	2.59	12.88	0.23	4.07

Method	Calibrator	RLU Calculated Means				Mean of the Calculated %CV
		Mean	Median	Min	Max	
RCS	Negative	54	46	24	127	14.4
	Positive	399	405	179	606	6.5
Manual	Negative	43	36	16	120	14.6
	Positive	295	309	167	415	4.7

CUTOFF CALCULATION

Once an assay has been verified according to the criteria stated above, the valid Positive Calibrator replicates will be utilized to establish the Cutoff RLU values for determining positive specimens. The Cutoff RLU values are calculated as follows:

Cutoff RLU Value = mean Positive Calibrator RLU

Example Cutoff Calculation:

	NC RLU Values	PC RLU Values
	97	312
	101	335
	91	307
Mean Value	96	318
%CV	4.9	4.7
Mean PC/Mean NC	N/A	3.31

Therefore, Cutoff RLU value is (mean PC) = 318

All specimen RLU values will be converted into a ratio to the appropriate Cutoff (CO) RLU Value by the *digene* assay analysis software. For example, all assays should be expressed as Specimen RLU/CO.

Note: RLU/CO values and positive/negative results for all specimens tested are reported in the *digene* assay analysis software data analysis report.

QUALITY CONTROL

Quality control samples are supplied with the *digene* HC2 GC-ID DNA Test. Consult the applicable *digene* assay analysis software user manual for instructions on how to input the Lot Numbers and expiration dates for the Quality Controls. These controls must be included in each assay, and the RLU/CO of each Quality Control must fall within the following acceptable ranges for the assay to be considered valid. **If the Quality Controls do not fall within these ranges, the assay is invalid and must be repeated.** Accordingly, no patient results should be reported for any invalid assay.

	QC CT	QC GC
Minimum RLU/CO	0	1.0
Maximum RLU/CO	0.9999	20.00
Maximum %CV	20.00	20.00

1. The Quality Controls provided in the kit are cloned CT and GC DNA targets, composed of the same plasmid construct for each individual organism (one for CT and one for GC), as is the Positive Calibrator provided with the *digene* HC2 GC-ID DNA Test.
2. This quality control material is not the same as GC organism in the sample matrix and will not act as an appropriate quality control for the *digene* Specimen Transport Medium or PreservCyt Solution.
3. The Positive Calibrator is used to normalize specimen results by establishing the cutoff RLU. The quality controls provided with this kit must be used for internal quality control. Additional quality controls may be tested according to guidelines or requirements of local, state, and/or country regulations or accrediting organizations.
4. To test the effectiveness of specimen lysis and denaturation, laboratories should, on a periodic basis, create specimen preparation controls by adding ≥ 5000 CFU/ml of *Neisseria gonorrhoeae* (auxotype 1, 5 or Type strain from ATCC) to a fresh tube of STM. Incubate the specimen for at least 1 hour at room temperature prior to testing in the same manner as a clinical specimen. An RLU/CO ≥ 2.50 should be obtained if the specimen is processed properly. Alternatively, commercially available specimen test panels containing GC organism can also be used for this purpose.
5. Acceptable ranges for the Calibrators and Quality Controls have been established only for QIAGEN-approved luminometers. The Negative Calibrator and Quality Controls monitor for substantial reagent failure and will not ensure precision of the assay.

INTERPRETATION OF SPECIMEN RESULTS

By the criteria of the *digene* HC2 GC-ID DNA Test:

1. Specimens with RLU/CO ratios of ≥ 2.50 are considered "Positive for *Neisseria gonorrhoeae* DNA." Organism viability and/or infectivity cannot be inferred because target DNA may persist in the absence of viable organisms.
2. Specimens with RLU/CO ratios < 1.00 do not contain *Neisseria gonorrhoeae* DNA or contain DNA below the assay's detection limit. These should be interpreted as "No *Neisseria gonorrhoeae* DNA detected." A negative result does not preclude *Neisseria gonorrhoeae* infection because results depend on adequate specimen collection and sufficient DNA to be detected.
3. Specimens with RLU/CO ratios of ≥ 1.00 and < 2.50 are considered equivocal. Results may be considered presumptively positive for *Neisseria gonorrhoeae* DNA. However, repeat testing of a new specimen from the patient or additional testing by an alternate test procedure is recommended due to the reduced predictive value of a positive result with these RLU/Cutoff values.*
4. It is recommended that positive results be confirmed by another method if the likelihood of *Neisseria* gonorrhoeae infection is uncertain or questioned when considering clinical or other laboratory findings. Analytical studies with this test have shown limited cross-reactivity to certain other DNA sequences that may cause a false-positive result. See *Analytical Specificity* for additional information.

* During the clinical evaluation of the *digene* HC2 GC-ID DNA Test, 3/17 results in this equivocal range were confirmed positive by GC culture testing; the remaining 14 were apparent false positives. In a subsequent evaluation, 5 specimens were observed with an initial RLU/CO between 1.00 and 2.50, three of which were GC culture positive. Repeat duplicate testing of these three specimens with the *digene* HC2 GC-ID DNA Test yielded results ≥1.00 RLU/CO. The remaining 2 specimens were negative by culture and both were also negative when repeated twice with the *digene* HC2 GC-ID DNA Test.

LIMITATIONS OF THE PROCEDURE

Refer to the *Rapid Capture System User Manual* for additional Limitations of the Procedure specific to the use of that system for high volume sample-throughput testing.

- For *In Vitro* diagnostic use only.
- The *digene* HC2 GC-ID DNA Test Procedure, Quality Control and Interpretation of Specimen Results must be followed closely to obtain reliable test results.
- The *digene* HC2 GC-ID DNA Test can only be used with cervical specimens collected using the *digene* HC2 DNA Collection Device and placed in STM, with cervical specimens collected with the *digene* Female Swab Specimen Collection Kit and placed in STM, or with specimens collected using a broom-type collection device and place in Hologic PreservCyt Solution.
- Results of this test should be interpreted only in conjunction with information available from clinical evaluation of the patient and from other procedures.
- The *digene* HC2 GC-ID DNA Test provides qualitative results. The numeric value (ratio) above the cutoff value determined for the patient specimen has not been demonstrated to correlate to the amount of GC DNA present in the patient specimen.
- A negative result does not exclude the possibility of *Neisseria gonorrhoeae* infection because detection is dependent on the number of organisms present in the specimen and may be affected by specimen collection methods, patient factors, stage of infection and/or infecting *Neisseria gonorrhoeae* strain.
- The *digene* HC2 GC-ID DNA Test is not intended to determine therapeutic success.
- The *digene* HC2 GC-ID DNA Test has only been validated for use with the Automated Plate Washer using the settings specified in the assay instructions. This validation study was conducted in-house and the data to support its use are on file at QIAGEN. Other plate washers or other plate washer settings are not acceptable for use with the *digene* HC2 GC-ID DNA Test.
- In order to minimize variability of the results obtained with the *digene* HC2 GC-ID DNA Test, it is necessary that laboratory personnel performing the assay achieve an acceptable level of technical proficiency. Each laboratory must also monitor technical proficiency with the assay. To accomplish this, it is suggested that commercially available specimen test panels containing GC organism or GC DNA be tested periodically, consistent with the establishments' Quality Procedures.

EXPECTED RESULTS

PREVALENCE

The prevalence of specimens positive for *Neisseria gonorrhoeae* varies depending on population characteristics such as age, sex, and risk factors. The prevalence of *Neisseria gonorrhoeae* observed in the clinical study population using the *digene* HC2 GC-ID DNA Test ranged from 1.1% to 13.0%. The prevalence was calculated assuming that the 17 specimens with equivocal results in the study were positive for GC DNA (Table 4). Eight of these 17 specimens were confirmed positive by GC culture or Polymerase Chain Reaction (PCR).

Table 4. Prevalence of *digene* HC2 GC-ID DNA Test Positive Results by Test Site.

Test Site	No. Positive/No. Tested	% Prevalence
1	60/460	13.0
2	34/302	11.3
3	23/324	7.1
4	10/390	2.6
5	4/349	1.1
Total	131/1825	7.2

POSITIVE AND NEGATIVE PREDICTIVE VALUES

The hypothetical positive and negative predictive values (PPV and NPV) for different prevalence rates using the *digene* HC2 GC-ID DNA Test were calculated using the overall sensitivity and specificity determined individually for specimens collected with the *digene* HC2 DNA Collection Device (cervical brush) and for specimens collected with the *digene* Female Swab Specimen Collection Kit (swab). Table 5 represents the hypothetical PPV and NPV for brush specimens (overall sensitivity 92.6% and specificity 98. 5%) and Table 6 represents the hypothetical PPV and NPV for swab specimens (overall sensitivity 93.0% and specificity 98.8%).

Table 5. *digene* HC2 GC-ID DNA Test Hypothetical Predictive Values at Different Prevalence Rates (Brush).

Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
5	92.6	98.5	76.5	99.6
10	92.6	98.5	87.3	99.2
15	92.6	98.5	91.6	98.7
20	92.6	98.5	76.3	99.60

Table 6. *digene* HC2 GC-ID Test Hypothetical Predictive Values at Different Prevalence Rates (Swab).

Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
5	93.0	98.8	79.8	99.7
10	93.0	98.8	88.3	99.4
15	93.0	98.8	91.6	99.1
20	93.0	98.8	93.3	98.7

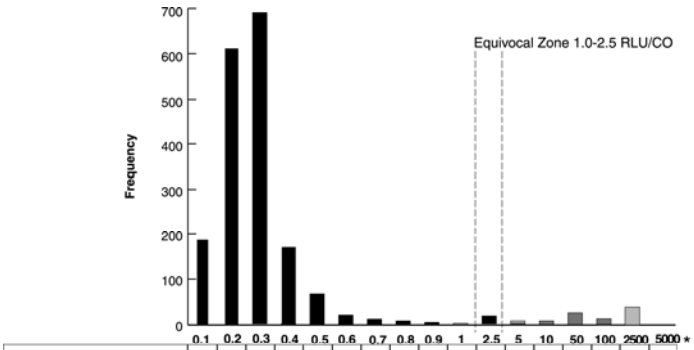
FREQUENCY DISTRIBUTION: *digene* HC2 GC-ID DNA TEST RLU/CO RESULTS

The distribution of the *digene* HC2 GC-ID DNA Test RLU/CO ratios observed during the multicenter clinical study is indicated below (Figure 1). These data include all specimens for which the *digene* HC2 GC-ID DNA Test was performed and GC culture results were available (n=1826). Interpretation of results was performed according to the following criteria. Specimens with RLU/CO values < 1.00 were considered negative. Specimens with RLU/CO values ≥ 2.50 were considered positive. Specimens with RLU/CO values ≥ 1.00 and < 2.50 were considered equivocal.

Distinct separation of the RLU/CO ratios is observed between *digene* HC2 GC-ID DNA Test-positive results and *digene* HC2 GC-ID DNA Test-negative results. Ninety-nine percent (1676/1690) of the *digene* HC2 GC-ID DNA Test-negative results have RLU/CO Values between an RLU/CO value of 0.0 and 0.5. Five (5/1690) of the *digene* HC2 GC-ID DNA Test-negative results yielded an RLU/CO between 0.6 and 0.8. Overall, less than one percent (< 0.9%, 17/1825) of the specimen results fall in the assay's equivocal

zone, 47% (8/17) of which were positive by GC Culture or PCR. Eighty-nine percent (93/104) of the *digene* HC2 GC-ID DNA Test-positive results have RLU/CO Values between an RLU/CO of 10-2500.

Figure 1. Frequency Distribution of the *digene* HC2 GC-ID DNA Test RLU/CO Results.



RLU/CO	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1	2.5	5	10	50	100	2500	5000 *
GC Culture Positive	0	3	1	0	2	1	1	0	0	0	3	8	7	33	15	41	0
GC Culture Negative	173	608	698	162	28	8	2	2	4	2	12	3	4	2	2	0	0

*Indicates the upper end of the range, inclusive of the value stated.

PERFORMANCE CHARACTERISTICS

CLINICAL TRIAL RESULTS BY SPECIMEN

digene HC2 GC-ID DNA Test performance characteristics were determined by comparing the assay results to results of Gonorrhea culture. One-thousand eight-hundred twenty-five (1825) specimens were tested from patients at 5 different sites including STD, Family Planning and OB/GYN clinics. PCR testing was performed for specimens that were *digene* HC2 GC-ID DNA Test-positive/culture-negative. *digene* HC2 GC-ID DNA Test results were NOT resolved by PCR test results, and, therefore, PCR had no impact on the calculations of the *digene* HC2 GC-ID DNA Test performance characteristics. Results from the clinical trial for specimens collected with the *digene* HC2 DNA Collection Device (cervical brush) are shown in Table 7 and specimens collected with the *digene* Female Swab Specimen Collection Kit (swab) in Table 8.

The performance characteristics of the *digene* HC2 GC-ID DNA Test were calculated applying both a 1.0 and a 2.5 cutoff without consideration of the presumptive-positive specimens falling in the equivocal zone described in the *Interpretation of Results* section of these instructions for use. Therefore, the performance of the *digene* HC2 GC-ID DNA Test may vary in your laboratory depending on the distribution of values that fall within the equivocal zone and the repeat results obtained when retesting presumptive positive (equivocal zone) specimens is performed. As a point of reference, less than 0.9% of the specimens (17/1825) tested during the Multicenter clinical study used to establish the *digene* HC2 GC-ID DNA Test's performance fell into this range. See the *Frequency Distribution of RLU/CO* results in the *Expected Results* section of these instructions for use for additional information.

Sufficient data have not been generated to accurately determine whether the sensitivity and positive predictive value of the *digene* HC2 GC-ID DNA Test using the *digene* Female Swab Specimen Collection Kit is equivalent to the sensitivity and positive predictive value observed with specimens collected using the *digene* HC2 DNA Collection Device. Because the use of the *digene* HC2 DNA Collection Device is contraindicated in the collection of cervical specimens from pregnant women, the ability of the test to detect the presence of GC DNA may be reduced in this population of patients or whenever a swab is used for specimen collection.
Performance estimates for the assay are based on specimens stored at 2-8°C or frozen and tested within 1-2 weeks of collection.

The clinical sensitivity and specificity of *digene* HC2 GC-ID DNA Test for detecting those patients with clinically active infection that can be transmitted to partners or cause GC-related sequelae has not been determined in comparison to all commercially-available Nucleic Acid Amplification (NAA) methods for

detection of GC DNA. In clinical studies, testing by a modified commercial NAA assay showed positivity in some *digene* HC2 GC-ID DNA Test-positive specimens obtained from culture negative patients. Estimated sensitivity is based on the number of *digene* HC2 GC-ID DNA Test-positive results found in patients who were culture positive for *Neisseria gonorrhoeae*. Therefore, the *digene* HC2 GC-ID DNA Test sensitivity can only be deduced relative to culture positivity that may have a sensitivity of 60-85%.

Table 7. *digene* HC2 GC-ID DNA Test Versus GC Culture Results for Brush Specimens. Performance characteristics calculated utilizing RLU/CO cutoff values of 1.0 and 2.5 are presented below; values stated parenthetically represent the performance considering the 2.5 RLU/CO Cutoff. The 95% Confidence Intervals are inclusive of both ranges when the point estimates differed at each of the RLU/CO cutoff values evaluated.

	Site[2]	*digene* HC2 GC-ID: Culture: n=	POS POS	POS NEG	NEG POS	NEG NEG	Sensitivity	PPV	Specificity	NPV	*digene* HC2 GC-ID+ Culture-PCR[1]+
Symptomatic											
	1	351	39 (38)	7 (3)	1 (2)	304 (308)	97.50 (95.00)	84.78 (92.68)	97.75 (99.04)	99.67 (99.35)	5/7 (2/3)
95% CI							83.1-99.9	80.1-98.5	97.2-99.8	98.2-100	
	2	188	13	2	4	169	76.47	86.67	98.83	97.69	1/2
95% CI							50.1-93.2	59.5-98.3	95.8-99.9	94.2-99.4	
	3	233	14	6 (3)	1	212 (215)	93.33	70.00 (82.35)	97.25 (98.62)	99.54	0[3]/6
95% CI							68.1-99.8	56.6-96.2	96.0-99.7	97.4-100	
	4	163	4	0	0	159	100.00	100.00	100.00	100.00	N/A
95% CI							39.8-100	39.8-100	97.7-100	97.7-100	
	All	**935**	**70 (69)**	**15 (8)**	**6 (7)**	**844 (851)**	**92.11 (90.79)**	**82.35 (89.61)**	**98.25 (99.07)**	**99.29 (99.18)**	**6[3]/15**
95% CI							**83.6-97.1**	**80.1-95.4**	**98.2-99.6**	**98.5-99.7**	
Asymptomatic											
	1	101	10 (9)	2	0 (1)	89	100.00 (90.00)	83.33 (81.82)	97.80	100.00 (98.89)	2/2
95% CI							69.2-100	51.6-97.9	92.3-99.7	95.9-100	
	2	12	2	0	0	10	100.00	100.00	100.00	100.00	N/A
95% CI							15.8-100	15.8-100	69.2-100	69.2-100	
	3	84	1 (0)	0	0 (1)	83	100.00 (0.00)	100.00	100.00	100.00 (98.81)	N/A
95% CI							2.5-100	2.5-100	95.7-100	95.7-100	
	4	226	4	2 (0)	1	219 (221)	80.00	66.67 (100.00)	99.10 (100.00)	99.55	1/2
95% CI							28.4-99.5	39.8-100	98.3-100	97.5-100	(N/A)
	5	1	0	0	0	1	N/A	N/A	100.00	100.0	N/A
95% CI									2.5-100	2.5-100	
	All	**424**	**17 (15)**	**4 (2)**	**1 (3)**	**402 (404)**	**94.44 (83.33)**	**80.95 (88.24)**	**99.01 (99.51)**	**99.75 (99.26)**	**3/4 (2/2)**
95% CI							**72.7-99.9**	**63.6-98.5**	**98.2-99.9**	**98.6-100**	
ALL											
	1	452	49 (47)	9 (5)	1 (3)	393 (397)	98.00 (94.00)	84.48 (90.38)	97.76 (98.76)	99.75 (99.25)	7/9 (4/5)
95% CI							89.4-100	79.0-96.8	97.1-99.6	98.6-100	
	2	200	15	2	4	179	78.95	88.24	98.90	97.81	1/2
95% CI							54.4-94.0	63.6-98.5	96.1-99.9	94.5-99.4	
	3	317	15 (14)	6 (3)	1 (2)	295 (298)	93.75 (87.50)	71.43 (82.35)	98.01 (99.00)	99.66 (99.33)	0[3]/6
95% CI							69.8-99.8	56.6-96.2	97.1-99.8	98.1-100	
	4	389	8	2 (0)	1	378 (380)	88.89	80.00 (100.00)	99.47 (100.00)	99.74	1/2 (N/A)
95% CI							51.8-99.7	63.1-100	99.0-100	98.5-100	
	5	1	0	0	0	1	N/A	N/A	100.00	100.00	N/A
95% CI									2.5-100	2.5-100	
	All	**1359**	**87 (84)**	**19 (10)**	**7 (10)**	**1246 (1255)**	**92.55 (89.36)**	**82.08 (89.36)**	**98.50 (99.21)**	**99.44 (99.21)**	**9[3]/19**
95% CI							**85.3-97.0**	**81.3-94.8**	**98.6-99.6**	**98.9-99.8**	

1 This information is provided for information only; specimen results were not resolved using PCR.

2 Site number 5 did not have any brush specimens from symptomatic patients.

3 In two cases PCR was not done.

N/A = Not Applicable

Table 8. *digene* HC2 GC-ID DNA Test versus GC Culture Results for Swab Specimens. Performance characteristics calculated utilizing RLU/CO cutoff values of 1.0 and 2.5 are presented below. Values stated parenthetically represent the performance considering the 2.5 RLU/CO Cutoff. The 95% Confidence Intervals are inclusive of both ranges when the point estimates differed at each of the RLU/CO cutoff values evaluated.

	Site[2]	*digene* HC2 GC-ID: Culture: n=	POS POS	POS NEG	NEG POS	NEG NEG	Sensitivity	PPV	Specificity	NPV	*digene* HC2 GC-ID+ Culture-PCR[1]+
Symptomatic											
	1	354	34 (31)	2 (3)	2 (5)	316 (315)	94.44 (87.18)	94.44 (91.18)	99.37 (99.06)	99.37 (98.44)	N/A
95% CI							81.34-99.32	81.34-99.32	97.75-99.92	97.75-99.92	
	2	92	13	2 (0)	1	76 (78)	92.86	86.67 (100)	97.44 (100)	98.70 (98.73)	0/2
95% CI							66.1-99.8	75.3-100	95.4-100	93.2-100	
	3	5	2	0	0	3	100	100	100	100	N/A
95% CI							15.8-100	15.8-100	29.2-100	29.2-100	
	5	162	0	3 (1)	0	159 (161)	N/A	0.00	98.15 (99.38)	100	1[3]/3
95% CI								2.5-100	96.6-100	97.7-100	
	All	**613**	**49 (46)**	**7 (4)**	**3 (6)**	**554 (557)**	**94.23 (88.46)**	**87.50 (92.00)**	**98.75 (99.29)**	**99.46 (98.93)**	**1[3]/5**
95% CI							**84.05-98.79**	**75.93-94.82**	**97.45-99.50**	**98.43-99.89**	
Asymptomatic											
	1	61	1	0	1	59	50.00	100	100	98.33	N/A
95% CI							1.26-98.74	2.50-100	93.94-100	91.06-99.96	
	2	10	2	0	0	8	100	100	100	100	N/A
95% CI							15.8-100	15.8-100	63.1-100	63.1-100	
	3	2	0	0	0	2	N/A	N/A	100	100	N/A
95% CI									15.8-100	15.8-100	
	4	1	0	0	0	1	N/A	N/A	100	100	N/A
95% CI									2.5-100	2.5-100	
	5	186	1	0	0	185	100	100	100	100	N/A
95% CI							2.5-100	2.5-100	98.0-100	98.0-100	
	All	**260**	**4**	**0**	**1**	**255**	**80.00**	**100**	**100**	**99.61**	**N/A**
95% CI							**28.36-99.49**	**39.76-100**	**98.56-100**	**97.84-99.99**	
ALL											
	1	415	35 (32)	5 (3)	3 (6)	372 (374)	92.11 (84.21)	87.50 (91.43)	98.67 (99.20)	99.20 (98.42)	N/A
95% CI							78.62-98.34	73.20-95.81	96.93-99.57	97.68-99.83	
	2	102	15	2 (0)	1	84 (86)	93.75	88.24 (100)	97.67 (100)	98.82 (98.85)	0/2
95% CI							69.8-99.8	63.6-100	91.9-100	93.6-100	
	3	7	2	0	0	5	100	100	100	100	N/A
95% CI							15.8-100	15.8-100	47.8-100	47.8-100	
	4	1	0	0	0	1	N/A	N/A	100	100	N/A
95% CI									2.5-100	2.5-100	
	5	348	1	3 (1)	0	344 (346)	100	25.00 (50.00)	99.14 (99.71)	100	1[3]/3
95% CI							2.5-100	1.3-98.7	98.4-100	98.9-100	
	All	**873**	**53 (50)**	**10 (4)**	**4 (7)**	**806 (812)**	**92.98 (87.72)**	**84.13 (92.59)**	**98.77 (99.51)**	**99.51 (92.59)**	**1[3]/5**
95% CI							**83.00-98.05**	**72.74-92.12**	**97.76-99.41**	**98.74-99.87**	

1 This information is provided for information only; specimen results were not resolved using PCR.
2 Site number 4 did not have any swab specimens from symptomatic patients.
3 In two cases PCR was not done.
N/A = Not Applicable

REPRODUCIBILITY

As part of the Multicenter Clinical Trial, a reproducibility study was performed to determine the assay-to-assay, day-to-day, site-to-site and total reproducibility of the *digene* HC2 GC-ID DNA Test using a panel composed of *Neisseria gonorrhoeae* DNA targets and *digene* HC2 GC-ID DNA Test-positive and *digene* HC2 GC-ID DNA Test-negative clinical specimens.

A 10-member panel of masked, denatured clinical and non-clinical specimens, consisting of 8 positive specimens and 2 negative specimens, was tested in replicates of six, twice per day over a three-day period at each of four sites (3 external sites and QIAGEN). Each site generated 36 data points for every target tested. All specimens were denatured and stored frozen prior to testing. 100% agreement was observed for the 1152 expected positive results (1152/1152) and 100% agreement was observed for the 288 expected negative results (288/288). Overall agreement was 100% (1440/1440), with a 95% confidence interval of 99.7-100 and kappa = 1.00. There was no significant assay-to-assay, day-to-day or site-to-site variability observed; therefore, the data from all assays at each site were combined and are presented below (Table 9).

Table 9. Reproducibility of the *digene* HC2 GC-ID DNA Test in a Multicenter Trial.

	Site 1		Site 2		Site 3		Site 4		Total		
Target Number	$\bar{X}$RLU /CO	% Agree	$\bar{X}$RLU /CO	% Agree	$\bar{X}$RLU /CO	% Agree	$\bar{X}$RLU /CO	% Agree	$\bar{X}$RLU /CO	Observed/ Expected	% Agree
1	2.5	100	2.1	100	2.7	100	2.6	100	2.5	144/144	100
2	4.8	100	4.2	100	5.0	100	5.2	100	4.8	144/144	100
3	29.4	100	23.3	100	30.1	100	30.4	100	28.3	144/144	100
4	51.5	100	43.0	100	52.1	100	54.1	100	50.2	144/144	100
5	2.5	100	2.0	100	2.5	100	2.5	100	2.4	144/144	100
6	4.7	100	3.5	100	4.9	100	4.8	100	4.5	144/144	100
7	14.0	100	10.6	100	13.9	100	14.1	100	13.2	144/144	100
8	16.7	100	12.7	100	17.4	100	18.2	100	16.3	144/144	100
9	0.2	100	0.2	100	0.2	100	0.2	100	0.2	144/144	100
10	0.2	100	0.2	100	0.2	100	0.2	100	0.2	144/144	100
TOTAL										1440/1440	100

A second proficiency/reproducibility study using whole *Neisseria gonorrhoeae* (GC) organism spiked into a mock clinical specimen matrix of epithelial cells was conducted at three external sites. The 25 specimens tested contained representatives of negative, low (at or near the limit of detection), and medium positives with 2 GC strains, mixed infections with *Chlamydia trachomatis* (CT) and specimens that contained blood. Twelve specimens were expected to be positive and thirteen specimens were expected to be negative. The percent agreement between observed and expected results of the *digene* HC2 GC-ID DNA Test at the three individual test sites and for all sites combined are shown in Table 10. Sensitivity, specificity, agreement and kappa values for each site are included in Table 11.

Table 10. *digene* HC2 GC-ID DNA Test % Agreement by Site.

Site	Observed vs. Expected	% Agreement*
1	25/25	100% (86.28%-100%)
2	25/25	100% (86.28%-100%)
3	25/25	100% (86.28%-100%)
Combine Sites	75/75	100% (95.20%-100%)

* Numbers in parentheses indicate 95% confidence intervals.

Table 11. Results of the *digene* HC2 GC-ID DNA Test Summary Statistics (Cutoff of 1.0).

Statistical Measure	Site 1	Site 2	Site 3	Over all
Sensitivity	100% (73.54%-100%)*	100% (73.54%-100%)	100% (73.54%-100%)	100% (90.26%-100%)
Specificity	100% (75.29%-100%)	100% (75.29%-100%)	100% (75.29%-100%)	100% (90.97%-100%)
Agreement	100% (86.28%-100%)	100% (86.28%-100%)	100% (86.28%-100%)	100% (95.20%-100%)
K	1.0	1.0	1.0	1.0

*Numbers in parentheses indicated 95% confidence intervals.

In routine proficiency testing, the 12 equivocal specimens presented in Table 10, all of which contained low concentrations of GC organism (~$5x10^4$ organisms/ml), would be interpreted according to the *Interpretation of Results* section of these instructions for use as presumptive positive. Therefore, the assay has demonstrated the ability to detect GC DNA in specimens with concentrations of organism detectable at or near the assay's limit of detection. Additional evidence of this was observed when testing an available panel that contained specimens with low numbers of organisms in a range intended to be detected by nucleic acid amplification assays. Testing at three external sites and at QIAGEN yielded 100% positive (or presumptive positive) results for the specimen in the panel containing GC organism. In two instances, the RLU/CO values fell into the assay's equivocal zone (see Table 12 below).

Table 12. CT and GC Specimen Panel Results.

		digene HC2 GC-ID DNA Test Result	
Site	RLU/CO	Interpretation	Expected Result
1	0.12	NEG	NEG
	10.45	POS	POS
	10.26	POS	POS
	9.74	POS	POS
	0.14	NEG	NEG
2	0.09	NEG	NEG
	9.31	POS	POS
	9.93	POS	POS
	9.69	POS	POS
	0.09	NEG	NEG
	0.11	NEG	NEG
	11.00	POS	POS
3	12.08	POS	POS
	9.45	POS	POS
	0.10	NEG	NEG
	0.07	NEG	NEG
	8.54	POS	POS
4	7.27	POS	POS
	8.09	POS	POS
	0.08	NEG	NEG

PRECISION

A precision study was performed at three sites to determine the within-assay and total precision of the *digene* HC2 GC-ID DNA Test using a panel of positive and negative masked, simulated clinical specimens. In addition, the intra- and inter-instrument precision observed with two separate luminometers was assessed using the same panel. The two luminometer models included the DML 2000, which is one of the luminometers recommended for use with the *digene* HC2 GC-ID DNA Test, and the MLX luminometer; the MLX luminometer was one of the luminometer models used during the clinical evaluation that is no longer available. One of the three sites experienced difficulties with other *digene* HC2 DNA Tests being performed as part of this study that were attributable to assay technique most likely caused by improper or inadequate training. Although the *digene* HC2 GC-ID DNA Test precision testing results were unaffected, the technician performing the testing was retrained in the proper assay technique.

Table 13 shows the performance of the *digene* HC2 GC-ID DNA Test for all sites combined (including the site that experienced technical problems prior to retraining the technician in the proper assay technique).

The assay demonstrated equivalent precision after technician retraining. However, for panel member 3 (which contained low concentrations of GC organism), the RLU/CO values observed were within or near the assay's equivocal zone of 1.0-2.5. For the purposes of these data analyses, all of those RLU/CO values that fell within the equivocal zone or exceeded 2.5 were interpreted as positive. Although not evident from this table, the qualitative results were 100% (54/54) (93.4%-100% 95%CI) in agreement with expected results at the three sites.

Table 13. Within-Instrument, Between-Instrument, Within-Assay, Total Precision Estimates For RLU/CO by Test and Target.

			Within Instrument		Between Instrument		Within Assay		Total	
Panel Member	n	Mean RLU/CO	Standard Deviation (SD)	(%CV)	(SD)	(%CV)	(SD)	(%CV)	(SD)	(%CV)
1	54	0.0974	0.0104	10.6818	0.0017	1.7328	0.0275	28.2556	0.0275	28.1978
2	54	0.0967	0.0111	11.5031	0.0015	1.5618	0.0338	34.9362	0.0342	35.4230
3	54	3.2335	0.1502	4.6462	0.0356	1.0997	0.3520	10.8869	0.3866	11.9551
4	54	3.8407	0.2078	5.4092	0.0525	1.3671	0.3401	8.8541	0.3487	9.0802
5	54	16.1676	1.0507	6.4986	0.1122	0.6940	2.1788	13.4766	2.1437	13.2589
6	54	18.0704	1.0539	5.8321	0.3456	1.9124	2.3701	13.1158	2.3316	12.9027

For the purposes of these data anaylses, all of those RLU/CO values that fell within the equivocal zone or exceeded 2.5 were interpreted as positive. An additional precision study was performed at QIAGEN to determine the total precision of the *digene* HC2 GC-ID DNA Test using the DML 2000. A six-member precision panel was prepared using a simulated clinical specimen matrix consisting of cultured epithelial cells suspended in *digene* Specimen Transport Medium (STM) and consisted of two negative specimens, two low-positive specimens and two mid-level-positive specimens, all containing a brush collection device. Each panel was tested in triplicate, two panels per plate, by two technicians over the course of 5 days. A freshly denatured panel was used per plate. The total precision results for the *digene* HC2 GC-ID DNA Test compiled for all five days of testing are presented in Table 14. Although not evident from these tables, the qualitative interpretation of results was 100% in agreement with the expected result (120/120; 96.97%-100% 95% CI), when using an RLU/CO of 1.0.

Table 14. Total Precision for *digene* HC2 GC-ID DNA Test.

Panel Member	n	Mean RLU/CO	SD	CV%	Mean -2xSD	Mean +2xSD
1	120	0.11	0.0361	32.28	0.04	0.18
2	120	0.11	0.0283	26.45	0.05	0.16
3	120	3.03	0.3212	10.62	2.38	3.67
4	120	4.06	0.4151	10.23	3.23	4.89
5	120	14.41	2.2239	15.44	9.96	18.85
6	120	13.34	1.7298	12.97	9.88	16.80

PRECISION WITH PRESERVCYT SPECIMENS

A multicenter study was conducted to characterize the laboratory-to-laboratory and day-to-day precision of the assay when testing PreservCyt Solution specimens. Two sites external to QIAGEN tested a twelve-member panel of simulated patient specimens collected in PreservCyt Solution. Each laboratory then tested the panel in triplicate, two times per day, over three days using the same manufactured lot of reagents. The twelve-member panel of simulated PreservCyt Solution specimens was prepared with varying amounts of GC (Auxotype 22; ATCC 27631) to create a panel as shown in Table 15.

Table 15. Simulated PreservCyt Solution Specimen Precision Panel for the *digene* HC2 GC-ID DNA Test.

Bulk Specimen	Panel Members*	Expected *digene* HC2 GC-ID DNA Test Result	Approximate RLU/CO
A	1P, 2P, 7P, 8P	Low GC-positive	~5
B	3P, 4P, 9P, 10P	Mid GC-positive	~10
C	5N, 11N	Negative	~0.20
D	6N, 12N	Negative	~0.20

*Specimen identifier indicates known *Neisseria gonorrhoeae* status [positive (P) or negative (N)]

For the purposes of data analysis, panel members derived from the same bulk specimen were combined.

Table 16. Qualitative Results by Bulk Specimen – *digene* HC2 GC-ID DNA Test Procedure.

Bulk Specimen Pool	GC Positive n (%)	Equivocal n (%)	Negative n (%)	Total
Negative (5N, 11N)	0 (0.0)	0 (0.0)	108 (100)	108
Negative (6N, 12N)	0 (0.0)	0 (0.0)	108 (100)	108
Total Negative	0 (0.0)	0 (0.0)	216 (100)	216
Low Positive (1P, 2P, 7P, 8P)	216 (100)	0 (0.0)	0 (0.0)	216
Mid Positive (3P, 4P, 9P, 10P)	216 (100)	0 (0.0)	0 (0.0)	216
Total Positive	432 (100)	0 (0.0)	0 (0.0)	432

Table 17. Standard Deviations (SD) and Coefficients of Variation (CV) for Precision By Laboratory and Day: *digene* HC2 GC-ID DNA Test in PreservCyt

Specimen	N	Mean RLU/CO	Within Run SD	Between Run SD	Between Day SD	Between Site SD	Total SD	%CV
Negative (5N, 11N)	108	0.201	0.037	0.019	0*	0.032	0.052	25.9
Negative (6N, 12N)	108	0.198	0.055	0.016	0.019	0.021	0.064	32.3
GC Mid Positive (3P, 4P, 9P, 10P)	216	7.981	0.906	1.203	0	0.243	1.526	19.1
GC Low Positive (1P, 2P 7P, 8P)	216	4.648	0.675	0.478	0.308	0	0.883	19.0

*Negative variance components were set to zero.

ANALYTICAL SENSITIVITY

The analytical sensitivity (limits of detection) of the *digene* HC2 GC-ID DNA Test was determined by directly testing a dilution series of a specimen panel consisting of 114 separate isolates of *Neisseria gonorrhoeae*. The 114 isolates represented 13 auxotypes, 5 serovars, 10 antibiotic resistant strains, 6 plasmidless strain isolates, and 2 uncharacterized isolates found discordant in the multicenter trial. Four-point dilution series of each of the isolates were tested once using the *digene* HC2 GC-ID DNA Test to establish the limits of detection for the test. The limit of detection for each *Neisserria auxotype* is summarized in Table 18. The detectable limit range stated was the dilution of each auxotype that was detected within or very near to the assay's equivocal zone of 1.0-2.5 RLU/CO.

The analytical sensitivity of the *digene* HC2 GC-ID DNA Test varied from 25 to 50,000 CFU/assay for the 114 Neisseria isolates tested, including auxotypes, serovars, plasmidless, and antibiotic-resistant strains. Only one of the six plasmidless strains and one of five of the *Neisseria gonorrhoeae* IA-5 serovars tested were detected at 50,000 CFU/assay; none of the other 112 isolates were detected at concentrations in excess of 5000 CFU/assay. The average detectable limit for all 114 isolates ranged from 974 to 2887 CFU/assay when taking into consideration isolate dilutions that fell both within the assay's equivocal zone and above 2.5 RLU/CO. The overall average limit of detection was 1931 CFU/assay (3.8×10^4 CFU/ml). Clinical specimens that contain organism at or near the limit of detection may need to be retested by an alternate test procedure or on a new specimen from the patient as defined in the *Interpretation of Results* section of these instructions for use.

Table 18. Summary of Detectable Limits of Sensitivity for GC Auxotypes, Serovars, Plasmidless, and Antibiotic-resistant Strains.

Auxotype	Detectable Concentration CFU/ml	CFUs/assay
N. gonorrhoeae Auxotype 1	1000	50
N. gonorrhoeae Auxotype 12	500-5000	25 - 250
N. gonorrhoeae Auxotype 16	10^3-10^4	50 - 500
N. gonorrhoeae Auxotype 22	10^4-10^5	500 - 5000
N. gonorrhoeae Auxotype 5	500-5000	25 - 250
N. gonorrhoeae Auxotype 9	5 x10^4	2500
N. gonorrhoeae Auxotype AHU (5 isolates)	10^4-10^5	500 - 5000
N. gonorrhoeae Auxotype Arg (5 isolates)	10^4-10^5	500 - 5000
N. gonorrhoeae Auxotype AU (5 isolates)	10^3-10^4	50 - 500
N. gonorrhoeae Auxotype PAU (5 isolates)	10^3-10^5	50 - 5000
N. gonorrhoeae Auxotype Pro (5 isolates)	10^4-10^5	500 - 500
N. gonorrhoeae Auxotype Proto (5 isolates)	10^3-10^4	50 - 500
N. gonorrhoeae Ciprofloxacin Intermedate (Cipl) (5 isolates)	10^3-10^5	50 - 5000
N. gonorrhoeae Ciprofloxacin Resistant (Cip R) (4 isolates)	10^3-10^4	50 - 500
N. gonorrhoeae CMRNG (5 isolates)	10^4-10^5	50 - 5000
N. gonorrhoeae Other-5423	10^4-10^5	50 - 5000
N. gonorrhoeae Other-5658	10^3-10^4	50 - 500
N. gonorrhoeae PenR (5 isolates)	10^4-10^5	500 - 5000
N. gonorrhoeae PenR (5 isolates)	10^3-10^6	50 - 50,000
N. gonorrhoeae Plasmidless strains (6 isolates)	10^4-10^5	500 - 5000
N. gonorrhoeae PPNG 3.05 (5 *isolates)*	10^4-10^5	500 - 5000
N. gonorrhoeae PPNG 3.2	10^3-10^5	50 - 5000
N. gonorrhoeae PPNG 4.4 (4 isolates)	10^4-10^5	500 - 5000
N. gonorrhoeae Serovar I A-1 or IA-2 *(5 isolates)*	10^4-10^6	500 - 50,000
N. gonorrhoeae Serovar I A-5 *(4 isolates)*	10^3-10^4	50 - 500
N. gonorrhoeae Serovar I B-1 (5 isolates)	10^3-10^5	50 - 5000
N. gonorrhoeae Serovar I B-4 or IB-15 (5 isolates)	10^3-10^5	50 - 5000
N. gonorrhoeae Spectinomycin Resistant (SpecR)	10^5	5000
N. gonorrhoeae TetR (5 isolates)	10^3-10^5	50 - 5000
N. gonorrhoeae TRNG American (5 isolates)	10^4-10^5	500 - 5000
N. gonorrhoeae TRNG Dutch (5 isolates)	10^4-10^5	500 - 5000
N. gonorrhoeae Type Strain	500-5000	25 - 250

ADDITIONAL CONSIDERATIONS FOR PRESERVCYT SPECIMENS

The limit of detection studies described in the previous section for STM were not repeated using PreservCyt Solution specimens because the analytical sensitivity of the assay is expected to be independent of either STM or PreservCyt Solution specimen type, specifically because PreservCyt Solution specimens are subjected to a conversion procedure (for details, reference the *digene* HC2 Sample Conversion Kit instructions for use), which renders PreservCyt Solution specimens similar in composition to STM specimens prior to use with the *digene* HC2 GC-ID DNA Test.

However, because the PreservCyt Solution specimen is subjected to a centrifugation step during the conversion procedure, it was necessary to evaluate any potential impact of centrifugation on the analytical sensitivity of the *digene* HC2 GC-ID DNA Test. To assess the potential impact of centrifugation on analytical sensitivity, eighty-eight (88) pairs of *Neisseria gonorrhoeae* DNA-negative STM and PreservCyt Solution specimens were prepared with matching amounts of *Neisseria gonorrhoeae* (Plasmidless Strain NRL 33151) organism. The paired specimens were tested and analytical sensitivity was estimated by comparing the mean RLU/CO values obtained [(PreservCyt:STM) x 100].

Table 19. Comparison of Analytical Sensitivity - *digene* HC2 GC-ID DNA Test - Paired PreservCyt Solution and STM Specimens.

	***digene* HC2 GC-ID DNA Test**		**PC:STM RLU/CO**
	STM	**PreservCyt**	
Number of Specimens	88	88	-
Mean RLU/CO	3.97	4.91	1.24
Median RLU/CO	4.01	4.93	1.23
Standard Deviation	0.34	1.00	-
Minimum RLU/CO	3.06	2.30	-
Maximum RLU/CO	4.77	7.10	-

An additional study provided a similar comparison with paired, simulated patient specimens. Patient specimens collected in PreservCyt solution were obtained from a site external to QIAGEN and screened by the *digene* HC2 GC-ID DNA Test to identify positive specimens. These positive patient specimens were then combined to generate a total of 10 concentrated specimen PreservCyt pools. From these pools, two aliquots were prepared and processed to form cell pellets. The cell pellets were resuspended in phosphate buffered saline (PBS). Aliquot A was prepared by adding the resuspended pellet to STM and Aliquot B was prepared by adding the resuspended pellet to PreservCyt. Both aliquots were tested with the *digene* HC2 GC-ID DNA Test with the following results:

Table 20. Comparison of Analytical Sensitivity - *digene* HC2 GC-ID DNA Test - Simulated (Pooled) Patient PreservCyt Solution Specimens Paired with STM.

	***digene* HC2 GC-ID DNA Test**		**PC: STM RLU/CO**
	STM	**PreservCyt**	
Number of Specimens	10	10	-
Mean RLU/CO	4.80	4.32	0.90
Median RLU/CO	2.66	2.47	0.93
Standard Deviation	5.44	5.08	-
Minimum RLU/CO	1.16	1.02	-
Maximum RLU/CO	18.97	17.26	-

ANALYTICAL SPECIFICITY

A battery of bacteria, viruses, plasmids, and human cellular material or blood products potentially found in the female anogenital tract were tested to determine if cross-reactivity would occur with the *digene* HC2 GC-ID DNA Test. All microorganisms were tested at concentrations of 10^5 and 10^7 organisms or CFU per ml, and when possible with 10^9 organisms or CFU per ml, unless otherwise indicated below. Purified DNA of viruses and plasmids were tested at a variety of concentrations as indicated below.
Below is a list of the bacteria tested.

Acinetobacter anitratus	*Neisseria caviae (2 isolates)* [e]
Acinetobacter calcoaceticus	*Neisseria cuniculi (3 isolates)* [f]
Acinetobacter lwoffi	*Neisseria cinera (6 isolates)*
Achromobacter xerosis	*Neisseria flavescens (4 isolates)*
Actinomyces israelii	*Neisseria species g **
Alcaligenes faecalis	*Neisseria lactamica (6 isolates)* [d]
Bacillus subtilus	*Neisseria meningitidis (Group A, B, C, W135, Y)*
Bacteroides fragilis	*Neisseria mucosa (6 isolates)* [d]
Bacteroides melaninogenicus	*Neisseria polysaccharea*
Branhamella catarrhalis (6 isolates)	*Neisseria sicca (6 isolates)*
Candida albicans	*Neisseria subflava*
Candida glabrata	*Neisseria subflava biovar flava (5 isolates)*
Chlamydia pneumoniae [b]	*Neisseria subflava biovar perflava (4 isolates)* [h]
Chlamydia psittaci [a] *(2 strains)*	*Peptostreptococcus anaerobius*
Chlamydia trachomatis [b] *(serovar B, Ba, E, J, L3)* [c]	*Peptostreptococcus asaccharalyicus*
Enterobacter cloacae	*Peptostreptococcus productus*
Enterococcus avium	*Proteus mirabilis*
Enterococcus faecalis	*Proteus vulgaris*
Escherichia coli (Clinical isolate) [†]	*Pseudomonas aeruginosa* [a]
Escherichia coli (HB101) [†]	*Salmonella minnesota*
Fusobacterium nucleatum	*Salmonella typhimurium*
Gardnerella vaginalis	*Serratia marcescens*
Gemella heamolysans	*Staphylococcus aureus (ProtA +)*
Haemophilus ducreyi	*Staphylococcus epidermidis*
Haemophilus influenzae	*Streptococcus agalactiae (Grp B)*
Kingella denitrificans [d]	*Streptococcus pyogenes (Grp A)*
Klebsiella pneumoniae	*Streptomyces griseus*
Lactobacillus acidophilus	*Treponema pallidum*
Mobiluncus curtisii	*Trichomonas vaginalis* [i]
Mobiluncus mulieris	*Ureaplasma urealyticum*
Moraxella lacunata	
Mycoplasma hominis	
Mycoplasma hyorhinis	

Concentrations tested (organisms/ml or CFU/ml for Neisseria species):

[a] 5×10^6, 5×10^7, 5×10^8, 8×10^4, 8×10^6, 8×10^8, 9×10^4, 9×10^6, 9×10^8
[b] 2×10^5, 2×10^7 and 2×10^8
[c] 1×10^5, 1×10^7 and 1×10^8
[d] 5×10^6, 5×10^7, 5×10^8
[e] 1.1×10^5, 1.1×10^7, 1.1×10^9
[f] 9.7×10^5, 9.7×10^6, 9.7×10^8
[g] 2×10^7, 2×10^8 and 2×10^9
[h] 4.8×10^4, 4.8×10^6, 4.8×10^8
[i] 1×10^5 and 1×10^6
[†] Both the *E. coli* strain used to grow plasmids (HB101) and a clinical isolate of *E. coli* were tested.
* ATCC *Neisseria* strain that has features of both *Neisseria gonorrhoeae* and *Neisseria meningitidis* (ATCC #43831).

All bacteria other than *Neisseria gonorrhoeae* potentially found in the urogenital tract, with the exception of the three commensal Neisseria strains and *Chlamydia psittaci,* tested negative with the *digene* HC2 GC-ID DNA Test. Only moderate cross-reactivity that would be interpreted as presumptive positive was observed with *Chlamydia psittaci* and *Neisseria lactamica*. Such cross-reactivity should not impact the interpretation of *digene* HC2 GC-ID DNA Test results of urogenital specimens. Organisms that demonstrated some degree of cross-reactivity are:

	Interpretation	Concentration at which Cross-reactivity Observed
Chlamydia psittaci (1 of 2 isolates)	Presumptive Positive*	1×10^7 organisms/ml
Neisseria lactamica (1 of 6 isolates)	Presumptive Positive*	1×10^9 CFU/ml
Neisseria meningitidis (Group Y, 1 of 2 isolates)	Positive	1×10^7 CFU/ml
Neisseria mucosa (1 of 6 isolates)	Positive	5×10^5 CFU/ml

* RLU/CO fell within the assay's equivocal zone of 1.00 to 2.50.

The three commensal Neisseria strains, *Neisseria lactamica*, *Neisseria meningitidis*, and *Neisseria mucosa*, are all primarily found in the nasopharynx and upper respiratory system. They are rarely, if at all, isolated from the urogenital system.[13,14] Furthermore, the cross-reactive Group Y *Neisseria meningitidis* isolate was determined to be lipopolysaccharide untypeable and is rarely found in the general population. *Chlamydia psittaci* may be detected from the skin of people who work with or handle avian species, but has not been detected in the anogenital tract.[15]

Moreover, not all isolates of the particular strain were cross-reactive with the *digene* HC2 GC-ID DNA Test, decreasing the likelihood that a false-positive result will be generated with a clinical specimen if that strain is present. For example, five of the six *Neisseria lactamica* or *Neisseria mucosa* isolates tested negative with the *digene* HC2 GC-ID DNA Test, as did one of the two *Chlamydia psittaci* strains. Thus, it is not expected that the cross-reactivity of the *digene* HC2 GC-ID DNA Test observed with the three commensal Neisseria strains and *Chlamydia psittaci* would lead to a false clinical interpretation of a positive result when testing anogenital specimens.

The following is a list of the viral DNA, plasmid DNA, and human cellular material or blood products tested and the concentrations that were tested:

Cytomegalovirus[a]
Epstein Barr Virus[b]
Hepatitis B Surface Antigen Positive Serum[c]
Herpes Simplex I[d]
Herpes Simplex II[d]
Human Immunodeficiency Virus (HIV)[b,g]
Human Genomic DNA[e]
Human Placental DNA[e]
Human Whole Blood[h]
Human Papillomavirus type 6 [f]
Human Papillomavirus type 11[f]
Human Papillomavirus type 16[f]
Human Papillomavirus type 18[f]
pBR322[i]
SV40 [j]
PGEM®3Z[j]
PGEM®3Zf(-)[j]
Human epithelial cells[k]

Concentrations tested:

[a] 1×10^5, 1×10^7, 1×10^9 viral particles/ml
[b] 1×10^5, 1×10^7, 1×10^8 viral particles/ml
[c] 2.9×10^8, 1.1×10^9 viral particles/ml
[d] 6.1×10^6, 2.4×10^7 viral particles/ml
[e] 2.7×10^2, 1.1×10^3 copies/ml
[f] 1.1×10^8, 4.6×10^8 viral particles/ml
[g] 2×10^6, 2×10^7, 2×10^8 viral particles/ ml
[h] 5%, 10%, 50%
[i] 2.1×10^8, 8.3×10^8 copies/ml
[j] 1 ng/ml, 4 ng/ml
[k] 1×10^5, 1×10^6, 1×10^7 cells/ml

None of the viruses tested showed cross-reactivity with the *digene* HC2 GC-ID DNA Test; however, cross-reactivity was observed with plasmids pBR322, pGEM® 3Z, and pGEM® 3Zf(-). All other DNA preparations tested, including human DNA, were negative. Human blood and epithelial cells did not cross-react with the *digene* HC2 GC-ID DNA Test. Cross-reactivity between pBR322 and the *digene* HC2 GC-ID DNA Test is not unexpected due to the manner in which the GC Probe is created. The presence of pBR322 homologous sequences has been reported in human genital specimens, and false-positive results could occur in the presence of high levels of bacterial plasmid. However, no specimens out of 103 tested from a U.S. Multicenter Clinical Study found positive for *Neisseria gonorrhoeae* with the *digene* HC2 GC-ID DNA Test were identified as false-positive due to cross-reactive pBR322 sequences. Thus, the likelihood of *digene* HC2 GC-ID DNA Test false-positive results with clinical specimens caused by cross-reactivity with homologous pBR322 sequences appears to be low. Although the *digene* HC2 GC-ID DNA Test has the potential to cross-react with *Chlamydia psittaci*, pBR322, pGEM, and several commensal Neisseria species, the likelihood of these organisms affecting the interpretation of the test is remote, as demonstrated by the results of the Multicenter Clinical Study.

HOMOLOGY OF PROBES TO TOTAL PLASMID AND GENOMIC DNA

The genomic probes are homologous to approximately 0.5% of the *Neisseria gonorrhoeae* genome. A breakdown for each probe in the *digene* HC2 GC-ID DNA Test follows:

Probe	Type	Approx. insert Size (bp)	% of Genome
pGC1	Genomic	6,400	0.34%
pGC2		3,300	0.17%
		9,700 (Total)	0.51%
pGC3	Cryptic Plasmid	4,200	N/A*

*This represents the entire sequence of the probe.

EFFECT OF BLOOD AND OTHER SUBSTANCES ON STM SPECIMENS

The effect of blood and other potentially interfering defined substances was evaluated in the *digene* HC2 GC-ID DNA Test. Whole blood, and one commercial brand of douche, anti-fungal cream and contraceptive jelly (agents that may commonly be found in cervical specimens) were added to positive specimens (clinical specimen pools) at concentrations that may be found in cervical specimens (see Table 21). No false-positive results were observed with any of the four agents at any concentration. A study of undefined substances present in a population of 100 negative clinical specimens showed that undefined substances do not appear to inhibit the generation of a positive signal within the *digene* HC2 GC-ID DNA Test when compared to the signal generated when testing for GC organism in STM.

Table 21. Effect of Interfering Substances on Test Results.

		digene HC2 GC-ID DNA Test Result					
		Pooled Clinical Specimens				Specimen Transport Media	
Interface Substance	Conc.	Negative		Positive*		Positive*	
		RLI/CO	Observed	RLU/CO	Observed	RLU/CO	Observed
None (Control)		0.15	NA	3.41	NA	2.70	NA
Blood	1%	0.21	+37%	3.17	-7%	3.21	+19%
	5%	0.19	+22%	3.11	-9%	3.05	+13%
Douche	1%	0.21	+37%	3.48	+2%	2.80	+4%
	5%	0.18	+20%	3.47	+2%	3.20	+18%
Anti-fungal cream	1%	0.19	+20%	3.60	+5%	2.95	+9%
	5%	0.20	+30%	3.52	+3%	3.09	+14%
Contraceptive Gel	1%	0.08	-54%	3.18	-7%	2.98	+10%
	5%	0.08	-54%	3.24	+5%	3.10	+15%

* Spiked with 10^3 CFU/ml auxotype 1 *Neisseria gonorrhoeae* organism.

EFFECT OF BLOOD AND OTHER SUBSTANCES ON PRESERVCYT SPECIMENS

Evaluations of specific interfering substances, as described in the previous section for STM specimens, were not conducted using PreservCyt Solution specimens. However, PreservCyt Solution specimens are not expected to exhibit different interference profiles than STM specimens because the anatomical site for the collection of endocervical specimens is identical for both PreservCyt Solution and STM specimens, and because a PreservCyt Solution specimen is subjected to a conversion process (as detailed in the *digene* HC2 Sample Conversion Kit instructions for use) that renders it comparable in composition with an STM specimen,

Residual Sample Conversion Buffer (SCB)[1] may be present in trace amounts in fully-converted PreservCyt Solution specimens. Therefore, an analytical study was completed to verify the analytical performance of the *digene* HC2 GC-ID DNA Test in the presence of varying amounts of SCB. Varying concentrations of plasmid GC DNA were prepared in STM. Excess volumes of SCB were then added to the specimens, and three aliquots from each specimen were tested to derive a mean RLU/CO for each specimen in the presence of either PreservCyt Solution or SCB. Comparison of these mean RLU/CO values for each specimen compared with the mean RLU/CO values for each STM control specimen resulted in no false-positive or false-negative results.

[1] Sample Conversion Buffer is a Buffered solution with Eosin Y and 0.05% (w/v) sodium azide, required for the conversion of a PreservCyt specimen. Refer to QIAGEN's *digene* HC2 Sample Conversion Kit instructions for use for specific details.

PRECISION AT THE CUTOFF OF THE *digene* HC2 GC-ID DNA TEST WITH CLINICAL SPECIMENS COLLECTED IN STM

The reproducibility of the *digene* HC2 GC-ID DNA Test with clinical specimens collected in STM was determined in a study using 30 clinical pools (15 positive and 15 negative) prepared by combining previously denatured and tested cervical brush specimens collected in STM. Specimens were tested in replicates of four on each of five days for a total of 20 replicates per specimen. Testing was performed using the *digene* HC2 GC-ID DNA Test. The mean RLU/CO value, 95% confidence intervals about the mean (95% CI) and percent positive results were calculated for each specimen over five days and are shown in Table 22.

Table 22. Mean RLU/CO with Confidence Intervals and Percent *digene* HC2 GC-ID DNA Test Positives (Descending Order by Mean RLU/CO).

No.	RLU/CO	95% CI	%Positive
1	1.92	1.31-2.00	100 (20/20)
2	1.22	1.16-1.29	100 (20/20)
3	1.21	1.16-1.25	100 (20/20)
4	1.21	1.16-1.25	90 (18/20)
5	1.17	1.03-1.28	100 (20/20)
6	1.14	1.09-1.18	90 (18/20)
7	1.08	1.04-1.12	80 (16/20)
8	1.05	1.00-1.09	70 (14/20)
9	1.05	1.01-1.09	70 (14/20)
10	1.02	0.98-1.06	65 (13/20)
11	1.00	0.96-1.03	65 (13/20)
12	1.00	0.97-1.03	45 (9/20)
13	1.00	0.96-1.03	40 (8/20)
14	0.98	0.95-1.02	45 (9/20)
15	0.91	0.89-0.94	10 (2/20)
16	0.90	0.87-0.92	0 (0/20)
17	0.87	0.84-0.91	5 (1/20)
18	0.86	0.83-0.89	0 (0/20)
19	0.84	0.82-0.85	0 (0/20)
20	0.82	0.79-0.85	0 (0/20)
21	0.79	0.75-0.82	0 (0/20)
22	0.77	0.78-0.80	0 (0/20)
23	0.76	0.74-0.79	0 (0/20)
24	0.75	0.78-0.79	0 (0/20)
25	0.73	0.70-0.75	0 (0/20)
26	0.56	0.54-0.59	0 (0/20)
27	0.56	0.54-0.59	0 (0/20)
28	0.56	0.53-0.59	0 (0/20)
29	0.54	0.52-0.56	0 (0/20)
30	0.12	0.11-0.13	0 (0/20)

Specimens with a mean RLU/CO of 20% or more above the cutoff were positive or presumptive positive 97% of the time, while specimens with an mean RLU/CO of 20% or more below the cutoff were negative 100% of the time. These results indicate that specimens at 20% or more away from the cutoff can be expected to yield consistent results with the *digene* HC2 GC-ID DNA Test.

Specimens with values close to the assay cutoff remained largely positive or negative; those that were above the assay cutoff but within 20% of it remained positive or presumptive positive 69.4% of the time. Those specimens below the cutoff but within 20% of it remained negative 91.4% of the time.

These results demonstrate that the *digene* HC2 GC-ID DNA Test yields reproducible results with clinical specimens collected in STM whose RLU/CO values are within 20% of the assay cutoff.

HISTORICAL INFORMATION

Historically, the Dynex Model MLX luminometer was used in addition to the DML 2000 to generate data and determine the performance characteristics of the *digene* HC2 GC-ID DNA Test. The MLX luminometer is no longer available for use, and only the DML 2000 is still used to generate results. The

following data were generated from the Multicenter Clinical Trial to determine the reproducibility of the Positive Calibrator and Negative Calibrator and are provided below as historical information.

To determine Positive Calibrator and Negative Calibrator reproducibility and estimate the frequency in which manual recalculations may be necessary, the results from the clinical evaluations involving 79 assays performed with the *digene* HC2 GC-ID DNA Test were compiled (Table 23). The results showed that the average %CV for these 79 assays was 5.79% and no assays had Negative Calibrator Mean values in excess of 150 RLUs. Considering all 3 replicates of the Positive Calibrator per assay, Calibrator reproducibility of greater than 20% CV was observed for only 1 out of 79 assays (1.3%) and the %CV recalculated. Following the recalculation, the assay's %CV remained under 15%, indicating that all of the assays were valid.

Table 23. Performance of the Positive Calibrator and Negative Calibrator. Combined Data from the Multicenter Clinical Trial and the Precision Study. (n = 79 assays)

				Mean of Calculated Means (RLU)		Mean of the Calculated %CVs	
Instrument	No. of assays	Mean of S/N ratios	Calibrator Type	Three Replicates	Adjusted for Outliers	Three Replicates	Adjusted for Outliers
DML 2000	9	7.71	Negative	40.300	34.470	18.960	12.240
			Positive	292.370	292.370	6.670	6.670
MLX*	70	4.52	Negative	0.076	0.070	13.830	12.360
			Positive	0.292	0.292	5.674	5.674

*No longer available for use.

EQUIVALENCE BETWEEN STM AND PRESERVCYT SOLUTION SPECIMENS

Equivalence between STM and PreservCyt Solution specimens was examined in a clinical evaluation of 1252 paired cervical specimens. A PreservCyt Solution specimen was processed according to the *digene* HC2 Sample Conversion Kit instructions for use and tested along with a paired STM specimen with the *digene* HC2 GC-ID DNA Test. The results of this evaluation are presented in Table 24. The clinical performance was established using PreservCyt Solution specimens with a residual volume greater than 6.5 ml. The testing of specimens with residual volumes from 4.0 - 6.5 ml should be validated by the laboratory.

Table 24. Summary of Statistical Data for *digene* HC2 GC-ID DNA Test Agreement amongst Paired Cervical Specimens Collected in STM and PreservCyt Solution.

Cohort	Kappa 95% CI	Positive Agreement (n/N) 95% CI	Negative Agreement (n/N) 95% CI	Overall Agreement (n/N) 95% CI
Equivocal Zone Data Exclusion	0.96 0.92, 0.99	98.00 (49/50) 89.35, 99.95	99.75 (1181/1184) 99.26, 99.95	99.68 (1230/1234) 99.17, 99.91
Equivocal Zone Retest Algorithm*	0.93 0.88, 0.98	91.80 (56/61) 81.90, 97.28	99.75 (1188/1191) 99.27, 99.95	99.36 (1244/1252) 98.74, 99.72

*Specimens in the 1.0 to 2.5 RLU/CO range were retested in duplicate. Specimen classification was then determined using a two of three rule.

The reproducibility of the *digene* HC2 GC-ID DNA Test was assessed as part of a clinical evaluation to demonstrate that equivalent *digene* HC2 GC-ID DNA Test results are obtained when a panel of 20 PreservCyt Solution specimens was tested over 3 days at three laboratories. The results of this reproducibility study are presented in Table 25 below.

Table 25. *digene* HC2 GC-ID DNA Test Percent Agreement – By Site.

Site	Observed vs. Expected*	% Agreement (95% CI)
1	60/60	100 (94.04, 100)
2	60/60	100 (94.04, 100)
3	59/60	98.33 (91.06, 99.96)
All Sites Combined	179/180	99.44 (96.94, 99.99)

*20 members x 3 days x 3 sites.

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TROUBLESHOOTING GUIDE

digene HC2 GC-ID DNA TEST

OBSERVATION	PROBABLE CAUSES	SOLUTIONS
Incorrect or no color change observed during denaturation.	Denaturation Reagent not added, or Denaturation Reagent not prepared properly.	1. Verify that the Denaturation Reagent contains the Indicator Dye and is a dark purple color. 2. Verify that Denaturation Reagent was added to the specimen by measuring the specimen volume (1.5 ml is expected). If the volume indicates that Denaturation Reagent was not added, make the appropriate addition, mix and proceed with the assay if the proper color change is then observed.
	Bloody specimen may mask the color change.	The exact color change described is not expected with these types of specimens; assay test results should not be adversely affected.
	Specimen pH may be unusually acidic.	The specimen may be unusually acidic, thus the expected color change will not occur. Collect a new specimen prior to the application of acetic acid to the cervix because improper specimen pH will adversely affect the test results.
Quality Controls give incorrect results	Incorrect software protocol chosen for test	If the software protocol is incorrect for the test being performed, the plate should be read again within 30 minutes after Detection Reagent 2 addition and with the correct protocol.
	Reverse placement of QC CT and QC GC	Retest Specimens.
Incorrect color change observed during hybridization.	• Inadequate mixing of Probe Mix with denatured Calibrators, Quality Controls, and/or specimens. • Probe Mix not added. • Incorrect volume of reagent added.	Shake Hybridization Microplate for an additional 2 minutes. If there are wells that still remain purple or gray add an additional 25 µl of Probe Mix and mix well. If upon probe addition and re-mixing the proper color change does not occur and the specimen did not contain blood or other materials, retest the specimen.
	Bloody specimen may mask the color change.	The exact color change described is not expected with these types of specimens; assay test results should not be adversely affected.
	Specimen had < 1000 µl *digene* Specimen Transport Medium (STM).	Check the volume of the original specimen. Volume should be 1425 µl ± 20 µl (after removing 75 µl). If volume is < 1405 µl, original specimen contained < 1000 µl STM. Obtain a new specimen.
Assay fails calibration verification criteria. No signal observed in Positive Calibrator, Quality Controls, or specimens.	No Probe added to Probe Diluent.	Prepare GC Probe Mix as described in the *Reagent Preparation and Storage* section of these instructions for use . Mix thoroughly. Label tube properly. Repeat assay using freshly prepared Probe Mix.
	Probe contaminated with RNase during preparation.	Use aerosol-barrier pipette tips when pipetting probe and wear powder-free gloves. Dilute Probe in sterile container. Only use clean, new disposable reagent reservoirs.
	Inadequate mixing of Probe Mix and Probe Diluent.	After adding Probe to Probe Diluent, mix very thoroughly by vortexing at high speed for at least 5 seconds. A visible vortex must be produced.
	Inadequate mixing of diluted Probe and denatured specimen.	After adding Probe Mix to denatured specimen, cover Hybridization Microplate and shake on Rotary Shaker I set at 1100 ± 100 rpm for 3 ± 2 minutes, as described in the Test Procedure, Hybridization section, step 6 of these instructions for use. Check for color change from purple to yellow in every well.
	Incorrect time or temperature during hybridization step.	Hybridize for 60 ± 5 minutes at 65 ± 2°C, as described in the Test Procedure, Hybridization section, step 7 of these instructions for use. Check temperature of Microplate Heater I. Ensure that the heater is set to heat specimens to correct temperature and was preheated for 1 hour prior to use.

digene HC2 GC-ID DNA TEST		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTIONS**
	Inadequate mixing during capture step.	Shake on Rotary Shaker I at 1100 ±100 rpm for 60 ± 5 minutes at 20-25°C, as described in the Test Procedure, Hybrid Capture section, step 7 of these instructions for use. Verify Rotary Shaker I speed by calibration as outlined in the Shaker Speed Calibration section of the Rotary Shaker I User Manual.
	• Failure to add correct amount of Detection Reagent 1. • Failure to incubate for specified time.	Pipette 75 µl Detection Reagent 1 into each well using an 8-channel pipettor. Incubate at 20-25°C for 30-45 minutes.
	• Failure to add correct amount of Detection Reagent 2. • Failure to incubate for specified time.	Pipette 75 µl Detection Reagent 2 into each well using an 8-channel pipettor. Incubate at 20-25°C for 15 to 30 minutes.
	Luminometer malfunction or incorrect programming.	Refer to the maintenance/service and troubleshooting sections in the applicable *digene* assay analysis software user guide for further instructions, or call QIAGEN Technical Services.
Elevated RLU values in Calibrators, Quality Controls and/or specimens (≥ 150 RLUs in many or all wells). Assay may fail validation criteria.	• Denaturation Reagent not added; or incorrect volume of reagent added; or inadequate mixing of Denaturation Reagent with Calibrators, Quality Controls, and/or specimens. • Inadequate water bath temperature and water level.	• Verify that the repeating pipettor is delivering accurately prior to adding Denaturation Reagent. Calibrated pipettors are essential. Add a half-volume of Denaturation Reagent to each tube and mix well. To avoid false-positive results, make sure liquid washes entire inner surface of tube (invert the tube one time if mixing manually). Calibrators, Quality Controls, and specimens should turn purple after addition of Denaturation Reagent. Check speed calibration of Multi-Specimen Tube Vortexer 2. • Check water level and temperature of water bath.
	• Light leak in the luminometer. • Seal is broken. • Door not sealed.	Perform a background reading (raw data measurement) of the luminometer by reading an empty microplate. A reading of greater than 50 RLUs indicates that a light leak may exist. Refer to the maintenance/service and troubleshooting sections in the applicable *digene* assay analysis software user guide for further instructions, or call QIAGEN Technical Services.
	Contamination of Detection Reagent 2 or Capture Microplate wells by Detection Reagent 1 or exogenous alkaline phosphatase.	Refer to Contamination Check in this Troubleshooting section.
	Contaminated Wash Buffer.	Refer to Contamination Check in this Troubleshooting section.
	Contaminated Automated Plate Washer.	Refer to Contamination Check in this Troubleshooting section.
	Inadequate washing of Capture Microplate wells after Detection Reagent 1 incubation.	Wash Microplate wells thoroughly with Wash Buffer 6 times, filling wells to overflow each time or using Automated Plate Washer . There should be no residual pink liquid visible in the wells after washing. See the Troubleshooting section of the *Automated Plate Washer User Manual* for instructions on testing for contamination or malfunctions.
	Detection Reagent 1 contamination of Microplate wells.	Ensure all work surfaces are clean and dry. Use care when using Detection Reagent 1. Avoid aerosols.
	Blotting hybridization solution on same area of Kimtowels Wipers or equivalent low-lint paper towels. Use of wrong blotting towels.	Do not reblot on same area of the Kimtowels Wipers or equivalent low-lint paper towels. Use Kimtowels Wipers or equivalent low-lint paper towels for blotting.

digene HC2 GC-ID DNA TEST		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTIONS**
	GC Quality Control material used as Positive Calibrator. Assay fails validation.	Ensure correct placement of Calibrators and Quality Controls.
Low PC/NC ratios or high number of low-positive specimens (>20% of the total specimens) with a RLU/CO ratio <2.0. Assay may fail validation criteria.	Inadequate specimen preparation.	Add the appropriate volume of Denaturation Reagent and mix thoroughly by vortexing. To avoid false-positive results, make sure liquid washes entire inner surface of tube by vortexing with the Multi-Specimen Tube Vortexer 2 method for at least 5 seconds (for the manual vortexer method, vortex for at least 5 seconds and invert tube one time). A distinct color change from clear to dark purple should be seen. Incubate for 45 ± 5 minutes at 65 ± 2°C. When using PreservCyt Solution specimens, these hybrids are likely to be present on the inside walls of the sample conversion tube. In order to prevent possible carryover of this non-denatured cellular material, the pipette tip must not touch the sides of the sample conversion tube during transfer of the denatured specimen to the microplate well used for GC Probe hybridization. Refer to the *digene* HC2 Sample Conversion Kit instructions for use for procedural details.
	Probe inadequately mixed or insufficient Probe added to assays.	Prepare Probe Mix as described. Mix thoroughly by vortexing, ensuring that a visible vortex is produced. Probe Mix must be added to wells with a multichannel or repeating pipettor to ensure accurate delivery.
	Inadequate volume of Probe Mix added to each hybridization microplate well.	Verify that the 8-channel pipettor is delivering accurately prior to adding Probe Mix to Hybridization Microplate. 25 µl of Probe Mix should be added to the denatured specimen at the bottom of each microwell. Verify that the 8-channel pipettor is delivering accurately prior to adding Probe Mix to the hybridization wells. Color change should be from dark purple to yellow upon addition and thorough mixing of Probe Mix.
	Loss of Detection Reagent 1 activity.	Store Detection Reagent 1 at 2-8°C. Use by the expiration date on the kit outer box label.
	Insufficient capture of RNA: DNA Hybrids.	The capture step should be performed using the Rotary Shaker I set at 1100 ± 100 rpm. Verify shaker speed as outlined in the Shaker Speed Calibration section of the Rotary Shaker I User Manual.
	Inadequate washing.	Wash Microplate wells thoroughly with Wash Buffer 6 times, filling the wells to overflow each time or using Automated Plate Washer.
	Contaminated Wash Buffer.	Refer to Contamination Check in this Troubleshooting section.
Series of positive specimens with RLU values approximately the same.	Contamination of Capture Microplate wells during assay manipulation.	Cover Capture Microplate wells during all incubations. Avoid exposing microplate wells to aerosol contamination while performing the assay. Wear powder-free gloves during manipulations.
	Detection Reagent 2 contamination.	Be careful not to contaminate the stock when pipetting Detection Reagent 2 into Capture Microplate wells. Avoid contamination of Detection Reagent 2 by aerosols from Detection Reagent 1 or from laboratory dust, etc.
	Automated Plate Washer malfunction.	Refer to Contamination Check in this Troubleshooting section or see the Troubleshooting Section of the *Automated Plate Washer User Manual* for instructions on testing for contamination or identifying malfunctions.
Wide % CVs between replicates.	Inaccurate pipetting (i.e., air bubbles, pipette not calibrated).	Check pipettor to ensure that reproducible volumes are being delivered. Calibrate pipettors routinely.
	Insufficient mixing.	Mix thoroughly at all steps. Vortex before and after denaturation incubation. Ensure that a visible vortex is produced.

digene HC2 GC-ID DNA TEST		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTIONS**
	Incomplete transfer of liquid from Hybridization Microplate to Capture Microplate wells.	Take care during transfer step from Hybridization Microplate to Capture Microplate to ensure reproducible volumes are transferred.
	Improper washing conditions.	Wash Microplate wells thoroughly with Wash Buffer 6 times, filling the wells to overflow each time or using Automated Plate Washer and proper Automated Plate Washer protocols.
	Detection Reagent 1 contamination of Microplate wells.	Ensure all work surfaces are clean and dry. Use care when using Detection Reagent 1. Avoid aerosols.
	Contamination of pipette tip with undenatured material during transfer of denatured specimen to the microplate well used for GC Probe hybridization.	The denaturation step of the specimen processing procedure must be performed as directed in these instructions. Improper specimen vortexing, tube inversion and agitation can result in incomplete denaturation of non-specific RNA:DNA hybrids endogenous to cervical specimens. When using PreservCyt Solution specimens in particular, these hybrids are likely to be present on the inside walls of the sample conversion tube. In order to prevent possible carryover of this non-denatured cellular material, the pipette tip must not touch the sides of the sample conversion tube during transfer of the denatured specimen to the microtube or microplate well used for GC Probe hybridization.
	Blotting on same area of Kimtowels Wipers over several rows.	Do not reblot on the same area of the Kimtowels Wipers.
False-positive results obtained from known negative specimens.	Detection Reagent 2 contaminated.	Be careful not to cross-contaminate specimens when adding Detection Reagent 2 between specimens. If only using part of a kit, aliquot the volume needed for that assay into a clean reagent reservoir prior to filling the pipettor.
	Detection Reagent 1 contamination of Microplate wells.	Wash Microplate wells thoroughly with Wash Buffer 6 times, filling to overflow each time or using Automated Plate Washer. There should be no residual pink liquid visible in the microplate wells after washing.
	Contamination of pipette tip with undenatured material during transfer of denatured specimen to the microplate well used for GC Probe hybridization.	The denaturation step of the specimen processing procedure must be performed as directed in these instructions. Improper specimen vortexing, tube inversion and agitation can result in incomplete denaturation of non-specific RNA:DNA hybrids endogenous to cervical specimens. When using PreservCyt Solution specimens in particular, these hybrids are likely to be present on the inside walls of the sample conversion tube. In order to prevent possible carryover of this non-denatured cellular material, the pipette tip must not touch the sides of the sample conversion tube during transfer of the denatured specimen to the microtube or microplate well used for GC Probe hybridization.
	Inadequate specimen preparation.	Add the appropriate volume of Denaturation Reagent and mix thoroughly by vortexing. To avoid false-positive results, make sure liquid washes entire inner surface of the tube by vortexing with the Multi- Specimen Tube Vortexer 2 method for at least 5 seconds (for the manual vortexer method, invert tube one time). A distinct color change from clear to dark purple should be seen. Incubate for 45 ± 5 minutes at 65 ± 2°C. When using PreservCyt Solution specimens in particular, these hybrids are likely to be present on the inside walls of the sample conversion tube. In order to prevent possible carryover of this non-denatured cellular material, the pipette tip must not touch the sides of the sample conversion tube during transfer of the denatured specimen to the microtube or microplate well used for GC Probe hybridization. Refer to the *digene* HC2 Sample Conversion Kit instructions for use for procedural details.
	Improper washing conditions.	Wash Microplate wells thoroughly with Wash Buffer 6 times, filling the wells to overflow each time or using Automated Plate Washer and proper Automated Plate Washer protocols.

digene HC2 GC-ID DNA TEST		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTIONS**
Elevated Negative Calibrator RLU values (> 150 RLUs). Remainder of assay performs as expected.	Detection Reagent 2 was incubated at a temperature greater than 20-25°C.	Test is invalid due to high Negative Calibrator values. Retest and ensure that Capture and Detection steps incubate at 20-25°C.
	Detection Reagent 2 was incubated longer than 30 minutes.	Read plate after 15 minutes of incubation (and no longer than 30 minutes of incubation) at 20-25°C.
	Detection Reagent 2 or Wash Buffer was contaminated with alkaline phosphatase or Detection Reagent 1.	Refer to Contamination Check in this Troubleshooting section.

CONTAMINATION CHECK

Reagent Evaluated	Contamination Check Procedure	Interpretation of Results
Note: Take care when pipetting Detection Reagent 2 to avoid contamination. Wear gloves and avoid touching pipette tips on any work surfaces.		
Detection Reagent 2	• Pipette 75 µl of the aliquoted, residual and or original vial of Detection Reagent 2 into a blank Capture Microplate well. • Incubate 20-25°C for 15 minutes. Avoid direct sunlight. • Read in the Microplate wells in the luminometer. **Note:** Testing the Detection Reagent 2 in replicates of 3 provides optimal assessment of performance.	• The Detection Reagent 2 Control should be < 50 RLUs. • If Detection Reagent 2 values are < 50 RLUs the Detection Reagent 2 can be used to repeat the assay. • If contaminated (>50 RLUs), obtain a new kit and repeat assay.
Wash Apparatus and/or Water Source	• Pipette 75 µl of Detection Reagent 2 into 4 separate Capture Microplate wells. • Label wells 1-4. • Well 1 serves as the Detection Reagent 2 control. • Pipette 10 µl of Wash Buffer from the wash bottle into well 2. • Allow Wash Buffer to flow through the washer tubing. • Pipette 10 µl of the Wash Buffer from the tubing into well 3. • Obtain an aliquot of the water used to prepare the Wash Buffer. Pipette 10 µl of the water into well 4. • Incubate 20-25°C for 15 minutes. Avoid direct sunlight. • Read the Microplate wells in the luminometer.	• The Detection Reagent 2 Control (well 1) should be < 50 RLUs. • Compare the RLU value from wells 2, 3 and 4 to the Detection Reagent 2 control RLU value (well 1). The individual RLU values for wells 2, 3 and 4 should not exceed 50 RLUs of the Detection Reagent 2 control RLU value (well 1). • Values exceeding 50 RLUs of the Detection Reagent 2 control indicate contamination. See *Reagent Preparation and Storage* for instructions on cleaning and maintenance of Wash Apparatus.
Automated Plate Washer	• Pipette 75 µl of Detection Reagent 2 into 5 separate Capture Microplate wells. • Label wells 1-5. • Well 1 serves as the Detection Reagent 2 control. • Pipette 10 µl of Wash Buffer from the plate washer bottle labeled *Wash* into well 2. • Pipette 10 µl of the rinse liquid from the plate washer bottle labeled *Rinse* into well 3. • Press the Prime key on the Plate Washer key pad, allowing Wash Buffer to flow through the lines. • Pipette 10 µl of the Wash Buffer from the trough into well 4. • Press the Rinse key on the Plate Washer key pad, allowing the rinse liquid to flow through the lines. • Pipette 10 µl of the Wash Buffer from the trough into well 5. • Cover and incubate 15 minutes at 20-25°C. Avoid direct sunlight. • Read the Microplate wells in the luminometer.	• The Detection Reagent 2 Control (well 1) should be < 50 RLUs. • Compare the RLU value from wells 2, 3, 4 and 5 to the Detection Reagent 2 control RLU value (well 1). The individual RLU values for wells 2, 3, 4 and 5 should not exceed 50 RLUs of the Detection Reagent 2 control RLU value (well 1). • Values exceeding 50 RLUs of the DR2 control indicate contamination of the Plate Washer. • See *Automated Plate Washer User Manual, Decontamination Procedure*.

QIAGEN CONTACT INFORMATION

Use the contact information sheet provided with this product to contact your local QIAGEN representative.

SUMMARY OF *digene* HC2 GC-ID DNA TEST

Important: *It is important to be thoroughly familiar with the detailed procedure before using this summary*.

	PROCEDURE	
Denaturation (For PreservCyt Solution specimens, see PreservCyt Solution Specimen Preparation Procedure)	**Manual Vortex Method** Create Plate Layout Label Hybridization Plate. Prepare Denaturation Reagent. ↓ Pipette Denaturation Reagent (volume is equivalent to half the specimen volume) into Calibrators, Quality Controls, and specimens. Vortex each specimen, Calibrators and Quality Control individually for 5 seconds at high speed and invert (see these instructions for use for details). ↓ Check that all tubes show a purple color. ↓ Incubate at 65 ± 2°C for 45 ± 5 minutes. ↓ Prepare GC Probe Mix. ↓ ↓ ↓	**Multi-Specimen Tube Vortexer 2 Method** Create Plate Layout. Label Hybridization Plate. Prepare Denaturation Reagent. ↓ Pipette Denaturation Reagent (volume is equivalent to half the specimen volume) into Calibrators, Quality Controls, and specimens. ↓ Check that all tubes show a purple color. ↓ Cover rack with film and lid. ↓ Vortex for 10 seconds at maximum speed. ↓ Incubate at 65 ± 2°C for 45 ± 5 minutes. ↓ Prepare GC Probe Mix. ↓
Hybridization	Mix denatured specimens well, and pipette 75 µl of denatured specimen, Calibrator, or Quality Control into microplate wells. ↓ Incubate for 10 minutes at 20-25°C. ↓ Pipette 25 µl GC Probe Mix into microplate wells. ↓ Cover microplate with a plate lid and shake on Rotary Shaker I at 1100 ±100 rpm for 3 ±2 minutes. *Check that all wells show yellow color*. (PreservCyt Solution specimens will turn pink.) ↓ Incubate at 65 ± 2°C for 60 ± 5 minutes. ↓ Prepare Capture Microplate. ↓	
Hybrid Capture	Transfer contents from each Hybridization Plate well to corresponding well in Capture Microplate using an 8-channel pipettor. ↓ Cover with a plate lid or sealer. ↓ Shake at 1100 ± 100 rpm at 20-25°C for 60 ± 5 minutes. Prepare Wash Buffer. ↓ Decant and blot Capture Microplate (see these instructions for use for details). ↓	
Hybrid Detection	Pipette 75 µl Detection Reagent 1 into each well of Capture Microplate. Cover Capture Microplate with plate lid or Parafilm or equivalent. Incubate at 20-25°C for 30 - 45 minutes. Wash plate using desired method. ↓	
Washing	**Manual Washing Method** Decant and blot Capture Microplate (see package insert for details). ↓ Wash 6 times. ↓ Blot on low-lint paper towels. ↓	**Automated Plate Washer Method** Place plate on washer and press “START/STOP” to begin. ↓ ↓ ↓ ↓ ↓ ↓
Signal Amplification	Pipette 75 µl Detection Reagent 2 into each well of Capture Microplate. **Cover with a plate lid. Incubate at 20-25°C for 15-30 minutes.** ↓	
Reading	Read Capture Microplate on QIAGEN-approved luminometer. ↓ **Validate assay and interpret specimen results.**	