Hybrid Capture® 2 CT-ID DNA Test

Instructions For Use

digene® HC2 CT-ID DNA Test

An *In Vitro* Nucleic Acid Hybridization Assay with Signal Amplification using Microplate Chemiluminescence for the Qualitative Detection of *Chlamydia trachomatis* (CT) 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. Updated CE registrar number
3. 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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Gaithersburg, MD 20878 USA

EC REP
QIAGEN GmbH
QIAGEN Str. 1
D-40724 Hilden
Germany

IVD

Σ 96

REF 5135-1330

L2171EN Rev. 5

CE 0197

The CE mark indicates that the *digene* HC2 CT-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) CT-ID DNA Test is an *In Vitro* nucleic acid hybridization assay with signal amplification using chemiluminescence for the qualitative detection of *Chlamydia trachomatis* (CT) DNA in cervical specimens collected with the *digene* HC2 DNA Collection Device [consisting of a cervical brush and *digene* Specimen Transport Medium (STM)] and 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 CT-ID DNA Test is indicated for use as an initial test to identify symptomatic or asymptomatic women as evidence of infection with *Chlamydia trachomatis*.

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

For *In Vitro* Diagnostic Use IVD

SUMMARY AND EXPLANATION

Chlamydiae are gram-negative organisms with a two-phase life cycle comprising morphologically distinct infectious and reproductive forms.[1] The *Chlamydia trachomatis* genome is relatively small, measuring approximately 1 x 10^6 base pairs.[2] The infectious form is an elementary body that cannot divide and serves only to carry the infection from one cell to another. Once inside a host cell, elementary bodies assemble into membrane bound vacuoles to produce the metabolically active chlamydial reproductive forms, or reticulate bodies. Replication is entirely host ATP dependent[3] and accomplished through binary fission within the refractile cytoplasmic inclusions, producing a new generation of elementary bodies that are then released to infect other cells. Chlamydiae have a membrane bound, genus-specific lipopolysaccharide that has served as a source of antigen for the production of diagnostic antibodies.

Conventional methods for the direct detection of *Chlamydia trachomatis* in clinical specimens include iodine or Giemsa staining of the organism followed by microscopic evaluation[4] or the more sensitive use of direct fluorescent antibody (DFA) staining.[5] However, these methods approach only 70-85% sensitivity when compared to optimal tissue culture techniques.[6] The most widely accepted procedure for Chlamydia detection is the infection of McCoy cells in cell culture. Fluorescein-conjugated antibodies are then used to detect intracytoplasmic inclusion bodies created by chlamydial reproductive elements in the infected cells.[7] Optimal cell culture has excellent sensitivity and specificity for the detection of Chlamydiae, but is a complex, expensive and time-consuming procedure. Results are generally not available for 48-72 hours post inoculation.[8] Enzyme immunoassays are also used to detect chlamydial antigens[6] and appear to be slightly more sensitive and slightly less specific than direct fluorescent antibody approaches.[9] Nucleic acid tests are also available for the detection of a variety of Chlamydia targets, including chromosomal DNA, mRNA, and the cryptic plasmid common to the vast majority of *Chlamydia trachomatis* strains. These methods vary in sensitivity and specificity, but in general approach or exceed the performance of culture methods.[10-12]

PRINCIPLE OF THE PROCEDURE

The *digene* HC2 CT-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 Chlamydia 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 Chlamydia DNA in the specimen. An RLU measurement less than a specified ratio to the positive Cutoff Value indicates the absence of Chlamydia DNA or Chlamydia DNA levels below the assay's detection limit.

The CT Probe contains a probe mixture specifically chosen to eliminate or minimize cross-reactivity with DNA sequences from human cells, other bacterial species, or Chlamydia species other than *Chlamydia trachomatis*. The CT Probe supplied with the *digene* HC2 CT-ID DNA Test is complementary to approximately 39,300 bp or 4% of the Chlamydia genomic DNA (1×10^6 bp).[3] One probe is complementary to 100% of the cryptic plasmid of 7,500 bp.

High-volume sample-throughput testing with the *digene* HC2 CT-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 CT-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 CT-ID DNA Test kit (REF 5135-1330). 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
CT Probe PROBE | CT CT 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
CT Positive Calibrator (PC) CAL | CT | + 1.0 pg/ml cloned CT 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 CT-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 CT-ID 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 (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 Washer

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 the *digene* HC2 CT-ID DNA Test 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 CT-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.[13,14,15,16] 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.[17,18]
5. Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with national and local regulations.[19,20]

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


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.
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 CT-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 are provided ready-to-use except Denaturation Reagent, CT Probe Mix and Wash Buffer.

Refer to the *Rapid Capture System User Manual* for the preparation of the CT 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>CT Probe Mix (Prepared from CT 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 CT 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 CT 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 CT 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 CT 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 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).
- Seal 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 CT-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 performed. See the *digene* HC2 DNA Collection Device instructions for use for additional specimen collection and handling procedures.

CERVICAL SPECIMENS IN *digene* 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 should 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 CT 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 CT; however, 5 contained a high level of GC 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 PreservCyt Solution for use in making Hologic ThinPrep Pap Test slides can be used with the *digene* HC2 CT-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 CT-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 CT-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 CT-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 CT-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 CT-ID DNA Test 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 the 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 CT. 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 Multi-Specimen Tube 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 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.[21] However, lab personnel should still adhere to national/local precautions.

CALIBRATORS, QUALITY CONTROLS, AND STM SPECIMEN PREPARATION PROCEDURE

Notes:

- Do not remove the 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, 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 Control, 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 and 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 CT 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 digene 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.

Reagent Preparation

Use either the Denaturation Reagent (DNR) provided with the *digene* HC2 CT-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 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 CT 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 sample, 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 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.

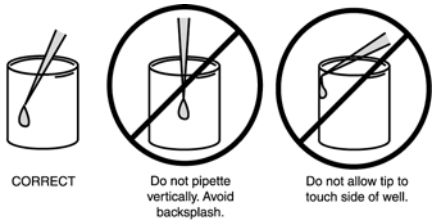
   Notes:

   - 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 the 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 the Microplate Heater I. Immediately remove the plate lid and place on a clean surface.
3. Transfer the entire contents (approximately 100 µl) of the Calibrators, Quality Controls, and specimens from the 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. Prepare Wash Buffer and check Automated Plate Washer rinse and waste reservoirs if applicable during this incubation. 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, it 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 Detection Reagent 1 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 spreadsheet.
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 CT-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 $\geq$ 10 and $\leq$ 150 in order to proceed. The coefficient of variation (%CV) for the Negative Calibrator replicates should be $\leq$ 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 $\leq$ 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 next step. 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 CT-ID DNA Test, the results generated with the *digene* Microplate Luminometer 2000 (DML 2000) during internal studies involving 63 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 106 assays was equal to or lower than 5.8% and the average of the %CV for the Negative Calibrator was equal to or lower than 11.2%. Although a maximum Negative Calibrator mean RLU value of 88 was obtained for manual assays, on average, 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 7.11. In this case, results are acceptable provided the NC RLU remains ≤ 250 and the PC/NC ratio is ≥ 2.0 and ≤ 20. 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	63	7.11	6.87	5.24	10.23	3.8	0.24
Manual	43	6.75	5.70	4.60	11.25	3.5	0.16

Method	Calibrator	RLU Calculated Means				Mean of the Calculated %CV
		Mean	Median	Min	Max	
RCS	Negative	52	50	29	84	9.2
	Positive	362	369	179	505	5.3
Manual	Negative	41	37	28	88	11.2
	Positive	275	274	135	428	5.8

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 CT-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	1.00	0
Maximum RLU/CO	20.00	0.9999
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 CT-ID DNA Test.
2. This quality control material is not the same as CT organism in the sample matrix and will not act as an appropriate quality control for the processing of *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, add >100,000 *C. trachomatis* elementary bodies (Serovars E or J recommended and available from ATCC, as ATCC VR348B and VR886, respectively) to a fresh tube of STM. Incubate for at least 1 hour prior to testing in the same manner as a normal clinical specimen. An RLU/CO $\geq$ 2.50 should be

obtained if the specimen is processed properly. Alternatively, commercially available specimen test panels containing CT organism can also be used for this purpose.

5. Acceptable ranges for the Negative Calibrator and Positive Calibrator have been established only for QIAGEN-approved luminometers. The Negative and Positive Calibrators monitor for substantial reagent failure and will not ensure precision of the assay cutoff.

INTERPRETATION OF SPECIMEN RESULTS

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

1. Specimens with RLU/CO ratios ≥ 2.50 are considered "Positive for *Chlamydia trachomatis* DNA." *Chlamydia trachomatis* 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 *Chlamydia trachomatis* DNA or contain DNA below the assay's detection limit. These should be interpreted as "No *Chlamydia trachomatis* DNA detected." A negative result does not preclude *Chlamydia trachomatis* infection because results depend on adequate specimen collection and sufficient DNA to be detected.
3. Specimens with RLU/CO ratios ≥ 1.00 and < 2.50 are considered equivocal. Results may be considered presumptively positive for *Chlamydia trachomatis* 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/CO values.*
4. It is recommended that positive results be confirmed by another method if the likelihood of *Chlamydia trachomatis* infection is uncertain or questioned. Analytical studies with this test have shown presumptive cross-reactivity to certain other DNA sequences that may cause a false-positive result. Although the frequency with which pBR322 and other DNA sequences are found in genital specimens has not been fully assessed, no pBR322 cross-reactivity was observed in a population of 1818 patients among which 106 CT positive specimens were tested for the presence of pBR322. This is a representative population and suggests that these findings may not reflect the frequency of occurrence of pBR322 in all tested populations. See Analytical Specificity for additional information.

* During the clinical evaluation of the *digene* HC2 CT-ID DNA Test, 7/14 results in this equivocal range were verified positive by culture, DFA or Polymerase Chain Reaction (PCR) testing; the remaining 7 were apparent false positive. However, these 7 false-positive specimens were among a total of only 11 specimens not found negative by the *digene* HC2 CT-ID DNA Test out of the 1643 specimens verified not to contain CT by culture (99.3% correctly identified relative to Culture/DFA when considering PCR test results). In a subsequent evaluation, these 7 initial positive specimens had an initial RLU/CO of 1.00-2.50 with 3 of these specimens negative by all other testing done (all 3 of these specimens were negative when repeated twice with the *digene* HC2 CT-ID DNA Test). For the remaining 4 specimens, all were culture/DFA/PCR positive and both repeat *digene* HC2 CT-ID DNA Test replicates were ≥1.00 RLU/CO.

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 CT-ID DNA Test Procedure, Quality Control and Interpretation of Specimen Results must be followed closely to obtain reliable test results.
- The *digene* HC2 CT-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 placed in PreservCyt Solution.
- Results of this assay should be interpreted only in conjunction with information available from clinical evaluation of the patient and from other procedures.

- The *digene* HC2 CT-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 CT DNA present in the patient specimen.
- A negative result does not exclude the possibility of *Chlamydia trachomatis* 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 *Chlamydia trachomatis* strain.
- As is true for all non-culture methods, a positive specimen cannot be interpreted as indicating the presence of viable *Chlamydia trachomatis*.
- The *digene* HC2 CT-ID DNA Test is not intended to determine therapeutic success.
- The *digene* HC2 CT-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 CT-ID DNA Test.
- In order to minimize variability of the results obtained with the *digene* HC2 CT-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 CT organism or CT DNA be tested periodically, consistent with the establishments' Quality Procedures.

EXPECTED RESULTS

PREVALENCE

The prevalence of specimens positive for *Chlamydia trachomatis* varies depending on population characteristics such as age, sex, and risk factors. The prevalence of *Chlamydia trachomatis* observed in the clinical study population using the *digene* HC2 CT-ID DNA Test ranged from 3.3% to 14.6%. The prevalence was calculated assuming that the 14 specimens with equivocal results in the study were positive for CT DNA (Table 4). Seven of these 14 specimens were verified positive by CT culture/DFA or CT PCR.

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

Test Site	No. Positive/No. Tested	% Prevalence
1	67/460	14.6
2	42/307	13.7
3	38/308	12.3
4	23/414	5.6
5	11/329	3.3
Total	181/1818	10.0

POSITIVE AND NEGATIVE PREDICTIVE VALUES

The hypothetical positive and negative predictive values (PPV and NPV) for different prevalence rates using the *digene* HC2 CT-ID DNA Test were calculated using the overall sensitivity and specificity relative to CT culture/DFA 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 97.71% and specificity 98.15%) and Table 6 represents the hypothetical PPV and NPV for swab specimens (overall sensitivity 87.50% and specificity 98.36%).

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

Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
5	97.7	98.2	73.5	99.9
10	97.7	98.2	85.4	99.7
15	97.7	98.2	90.3	96.6
20	97.7	98.2	93.0	99.4

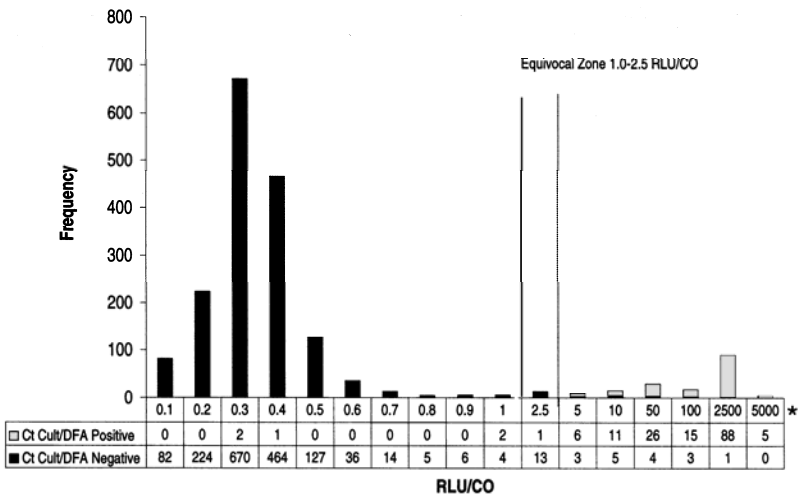
Table 6. *digene* HC2 CT-ID Test Hypothetical Predictive Values at Different Prevalence Rates (Swab).

Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
5	87.5	98.4	73.4	99.4
10	87.5	98.4	83.2	98.9
15	87.5	98.4	87.2	98.4
20	87.5	98.4	89.3	97.9

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

The distribution of the *digene* HC2 CT-ID DNA Test RLU/CO ratios observed during the multicenter clinical study are indicated below (Figure 1). These data include specimens for which the *digene* HC2 CT-ID DNA Test was performed and CT culture/DFA results were available (n=1818). 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/Cutoff Value ratios ≥ 1.00 and < 2.50 were considered equivocal.

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



	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 *
□ Ct Cult/DFA Positive	0	0	2	1	0	0	0	0	0	2	1	6	11	26	15	88	5
■ Ct Cult/DFA Negative	82	224	670	464	127	36	14	5	6	4	13	3	5	4	3	1	0

Distinct separation of the RLU/CO ratios is observed between *digene* HC2 CT-ID DNA Test positive results and *digene* HC2 CT-ID DNA Test negative results. Ninety-nine percent (99%, 1620/1637) of the *digene* HC2 CT-ID DNA Test negative results have RLU/CO Values between 0 and 0.7. Overall, < 1% (14/1818) of the specimen results fall in the assay's equivocal zone, 7.1% (1/14) of which were positive by CT Culture/DFA and an additional 6 of which (46%) were positive by CT PCR. Eighty-five percent (85%, 142/167) of the *digene* HC2 CT-ID DNA Test positive results have RLU/CO Values between 10 and 5000.

PERFORMANCE CHARACTERISTICS

CLINICAL TRIAL RESULTS BY SPECIMEN

digene HC2 CT-ID DNA Test performance characteristics were determined by comparing the assay results to results of Chlamydia culture and DFA. One-thousand eight-hundred and eighteen (1818) specimens were collected and later tested from patients at 5 different sites including STD, Family Planning and OB/GYN clinics. DFA testing was performed on the sediment of the CT culture transport medium after centrifugation for specimens that were *digene* HC2 CT-ID DNA Test-positive/culture-negative. PCR testing was then performed on *digene* HC2 CT-ID DNA Test-positive/culture-negative/DFA-negative specimens. *digene* HC2 CT-ID DNA Test results were NOT resolved by PCR test results, and, therefore, PCR had no impact on the calculations of the *digene* HC2 CT-ID DNA Test performance characteristics. Two different models of luminometer (Dynex Models MLX and ML2200) were used to generate the data used to determine the performance characteristics of the *digene* HC2 CT-ID DNA Test. 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.

Table 7. *digene* HC2 CT-ID DNA Test versus CT Culture/DFA Results for Brush Specimens.

Performance Characteristics calculated utilizing RLU/CO cutoff values of 1.0 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	CT-ID : Culture: DFA: n	POS POS N/A	POS NEG POS	POS NEG NEG[1]	NEG POS N/A	NEG NEG N/A[3]	Sensitivity	Specificity	NPV	PPV	CT-ID+ Cul- DFA- PCR[2]+
Symptomatic											
1	351	42	5	7 (4)	2	295 (298)	95.92	97.68 (98.68)	99.33	87.04 (92.16)	5/7 (3/4)
95% CI							86.0-99.5	95.3-99.6	97.6-99.9	75.1-97.8	
2	192	11	5	6 (5)	0	170 (171)	100.00	96.59 (97.16)	100	72.73 (76.19)	6/6 (5/5)
95% CI							79.4-100	92.7-99.1	97.9-100	49.8-91.8	
3	219	34	0	3 (1)	1	181 (183)	97.14	98.38 (99.46)	99.45 (99.46)	91.89 (97.14)	1/2[4] (1/1)
95% CI							81.5-100	94.4-100	97.0-100	78.1-99.9	
4	177	6	3 (2)	0	0 (1)	168	100.00 (88.89)	100.00	100.00 (99.41)	100.00	N/A
95% CI							51.8-100	97.8-100	96.8-100	63.1-100	
All	**939**	**93**	**13 (12)**	**16 (10)**	**3 (4)**	**814 (820)**	**97.25 (96.33)**	**98.07 (98.80)**	**99.63 (99.51)**	**86.89 (91.30)**	**12/15[4] (9/10)**
95% CI							**90.9-99.4**	**96.9-98.9**	**98.8-99.9**	**79.6-95.8**	
Asymptomatic											
1	101	8	0	2 (0)	0	91 (93)	100.00	97.85 (100.00)	100.00	80.00 (100.00)	0/2 (N/A)
95% CI							63.1-100	92.5-100	96.0-100	44.4-100	
2	12	1	0	1	0	10	100.00	90.91	100.00	50.00	1/1
95% CI							2.50-100	58.7-99.8	69.2-100	1.3-98.7	
3	81	3	0	0	0	78	100.00	100.00	100.00	100.00	N/A
95% CI							29.2-100	95.4-100	95.4-100	29.2-100	
4	236	9	1	4 (2)	0	222 (224)	100.00	98.23 (99.12)	100.00	71.43 (83.33)	3/4[4] (1/1[4])
95% CI							69.2-100	95.5-99.9	98.4-100	41.9-97.9	
5	1	0	0	0	0	1	N/A	100.00	100.00	N/A	N/A
95% CI							2.5-100	2.5-100	2.5-100		
All	**431**	**21**	**1**	**7 (3)**	**0**	**402 (406)**	**100.00**	**98.29 (99.27)**	**100**	**75.86 (88.00)**	**4/7[4] (2/3[4])**
95% CI							**84.6-100**	**96.5-99.9**	**99.1-100**	**56.5-97.5**	
Total Patient Population											
95% CI 1	452	50	5	9 (4)	2	386 (391)	96.49	97.72 (98.99)	99.48 (99.49)	85.90 (93.22)	5/9 (3/4)
							87.9-99.6	95.7-99.7	98.2-99.9	75.0-98.1	
95% CI 2	204	12	5	7 (6)	0	180 (181)	100.00	96.26 (96.79)	100.00	70.83 (73.91)	7/7 (6/6)
							80.5-100	92.4-98.8	98.0-100	48.9-89.8	
95% CI 3	300	37	0	3 (1)	1	259 (261)	97.37	98.86 (99.62)	99.62	92.50 (97.37)	1/2[4] (1/1)
							86.2-99.9	96.7-100	97.9-100	79.6-99.9	
95% CI 4	413	15	4 (3)	4 (2)	0 (1)	390 (392)	100.00 (94.74)	98.98 (99.49)	100.00 (99.75)	82.61 (90.00)	3/3[4] (1/1[4])
							74.0-100	97.4-99.9	98.6-100	61.2-98.8	
95% CI 5	1	0	0	0	0	1	N/A	100.00	100.00	N/A	N/A
								2.5-100	2.5-100		
All	**1370**	**114**	**14 (13)**	**23 (13)**	**3 (4)**	**1216 (1226)**	**97.71 (96.85)**	**98.15 (98.95)**	**99.75 (99.67)**	**84.77 (90.71)**	**16/21[5] (11/12[4])**
95% CI							**92.4-99.5**	**97.2-99.4**	**99.2-100**	**78.0-95.0**	

1 In two cases, DFA was required but not done.
2 This information is provided for information only; specimen results were not resolved using PCR.
3 One *digene* HC2 CT-ID DNA Test negative culture negative specimen was unnecessarily tested by DFA and gave a positive result. This result was included in the performance calculations as a *digene* HC2 CT-ID DNA Test false negative.
4 PCR was not done on one specimen.
5 In one case, DFA was required but not performed.
NA = Not Applicable.

Table 8. *digene* HC2 CT-ID DNA Test versus CT Culture/DFA Results for Swab Specimens.
Performance Characteristics calculated utilizing RLU/CO cutoff values of 1.0 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	CT-ID: Culture: DFA: n	POS POS N/A	POS NEG POS	POS NEG NEG[1]	NEG POS N/A	NEG NEG N/A[3]	Sensitivity	Specificity	NPV	PPV	CT-ID+ Cul- DFA- PCR[2]+
Symptomatic												
	1	358	31 (28)	0	5 (3)	7 (10)	315 (317)	81.58 (73.68)	98.44 (99.06)	97.83 (96.94)	86.11 (90.32)	N/A
95% CI								65.67-92.26	96.39-99.49	95.57-99.12	70.50-95.33	
	2	94	10	1	3 (1)	1	79 (81)	91.67	96.34 (98.78)	98.75 (98.78)	78.57 (91.67)	2/3 (1/1)
95% CI								61.5-99.8	89.6-100	93.2-100	49.2-100	
	3	5	1	0	0	0	4	100.00	100.00	100.00	100.00	N/A
95% CI								0.84-90.6	47.8-100	29.0-96.3	2.5-100	
	5	152	7	0	2 (1)	0	143 (144)	100.00	98.62 (99.31)	100.00	77.78 (87.50)	0/0[4] (0/0[3])
95% CI								59.0-100	95.1-100	97.5-100	40.0-99.7	
	All	**609**	**49 (46)**	**1**	**10 (5)**	**8 (11)**	**541 (546)**	**86.21 (81.03)**	**98.19 (99.09)**	**98.54 (98.03)**	**83.33 (90.38)**	**2/3[4] (1/1[3])**
95% CI								**74.62-93.85**	**96.69-99.13**	**97.15-99.37**	**71.48-91.71**	
Asymptomatic												
	1	61	4 (3)	0	2 (1)	0 (1)	55 (56)	100 (75.00)	96.49 (98.25)	100 (98.25)	66.67 (75.00)	N/A
95% CI								39.76-100	87.89-99.57	93.51-100	22.28-95.67	
	2	10	0	0	0	0	10	N/A	100.00	100.00	N/A	N/A
95% CI									69.2-100	69.2-100		
	3	2	0	0	1	0	1	N/A	50.00	100.00	0.00	N/A[3]
95% CI									1.3-98.7	2.5-100	0-97.5	
	4	1	0	0	0	0	1	N/A	100.00	100.00	N/A	N/A
95% CI									2.5-100	2.5-100		
	5	176	2	0	0	0	174	100.00	100.00	100.00	100.00	N/A
95% CI								15.8-100	97.9-100	97.9-100	15.8-100	
	All	**250**	**6 (5)**	**0**	**3(2)**	**0 (1)**	**241 (242)**	**100 (83.33)**	**98.77 (99.18)**	**100 (99.59)**	**66.67 (71.43)**	**N/A[3]**
95% CI								**54.07-100**	**96.45-99.75**	**98.48-100**	**29.93-92.51**	
Total Patient Population												
	1	419	35 (31)	0	7 (4)	7 (11)	370 (373)	83.33 (73.81)	98.14 (98.94)	98.14 (97.14)	83.33 (88.57)	N/A
95% CI								68.64-93.03	96.21-99.25	96.21-99.25	68.64-93.03	
	2	104	10	1	3 (1)	1	89 (91)	91.67	96.74 (98.78)	98.89 (91.67)	78.57 (98.78)	2/3 (1/1)
95% CI								61.5-99.8	90.8-100	94.0-100	49.2-99.8	
	3	7	1	0	1	0	5	100.00	83.33	100.00	50.00	N/A[3]
95% CI								2.5-100	35.9-99.6	47.8-100	1.3-98.7	
	4	1	0	0	0	0	1	N/A	100.00	100.00	N/A	N/A
95% CI									2.5-100	2.5-100		
	5	328	9	0	2 (1)	0	317 (318)	100.00	99.37 (99.69)	100.00	81.82 (90.00)	N/A[4]
95% CI								66.4-100	97.8-100	98.8-100	48.2-99.8	
	All	**859**	**55 (51)**	**1**	**13 (7)**	**8 (12)**	**782 (788)**	**87.50 (81.25)**	**98.36 (99.12)**	**98.99 (98.50)**	**81.16 (88.14)**	**2/3[5] (1/1[4])**
95% CI								**76.85-94.45**	**97.22-99.13**	**98.01-99.56**	**69.94-89.57**	

1 Any specimens that required DFA but it was not performed, were placed in this category.
2 This information is provided for information only; specimen results were not resolved using PCR.
3 In one case, PCR was not done.
4 In two cases, PCR was not done.
5 In three cases, PCR was not done.
NA = Not Applicable

The performance characteristics of the *digene* HC2 CT-ID DNA Test were calculated applying both a 1.0 and 2.5 cut-off without consideration of the presumptive positive specimens falling in the equivocal zone described in the "Interpretation of Results" section of these instructions of use. Therefore, the performance of the *digene* HC2 CT-ID DNA Test may vary in your laboratory depending on the distribution of values that fall within the equivocal zone. Repeat testing of presumptive-positive (equivocal zone) specimens may be performed as recommended in the *Interpretation of Results* section of these instructions for use (Criteria 3). As a point of reference, less than 0.8% of the specimens (14/1818) tested during the Multicenter Clinical Study used to establish the *digene* HC2 CT-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 of use for additional information.

Sufficient data have not been generated to estimate equivalent sensitivity and positive predictive value of the *digene* HC2 CT-ID DNA Test using the *digene* Female Swab Specimen Collection Kit compared 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 CT DNA may be reduced in this population of patients or whenever a *digene* Female Swab Specimen Collection Kit is used for specimen collection.

The clinical sensitivity and specificity of the *digene* HC2 CT-ID DNA Test for detecting those patients with clinically active infection that can be transmitted to partners or cause Chlamydia-related sequelae has not been determined in comparison to commercially-available Nucleic Acid Amplification (NAA) methods for the detection of CT DNA. In clinical studies, testing with a modified commercial NAA assay showed positivity in 12 *digene* HC2 CT-ID DNA Test positive and 6 presumptive positive specimens obtained from 24 CT culture -DFA negative patients; however, the 1637 *digene* HC2 CT-ID DNA Test negative specimens in that study and 5 of the *digene* HC2 CT-ID DNA Test positive/CT culture-DFA negative specimens were not tested using this modified NAA. Estimated sensitivity is based on the number of *digene* HC2 CT-ID DNA Test positive results found in patients who were culture or DFA positive for *Chlamydia trachomatis*. Therefore, the *digene* HC2 CT-ID DNA Test sensitivity can only be deduced relative to culture/DFA positivity, which may have a sensitivity of 60-85%. In addition, several studies have been conducted by various independent research groups showing the performance of the *digene* HC2 CT-ID DNA Test compared to commercially available and research NAA tests.[22]

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 CT-ID DNA Test using a panel composed of *Chlamydia trachomatis* DNA targets and *digene* HC2 CT-ID DNA Test positive and *digene* HC2 CT-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. 99.9% agreement was observed for the 1152 expected positive results (1151/1152) and 99.6% agreement was observed for the 288 expected negative results (287/288). Overall agreement was 99.9% (1438/1440), with a 95% confidence interval of 99.5-99.9 and kappa = 0.996. 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 CT-ID DNA Test in a Multicenter Trial.

Target Number	Site 1 $\bar{X}$RLU /CO	Site 1 % Agree	Site 2 $\bar{X}$RLU /CO	Site 2 % Agree	Site 3 $\bar{X}$RLU /CO	Site 3 % Agree	Site 4 $\bar{X}$RLU /CO	Site 4 % Agree	Total $\bar{X}$RLU /CO	Total Observed/ Expected	Total % Agree
1	3.7	100	3.2	100	4.1	100	4.2	100	3.8	144/144	100
2	6.7	100	6.0	100	7.4	100	9.8	100	7.5	144/144	100
3	34.2	100	29.3	100	38.6	100	42.8	100	36.2	144/144	100
4	61.9	100	55.0	100	69.4	100	79.1	100	66.4	144/144	100
5	2.7	100	2.5	100	3.2	100	3.4	100	3.0	144/144	100
6	6.4	100	5.4	100	7.4	100	7.4	100	6.6	144/144	100
7	13.9	100	12.0	100	16.0	100	16.3	100	14.5	144/144	100
8	17.3	100	14.8	100	19.2	97.2	23.2	100	18.6	143/144	99.3
9	0.3	100	0.2	100	0.2	100	0.2	100	0.2	144/144	100
10	0.3	100	0.3	97.2	0.3	100	0.2	100	0.3	143/144	99.3
TOTAL										1438/1440	99.9

A second proficiency/reproducibility study using whole *Chlamydia trachomatis* (CT) organism spiked into a mock clinical specimen matrix of epithelial cells was conducted at three external sites. The specimens tested contained representatives of negative, low (at or near the limit of detection), and medium positives with 2 CT serovars, mixed infections with *Neisseria gonorrhoeae* (GC) 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 CT-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 CT-ID DNA Test Reproducibility Study Results.

		Observed vs. Expected			% Agreement		
				Positive	All Specimens		Excluding Equivocal Specimens
Site	n	Negative	Equivocal	Positive (≥2.5)	@1.0 Cutoff*	@2.5 Cutoff	@2.5 Cutoff
1	25	13	7	5	25/25 (100%)	18/25 (72%)	18/18 (100%)
2	25	13	3	9	25/25 (100%)	22/25 (88%)	22/22 (100%)
3	25	13	2	10	25/25 (100%)	23/25 (92%)	23/23 (100%)
Total	75	39	12	24	75/75 (100%)	63/75 (84%)	63/63 (100%)

*The same values were obtained when interpreting results as "presumptive positive" at a 2.5 cutoff.

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

Statistical Measure	Site 1	Site 2	Site 3	Site 4
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 11, all of which contained low concentrations of CT 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 CT 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 CT 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.

Site	Specimen ID	*digene* HC2 CT/GC DNA Test Result RLU/CO	Interpretation	Expected Result
1	1	3.63	**POS**	**POS**
	2	0.14	NEG	NEG
	3	0.17	NEG	NEG
	4	0.14	NEG	NEG
	5	0.21	NEG	NEG
2	1	1.79	**EQUIV***	**POS**
	2	0.11	NEG	NEG
	3	0.10	NEG	NEG
	4	0.09	NEG	NEG
	5	0.14	NEG	NEG
3	1	3.24	**POS**	**POS**
	2	0.15	NEG	NEG
	3	0.14	NEG	NEG
	4	0.14	NEG	NEG
	5	0.13	NEG	NEG
4	1	1.87	**EQUIV***	**POS**
	2	0.15	NEG	NEG
	3	0.53	NEG	NEG
	4	0.14	NEG	NEG
	5	0.15	NEG	NEG

*Interpreted as presumptive positive.

PRECISION

A precision study was performed at three sites to determine the within-assay and total precision of the *digene* HC2 CT-ID DNA Test using a panel of positive and negative masked, simulated, clinical STM 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 CT-ID DNA Test, and the MLX luminometer; the MLX was one of the luminometer models used during the clinical evaluation that is no longer available. Upon initial testing, two of the sites yielded acceptable results. However, one site experienced difficulties that were attributable to assay technique due most likely to technical error caused by improper or inadequate training. The technician who performed the testing at this site had been trained in proper assay technique; however, this person had not performed any *digene* HC2 CT-ID DNA Test testing for over 6 months.

Table 13 shows the performance of the *digene* HC2 CT-ID DNA Test including the site that experienced technical problems. The technician was retrained in the proper assay technique and the testing was repeated. The precision data showing significant improvement in assay performance are shown in Table 14.

Table 13. Within Instrument, Between Instrument, Within Assay, Total Precision Estimates For RLU/CO by Target Prior to Technologist Retraining.

			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	17.6152	2.7418	15.5647	0.6011	3.4123	45.8628	260.3593	53.8172	305.5160
2	54	6.9076	0.8102	11.7297	0.2198	3.1819	17.9588	259.9861	20.9987	303.9941
3	54	3.0293	0.0969	3.1981	0.0930	3.0685	0.6870	22.6801	0.6739	22.2459
4	54	5.4674	0.3348	6.1231	0.1485	2.7156	10.0455	183.7341	11.4415	209.2673
5	54	13.6956	0.4045	2.9536	0.5280	3.8555	1.7475	12.7599	1.8065	13.1904
6	54	16.9526	0.7011	4.1359	0.6187	3.6497	22.1095	130.4199	25.9379	153.0027

The precision results for the combined sites are shown in Table 14. 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 after proper training of all technicians performing the *digene* HC2 CT-ID DNA Test.

Table 14. Within Instrument, Between Instrument, Within Assay, Total Precision Estimates For RLU/CO by Target After Technologist Retraining.

			Within Instrument		Between Instrument		Within Assay		Total	
Panel Member	n	Mean	Standard Deviation (SD)	(%CV)	(SD)	(%CV)	(SD)	(%CV)	(SD)	(%CV)
1	54	0.1441	0.0224	15.5507	0.0000	0.0000	0.0603	41.8765	0.0629	43.6874
2	54	0.1256	0.0212	16.8771	0.0000	0.0000	0.0210	16.7125	0.0234	18.6069
3	54	2.7720	0.0996	3.5933	0.0888	3.2046	0.4732	17.0719	0.4749	17.1332
4	54	1.8643	0.0647	3.4683	0.0635	3.4051	0.4015	21.5358	0.3956	21.2227
5	54	13.2050	0.4129	3.1266	0.5281	3.9989	1.7018	12.8873	1.6604	12.5743
6	54	7.8674	0.2725	3.4633	0.3946	5.0157	1.5361	19.5250	1.5118	19.2160

For panel members 3 and 4, both of which contained low concentrations of CT 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.

An additional precision study was performed at QIAGEN to determine the total precision of the *digene* HC2 CT-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 CT-ID DNA Test compiled for all five days of testing are presented in Table 15. Although not evident from these tables, the qualitative interpretation of results was 100% in agreement with the expected result (120/120; 97.0-100% 95% CI), when using an RLU/CO of 1.00.

Table 15. Total Precision for *digene* HC2 CT-ID DNA Test.

Panel Member	n	Mean RLU/CO	SD	CV%	Mean -2xSD	Mean +2xSD
1	120	0.15	0.0326	21.24	0.09	0.22
2	120	0.16	0.0479	29.25	0.07	0.26
3	120	3.07	0.7078	23.05	1.66	4.49
4	120	4.00	0.5585	13.97	2.88	5.12
5	120	11.61	1.6955	14.60	8.22	15.00
6	120	12.01	1.9818	16.50	8.05	15.98

PRECISION WITH PRESERVCYT SOLUTION 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 CT (Serovar D; ATCC VR885) to create a panel, as shown in Table 16.

Table 16. Precision Panel Composition.

Bulk Specimen	**Panel Members***	**Expected *digene* HC2 CT/GC DNA Test Result**	**Approximate RLU/CO**
A	1P, 2P, 7P, 8P	Low CT- positive	~5
B	3P, 4P, 9P, 10P	Mid CT-positive	~10
C	5N, 11N	Negative	~0.20
D	6N, 12N	High Negative	~0.70

*Specimen identifier indicates known *C. trachomatis* status [positive (P) or negative (N)]

In accordance with the NCCLS guideline EP-12A for the evaluation of qualitative *in vitro* diagnostic tests, panel members 6N and 12N that were both derived from specimen bulk "D" were included to assess precision just below the negative assay cutoff value of 1.0 RLU/CO.

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

Table 17. *digene* HC2 CT-ID DNA Test Procedure Qualitative Results by Bulk Specimen.

Bulk Specimen Pool	CT Positive n (%)	Equivocal n (%)	Negative n (%)	Total
Negative (5N, 11N)	0 (0.0)	0 (0.0)	108 (100)	108
High Negative (6N, 12N)	0 (0.0)	12 (11.2)	90 (88.8)	108
Total Negative	0 (0.0)	12 (5.6)	204 (94.4)	216
CT Low Positive (1P, 2P, 7P, 8P)	216 (100)	0 (0.0)	0 (0.0)	216
CT 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 18. Standard Deviations (SD) and Coefficients of Variation (CV) for Precision By Laboratory and Day: *digene* HC2 CT-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	0.215	0.038	0.020	0.010	0.037	0.058	27.0
High Negative (6N, 12N)	108	0.648	0.304	0.210	0*	0	0.370	57.1
CT Mid Positive (2P,3P,8P,9P)	216	12.64	1.444	0.733	1.013	1.070	2.189	17.3
CT Low Positive (1P, 2P, 7P, 8P)	216	4.637	0.490	0.485	0.285	0.288	0.800	17.3

*Negative variance components were set to zero.

ANALYTICAL SENSITIVITY

The analytical sensitivity (limits of detection) of the *digene* HC2 CT-ID DNA Test was determined by directly testing dilutions of a nonclinical panel consisting of 15 serovars of *Chlamydia trachomatis* as well as *Chlamydia psittaci* and *Chlamydia pneumoniae*. Four-point dilution series of each of the serovars were tested using the *digene* HC2 CT-ID DNA Test to determine the organism load estimate of the highest dilution yielding a positive result with the *digene* HC2 CT-ID DNA Test. Each concentration of each target type was tested in triplicate following the *digene* HC2 CT-ID DNA Test instructions for use.

The limit of detection for each Chlamydia serovar is summarized in Table 19. The detectable limit stated was the dilution of each serovar that was detected within or above the assay's equivocal zone of 1.0-2.5 RLU/CO. The detectable limit ranged from 1,000 to 500,000 EBs/mL depending on the serovar tested. Detection of 50 to 25,000 EBs in each test is equivalent to 1,000 to 500,000 EBs in the original specimen (per mL STM).

The most common CT serovars in the United States for asymptomatic women less than 30 years old are E, I, and D (in decreasing order).[23] For women aged 17-68 who were attending an inner city gynecological clinic, the most prevalent CT serovars encountered were F, E and G (in decreasing order). It is important to note that for all of the most commonly encountered CT serovars except serovar E, the *digene* HC2 CT-ID DNA Test lower limit of detection was 50 EBs/assay; serovar E has a higher limit of detection (2500 EBs/assay) as described earlier. The authors of this paper further suggest that certain serovars might be associated with symptomatic (i.e., serovar G) or asymptomatic (i.e., serovars D and I) infections. Again, for these serovars, the *digene* HC2 CT-ID DNA Test demonstrated a lower limit of detection of 50 EBs/assay.

Table 19. Summary of Detectable Limits of Sensitivity for CT Serovars.

Serovar	Detectable Concentration	
	EBs/ml	EBs/test
A	1000 - >10,000	50 - >500
B	10,000 - 100, 000	500 - 5000
Ba	5000 - 50,000	250 - 2500
C	10,000	500
D	1000 - 10,000	50 - 500
E	50,000	2500
F	1000	50
G	1000 - 10,000	50 - 500
H	10,000 - 100,000	500 - 5000
I	1000 - 10,000	50 - 500
J	5000 - 500,000	2500 - 25,000
K	20,000	1000
L1	2000	100
L2	2000 - 20,000	100 - 1000
L3	10,000	500

ADDITIONAL CONSIDERATIONS FOR PRESERVCYT SOLUTION 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 CT-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 CT-ID DNA Test. To assess the potential impact of centrifugation on analytical sensitivity, eighty-eight (88) pairs of *C. trachomatis* DNA-negative STM and PreservCyt Solution specimens were prepared with matching amounts of CT (serovar G) organism. The paired specimens were tested and analytical sensitivity was estimated by comparing the mean RLU/CO values obtained [(PreservCyt:STM) x 100].

A paired-T test of the data in Table 20 demonstrates that the analytical sensitivity of the *digene* HC2 CT-ID DNA Test is not statistically different (p = 0.33) when testing cervical specimens from either PreservCyt Solution or STM.

Table 20. Comparison of Analytical Sensitivity - *digene* HC2 CT-ID DNA Test - Paired PreservCyt Solution and STM Specimens.

	digene HC2 C**T-ID DNA Test RLU/CO**	
	STM	**PreservCyt**
Number of Specimens	88	88
Mean RLU/CO	3.38	3.48
Median RLU/CO	3.41	3.44
Standard Deviation	0.41	0.54
Maximum RLU/CO	4.42	5.01
Minimum RLU/CO	2.44	2.27

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 CT-ID DNA Test to identify positive specimens. These positive patient specimens were then combined to generate a total of 10 concentrated specimen PreservCyt Solution 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 Solution. Both aliquots were tested with the *digene* HC2 CT-ID DNA Test with the following results:

A paired-T test of the data in Table 21 demonstrates that the analytical sensitivity of the *digene* HC2 CT-ID DNA Test is not statistically different (p = .98) when testing cervical specimens from either PreservCyt Solution or STM.

Table 21. Comparison of Analytical Sensitivity - *digene* HC2 CT-ID DNA Test - Simulated Patient PreservCyt Solution Specimens Paired with STM.

	***digene* HC2 CT-ID DNA Test**	
	STM (Aliquot A)	**PreservCyt (Aliquot B)**
Number of Specimens	10	10
Mean RLU/CO	30.92	24.90
Median RLU/CO	3.56	3.00
Standard Deviation	47.27	38.91
Maximum RLU/CO	125.62	115.08
Minimum RLU/CO	1.15	1.26

ANALYTICAL SPECIFICITY

A battery of bacteria, viruses and plasmids potentially found in the female anogenital tract were tested to determine if cross-reactivity would occur with the probes used in the *digene* HC2 CT-ID DNA Test. All microorganisms were tested at concentrations of 10^5 and 10^7 organisms per ml, and when possible at 10^9 organisms per ml. Purified DNAs of viruses and plasmids were tested at a concentration of 4 ng per ml.

The bacteria tested with the *digene* HC2 CT-ID DNA Test are presented in Table 22. All bacteria except *Chlamydia psittaci* tested negative with the *digene* HC2 CT-ID DNA Test. *Chlamydia psittaci* may be detected from the skin of some people who work with or handle avian species, but has not been detected in the anogenital tract.[24] Thus, the cross-reactivity observed between *Chlamydia psittaci* and the CT Probe would not be expected to cause a clinically confusing result for anogenital specimens.

The CT Probe did not cross-react with *Neisseria gonorrhoeae*, demonstrating that the probe in the *digene* HC2 CT-ID DNA Test do not cross-react with targets of the GC-ID Probe in the *digene* HC2 GC-ID DNA Test.

Table 22. Microorganisms Tested for Cross-reactivity.

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

[a] Concentrations tested were $1x10^5$, $1x10^7$ and $1x10^8$ organisms/ml.
[b] Both the *E. coli* strain used to grow plasmids (HB101) and a clinical isolate of *E. coli* were tested.
[c] Concentrations tested were $2x10^5$, $2x10^7$ and $2x10^8$ organisms/ml.
[d] Concentrations tested were $2x10^7$, $2x10^8$ and $2x10^9$ organisms/ml.
[e] Concentrations tested were $1x10^5$ and $1x10^6$ organisms/ml.
* ATCC Neisseria strain that has features of both *Neisseria gonorrhoeae* and *Neisseria meningitidis* (ATCC #43831).

The viral or plasmid DNA or human serum tested with the *digene* HC2 CT-ID DNA Test are presented in Table 23. Presumptive cross-reactivity was observed with plasmid vectors pBR322, pGEM® 3Zf and pGEM® 3Zf(-). The presence of these homologous sequences has been reported in human genital

specimens, and false-positive results could occur in the presence of high levels of these bacterial plasmids. Of 106 clinical specimens found positive with the *digene* HC2 CT-ID DNA Test among a total population of 1818 patients, two were identified as having pBR322; however, one specimen was positive by CT culture and DFA and the other by CT DNA PCR. Thus, false-positive results due to homologous pBR322, pGEM3Z and pGEM3Z(-) sequences in these 106 clinical specimens did not occur. The frequency of finding these plasmids in female genital tract specimens has not been fully determined. This is a representative population and may not reflect the frequency of occurrence of pBR322 in all tested populations.

Table 23. Viral or Plasmid DNA or Human Serum Tested for Cross-reactivity.

Cytomegalovirus	Human Whole Blood
Epstein Barr Virus	Human Papillomavirus type 6
Hepatitis B Surface Antigen Positive Serum	Human Papillomavirus type 11
Herpes Simplex I	Human Papillomavirus type 16
Herpes Simplex II	Human Papillomavirus type 18
Human epithelial cells	pGEM®3Z
Human Immunodeficiency Virus (HIV)[a]	pGEM®3Zf(-)
Human Genomic DNA	pBR322
Human Placental DNA	SV40

[a] Concentrations tested were 2×10^6, 2×10^7, and 2×10^8 organisms/ml.

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 CT-ID DNA Test. Whole blood, douche, anti-fungal cream and contraceptive jelly (agents that may commonly be found in cervical specimens) were added at concentrations of 1% and 5% to negative and positive specimens in STM (clinical specimen pools and nonclinical specimens). 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 117 negative clinical specimens showed that undefined substances may slightly, but not materially, increase the signal of *Chlamydia trachomatis* DNA detected by the *digene* HC2 CT-ID DNA Test. This effect is not of concern as it is the opposite of an inhibitory effect.

EFFECT OF BLOOD AND OTHER SUBSTANCES ON PRESERVCYT SOLUTION 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 CT-ID DNA Test in the presence of varying amounts of SCB. Varying concentrations of plasmid CT 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.

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

The reproducibility of the *digene* HC2 CT-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 CT-ID DNA Test. The mean RLU/CO value, 95% confidence intervals about the

[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 Solution specimen. Refer to QIAGEN's *digene* HC2 Sample Conversion Kit instructions for use for specific details.

mean (CIs) and percent positive results were calculated for each specimen over five days and are shown in Table 24.

Table 24. Mean RLU/CO with Confidence Intervals and Percent *digene* HC2 CT-ID DNA Test Positives (Descending Order by Mean RLU/CO).

No.	RLU/CO	95% CI	%Positive
1	2.14	2.06-2.22	100 (20/20)
2	1.43	1.35-1.51	100 (20/20)
3	1.41	1.36-1.47	100 (20/20)
4	1.37	1.26-1.48	90 (18/20)
5	1.31	1.24-1.39	100 (20/20)
6	1.29	1.21-1.36	100 (20/20)
7	1.28	1.20-1.36	95 (19/20)
8	1.19	0.94-1.62	90 (18/20)
9	1.18	1.00-1.37	75 (15/20)
10	1.17	0.62-1.71	30 (6/20)
11	1.15	1.10-1.20	95 (19/20)
12	1.08	1.02-1.13	75 (15/20)
13	1.05	1.00-1.09	65 (13/20)
14	1.04	0.99-1.09	70 (14/20)
15	1.02	0.97-1.06	60 (12/20)
16	0.99	0.95-1.04	45 (9/20)
17	0.93	0.87-1.00	30 (6/20)
18	0.93	0.88-0.99	35 (7/20)
19	0.91	0.85-0.96	25 (5/20)
20	0.91	0.85-0.97	25 (5/20)
21	0.90	0.87-0.93	10 (2/20)
22	0.90	0.84-0.95	25 (5/20)
23	0.86	0.76-0.96	5 (1/20)
24	0.85	0.81-0.88	5 (1/20)
25	0.82	0.77-0.88	10 (2/20)
26	0.80	0.78-0.82	0 (0/20)
27	0.48	0.46-0.50	0 (0/20)
28	0.48	0.46-0.50	0 (0/20)
29	0.45	0.42-0.47	0 (0/20)
30	0.24	0.22-0.25	0 (0/20)

Specimens with a mean RLU/CO of 20% or more above the cutoff were positive 98% of the time, while specimens with a 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 CT-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 70% of the time. Those specimens below the cutoff but within 20% of it remained negative 79% of the time.

These results demonstrate that the *digene* HC2 CT-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 CT-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, the results from the clinical evaluations involving 81 assays performed with the *digene* HC2 CT-ID DNA Test were compiled (Table 25). The results showed that the average %CV for these 81 assays was 6.4% and no assays had Negative Calibrator Mean values in excess of 150 RLUs. Positive Calibrator reproducibility in excess of

25% CV was observed for only 2 out of the 81 assays (2.5%). None of the test assays' %CV remained greater than 25% indicating that all of the assays were valid.

Table 25. Performance of the Positive Calibrator and Negative Calibrator. Combined Data from the Multicenter Clinical Trial and the Precision Study (n = 81 assays).

				Mean of Calculated Mean (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	5.49	Negative	44.89	39.15	26.10	13.75
			Positive	231.41	231.41	7.35	7.35
MLX*	72	5.33	Negative	0.075	0.074	16.59	12.90
			Positive	0.265	0.263	6.34	4.86

*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 1231 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 CT-ID DNA Test. The results of this evaluation are presented in Table 26. 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 26. Summary of Statistical Data for *digene* HC2 CT-ID DNA Test Agreement amongst Paired Cervical Specimens Collected in STM and PreservCyt Solution.

Data Analysis 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	0.92 (0.88, 0.96)	92.16 (94/102) 85.13, 96.55	99.36 (1092/1099) 98.69, 99.74	98.75 (1186/1201) 97.95, 99.30
Equivocal Zone Retest Algorithm*	0.90 (0.86, 0.94)	90.09 (100/111) 82.96, 94.95	99.20 (1111/1120) 98.48, 99.63	98.30 (1211/1231) 97.50, 99.00

*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 CT-ID DNA Test was assessed as part of a clinical evaluation to demonstrate that equivalent *digene* HC2 CT-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 27 below.

Table 27. *digene* HC2 CT-ID DNA Test Percent Agreement – By Site

Site	**Observed vs. Expected[a]**	**% Agreement (95% CI)**
1	60/60	100 (94.04-100)
2	60/60	100 (94.04-100)
3	60/60	100 (94.04-100)
Combined Sites	180/180	100 (97.97-100)

[a]20 members x 3 days x 3 sites

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TROUBLESHOOTING GUIDE

***digene* HC2 CT-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 Calibrator, Quality Control, 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 CT 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.

digene HC2 CT-ID DNA TEST		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTIONS**
	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.
	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 4 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, 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 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). Calibrator, 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.

digene HC2 CT-ID DNA TEST		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTIONS**
	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.
	CT 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 CT Probe hybridization. Refer to the *digene* HC2 Sample Conversion Kit instructions for use for procedural details.
	Probe inadequately mixed or insufficient Probe Mix 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 microplate well. 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 overflowing 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 *Automated Plate Washer User Manual* for instructions on testing for contamination or identifying malfunctions.

***digene* HC2 CT-ID DNA TEST**		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTIONS**
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.
	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 CT probe hybridization.	The denaturation step of the specimen processing procedure must be performed as directed in these instructions for use. 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, 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 CT 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 CT 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 CT 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 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, 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 CT 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 CT-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 the test 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 Detection Reagent 2 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 CT-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 CT Probe Mix. ↓ ↓ ↓	**Multi-Specimen Tube Vortexer 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 CT Probe Mix. ↓
Hybridization	**Microplate Heater I Method** Mix denatured specimen well, and pipette 75 µl of denatured Calibrator, Quality Control, or specimen into microplate wells. ↓ Incubate for 10 minutes at 20-25°C. ↓ Pipette 25 µl CT 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 a 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. Go to the next step. ↓ ↓ ↓ ↓ ↓
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-approve luminometer. ↓ Validate assay and interpret specimen results.	