Instructions For Use

hc2 CT-ID DNA Test Version 2.0

A Nucleic Acid Hybridization Microplate Assay with Signal Amplification for the Chemiluminescent Detection of *Chlamydia trachomatis* (CT) DNA in Cervical Specimens

For use with:

hc2 DNA Collection Device
HC Female Swab Specimen Collection Kit™

KEY CHANGES FROM PREVIOUS PACKAGE INSERT REVISION
1. Dimensions of the Specimen Rack have changed (see *Materials Required but Not Supplied*).

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

IVD

REF 5135-1220

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NAME AND INTENDED USE

The Hybrid Capture® 2 (hc2) CT-ID DNA Test is an *in vitro* nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of *Chlamydia trachomatis* DNA in cervical specimens collected using the hc2 DNA Collection Device (cervical brush and Specimen Transport Medium™ [STM]) and in cervical specimens collected using the Female Swab Specimen Collection Kit™ (Dacron® swab and STM). The hc2 CT-ID DNA Test is indicated for use with symptomatic or asymptomatic women as evidence of infection with *Chlamydia trachomatis*.

The hc2 CT-ID DNA Test may be used alone or as a supplemental test to the hc2 CT/GC DNA Test to detect *Chlamydia trachomatis* DNA in specimens that are positive by the hc2 CT/GC DNA Test.

For high-volume sample-throughput testing, the hc2 CT-ID DNA Test may be performed using the Rapid Capture® System (RCS) Application.

For *In Vitro* Diagnostic Use IVD

SUMMARY AND EXPLANATION

Chlamydia trachomatis is considered to be the most common sexually transmitted bacterial pathogen in developed countries. The annual incidence in the United States is currently estimated at four million new cases, with infections spreading most rapidly among young, sexually active individuals. *Chlamydia trachomatis* infection now accounts for more than one-half of all cases of nongonococcal urethritis (NGU).[1,2] This high prevalence is of concern because asymptomatic infections are common in both men and women, particularly young women where the failure to provide effective treatment during the early stages of infection can lead to serious consequences such as Pelvic Inflammatory Disease (PID) and infertility. The annual number of outpatient visits to physicians for symptomatic PID in the United States alone is over 2.5 million; more than 250,000 women are hospitalized every year with over 100,000 surgical procedures performed due to PID.[3,4] The direct and indirect costs of chlamydial illnesses exceed $2.4 billion per year according to several recent studies.[5,6] These developments have prompted the Centers for Disease Control and Prevention to recommend routine *Chlamydia trachomatis* testing of sexually active, asymptomatic adolescent girls during gynecological examinations, and to further suggest that young adult women aged 20-24 also be screened, particularly if they do not use barrier contraceptives or have new or multiple partners.[7]

Chlamydiae are gram-negative organisms with a two-phase life cycle comprising morphologically distinct infectious and reproductive forms.[8] The *Chlamydia trachomatis* genome is relatively small, measuring approximately 1×10^6 base pairs.[9] 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[10] and is 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[11] or the more sensitive use of direct fluorescent antibody (DFA) staining.[12] However, these methods approach only 70-85% sensitivity when compared to optimal tissue culture techniques.[1] 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.[13] 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.[14] Enzyme immunoassays are also used to detect chlamydial antigens[1] and appear to be slightly more sensitive and slightly less specific than direct fluorescent antibody approaches.[15] 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.[16-18]

PRINCIPLE OF THE PROCEDURE

The hc2 CT-ID DNA Test using 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 cocktail. 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 detection limit of the assay.

The CT Probe Cocktail 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 Cocktail supplied with the hc2 CT-ID DNA Test is complementary to approximately 39,300 bp or 4% of the *Chlamydia* genomic DNA (1 x 10^6 bp).[10] One probe is complementary to 100% of the cryptic plasmid of 7,500 bp.

High-volume sample-throughput testing with the 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 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 hc2 CT-ID DNA Test kit (REF 5135-1220). The number of patient results will vary, depending on the number of uses per kit:

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

1 x 0.35 ml **Indicator Dye** **INDIC**
Contains 0.05% (w/v) sodium azide.

1 x 50 ml **Denaturation Reagent** **REAG** | **DENAT**
Dilute sodium hydroxide (NaOH) solution.

1 x 5 ml **Probe Diluent** **DIL** | **PROBE**
Buffered solution with 0.05% (w/v) sodium azide.

1 x 200 µl **CT Probe** **PROBE** | **CT**
CT RNA probe cocktail in buffered solution.

1 x 2 ml **Negative Calibrator** **CAL** | **-**
Carrier DNA in STM with 0.05% (w/v) sodium azide.

1 x 1 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 **Capture Microplate** **PLATE** | **CAPTURE**
Coated with Goat polyclonal anti-RNA:DNA hybrid antibodies.

1 x 12 ml **Detection Reagent 1** **REAG** | **DET** | **1**
Alkaline phosphatase-conjugated antibodies to RNA:DNA hybrids in buffered solution with 0.05% (w/v) of sodium azide.

1 x 12 ml **Detection Reagent 2** **REAG** | **DET** | **2**
CDP-Star® with Emerald II (chemiluminescent substrate).

1 x 100 ml **Wash Buffer Concentrate** **BUF** | **WASH** | **X 30**
Contains 1.5% (w/v) sodium azide.

*See *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 ("hc2 System"), consisting of a Digene-approved luminometer ("luminometer"), Digene-approved personal computer and computer peripherals (monitor, keyboard, mouse, printer, and printer cable), hc2 System Software ("Digene assay analysis software"), hc2 System Assay Protocols for CT-ID, LumiCheck™ Plate Software, and *Digene Hybrid Capture® 2 System User Guide* or the above-listed equipment with Digene Qualitative Software version 1.3 or earlier ("Digene assay analysis software") and *Digene Qualitative Software User Manual*
Hybrid Capture System Rotary Shaker I
Hybrid Capture System Microplate Heater I
Hybrid Capture System Automated Plate Washer or Wash Apparatus
Hybrid Capture System Multi-Specimen Tube Vortexer 2; Digene Specimen Rack and Rack Lid (optional)[B]
EXPAND-4™ Pipettor and Stand (optional)[C]
Hybrid Capture 2 DNA Collection Device (consists of cervical brush and Specimen Transport Medium)[D]
Hybrid Capture Female Swab Specimen Collection Kit (consists of 2 Dacron swabs and Specimen Transport Medium)[D]
Tube Sealer Dispenser and cutting device (optional, used with the Multi-Specimen Tube Vortexer)
Rapid Capture® System (optional for high-volume sample-throughput testing)[E]
Specimen Collection Tubes
Specimen Collection Tube Rack (to fit specimen collection tubes)
Specimen Tube Rack
Hybridization Microplate
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 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 one Multi-Specimen Tube Vortexer Rack (36 x 21 x 9 cm) or two specimen racks (each 31.7 x 15.2 x 6.4 cm)[B]
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
Eight-channel Pipettor capable of delivering 25-75 µl
Timer
Sodium hypochlorite solution, 0.5% (or 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 eight-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

[A] Only equipment and accessories validated with hc2 CT/GC DNA Tests are available from Digene. Refer to local Digene Representative.
[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 hc2 CT-ID DNA Test were established only with the collection kits indicated.
[E] Refer to *Rapid Capture System User Guide* for instructions specific to the use of that system for high-volume sample-throughput testing with this assay.

WARNINGS AND PRECAUTIONS

READ ALL INSTRUCTIONS CAREFULLY BEFORE USING THE TEST.
Refer to the Glossary of Symbols for explanations of symbols used in labeling.

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 OSHA Standard on Bloodborne Pathogens.[19] Biosafety Level 2[20] or other appropriate biosafety practices[21,22] should be used for 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% sodium hypochlorite, or other suitable disinfectant.[23,24]
5. Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with local regulations.[25,26]

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% sodium hydroxide solution, (3) allow to stand for 16 hours, and (4) flush well with water.

Caution: The Probe Diluent may cause reversible eye irritation. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).


T

The Wash Buffer Concentrate contains sodium azide and is classified per applicable European Community (EC) directives as: Toxic (T). The following are appropriate risk (R) and safety (S) phrases.
R25: Toxic if swallowed.
R32: Contact with acids liberates very toxic gas.
S35: This material and its container must be disposed of in a safe way.
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

The Denaturation Reagent contains sodium hydroxide and is classified per applicable European Community (EC) directives as: Corrosive (C). The following are the appropriate risk (R) and safety (S) phrases.
R35: Causes severe burns.
S35: This material and its container must be disposed of in a safe way.
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).

Refer to the *Rapid Capture System User Guide* 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 on the outer box label.
4. These components have been tested as a unit. **Do not** interchange components from other sources or from different lots.
5. 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 disposable pads **and wear powder-free gloves when performing all assay steps.**
6. Care should be taken 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.**
7. Protect Detection Reagent 2 from prolonged exposure to direct light. Use reagent immediately after aliquoting and avoid direct sunlight.
8. Care should be taken to deliver the correct volumes of reagents to the reaction tubes and microplates at all steps and to mix well after each reagent addition. 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.
9. Multichannel pipetting should be performed using the reverse pipetting technique for dispensing Detection Reagents 1 and 2. Check each pipette tip on the multichannel pipettor for proper fit and filling. See specific directions for use.
10. Care should be taken during washing to ensure that each microwell is thoroughly washed. 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.
11. Allow 60 minutes for the Microplate Heater I to equilibrate to temperature from a cold start. Not allowing for this warm-up period could result in melting of the Hybridization Microplate. Consult *Microplate Heater I Operator's 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 *Rapid Capture System User Guide* 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 five 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 three 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: Extreme care should be taken at this step to prevent RNase contamination of Probe and Probe Mix. Use aerosol-barrier pipette tips for pipetting Probe. Probe Diluent is viscous. Care should be taken 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 run 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 clean, closed container until ready for use. Unused Probe Mix should be discarded.</td></tr>
</table>

Wash Buffer	**PREPARE DURING CAPTURE STEP:** **For the Hybrid Capture System Automated Plate Washer**, the Wash Buffer can be prepared as described below and stored in a clean, closed 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 Operator's 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: 33.3 ml; Amount of Distilled or Deionized Water: 966.7 ml; Final Volume of Wash Buffer: 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 run, 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 Operator's Manual* for additional *Care and Maintenance Instructions*. **For the manual plate washing method:** • Dilute 100 ml Wash Buffer Concentrate with 2.9 L of distilled or deionized water and mix well (final volume should be 3 L). • Close the container to prevent contamination or evaporation. 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. 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 15-30°C before using.

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 eight-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: 96/12; Volume Detection Reagent 1 or 2: 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 hc2 DNA Collection Device (cervical brush and Specimen Transport Medium) or the HC Female Swab Specimen Collection Kit (Dacron® swab and Specimen Transport Medium) are the only specimens recommended for use with the 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. Cervical specimens should be collected prior to the application of acetic acid or iodine if colposcopic examination is performed. *The specimen collection device must not be removed from the tube containing Specimen Transport Medium prior to recapping after collection.*

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 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 Specimen Transport Medium cannot be used for culture or other testing methods.

Specimen stability for two 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 five high-positive specimens that exceeded 10 times the LOD. The remaining 10 specimens were negative for CT. However, five 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. 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 15-30°C.
   - When removing specimens from the freezer for testing, replace caps immediately with Specimen Collection Tube Screw Caps.
2. The hc2 DNA Collection Device must not be used for pregnant women. Collect specimens from pregnant women using the HC Female Swab Specimen Collection Kit only.

TEST PROCEDURE

Specimens may contain infectious agents and should be handled accordingly. The hc2 CT-ID DNA Test can be performed manually as instructed in this package insert or using the Rapid Capture System instrument for high-volume sample-throughput testing.

High-Volume Sample-Throughput Testing Using the RCS Application

The Rapid Capture System is a general use automated pipetting and dilution system that can be used with the 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 done for the manual method of the 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 luminometer system common to both the manual and RCS methods. Each of the hc2 CT-ID DNA Test's procedural steps is performed in the exact sequence as the manual test procedure. The RCS Application allows for the staggered processing of up to four microplates, each plate containing specimens and the required assay Calibrators and Quality Controls. Because the required accessories for the hc2 CT-ID DNA Test and procedural steps are the same, the assay can also be performed manually as described in the next section.

When using the Rapid Capture System, refer to *Rapid Capture System User Guide* provided with the instrument, in addition to this package insert, for necessary procedural and descriptive information.

Manual Method

Setup

1. Allow 60 minutes for the Microplate Heater I to equilibrate to 65 ± 2°C from a cold start. See the *Microplate Heater I Operator's Manual* for details. Confirm a water bath is at 65°C and the water level is high enough to immerse the entire volume in the specimen tubes.
2. 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.
3. Use the Digene assay analysis software with Digene assay protocols for CT-ID to create the assay plate layout. Consult the applicable Digene assay analysis software user guide for details.
4. The Calibrators and Quality Controls must be prepared **fresh** for each run. Mix the Calibrators and Quality Controls well. If using the Multi-Specimen Tube Vortexer, 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.
5. Remove and discard caps from Calibrators, Quality Controls, specimens to be tested. **The Negative Calibrator and Positive Calibrators 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 run in an eight-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. Digene assay analysis software determines the calibrator positions in the microplate. Consult the applicable Digene assay analysis software user guide for proper Calibrator/specimen setup in the software.
6. Caps removed from the specimen tubes are considered potentially infectious. Dispose of in accordance with national/local regulations.

EXAMPLE LAYOUT FOR A RUN OF 24 MICROWELLS:

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

Note: Digene assay analysis software will evaluate both Calibrator and Quality Control results based on their location in the plate to verify the assay run. Proper placement of Calibrators and Quality Controls and selection of the proper software protocol are essential for valid results.

Denaturation

Notes:

- **Warning**: 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.
- Do not remove specimen collection device prior to denaturation.
- 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 three months. A maximum of three freeze-thaw cycles may be performed with a maximum of two 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.**
- To avoid false-positive results, it is critical that all Calibrator, Quality Control, and specimen material come into contact with Denaturation Reagent. Mixing after Denaturation Reagent addition is a critical step. **Make sure the Multi-Specimen Tube Vortexer is set to 100 (maximum speed) and a visible vortex of liquid is observed during mixing such that the liquid washes the entire inner surface of the tube. If performing manual vortexing, make sure that each Calibrator, Quality Control, and specimen is mixed individually by vortexing each for at least five seconds at full speed such that the liquid vortex washes the entire inner surface of the tube followed by inverting the tube one time.**
- Following denaturation and incubation, the specimens are no longer considered infectious.[27] However, lab personnel should still adhere to national/local precautions.

1. Pipette Denaturation Reagent with Indicator Dye into each Calibrator, Quality Control, or 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 local, state and Federal regulations.

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

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

   Multi-Specimen Tube Vortexer Method

   a) Cover the Calibrators/Quality Controls/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 cutter device.
   c) Place the rack on the Multi-Specimen Tube Vortexer, 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 Calibrator, Quality Controls, and specimen tubes with clean specimen collection tube screw caps and mix each tube thoroughly by vortexing individually, at high speed, for five seconds.
   b) Invert each specimen tube one time to wash the inside of the tube, cap and rim, then return tube to rack.

3. 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.*

4. 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, or stored as described in **Notes** above). Prepare CT Probe Mix during this incubation. See *Reagent Preparation and Storage* section.

Hybridization

Notes:

- The CT Probe Mix is viscous. Care should be taken 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 60 minutes prior to use. See the *Microplate Heater I Operator's 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 is being used, vortex the entire rack for a minimum of five seconds on the maximum speed setting. Alternatively, vortex each tube individually for at least five seconds.

3. Pipette 75 µl of each Calibrator, Quality Control, or specimen into the **bottom** of the Hybridization Microplate well following the template 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. It is not necessary to 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.

Notes:

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

- **Important**: Some specimens may contain blood or other biological material that may mask the color changes upon addition of Probe Mix. 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.

4. After transferring the last specimen, cover with Parafilm or plastic 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 eight-channel pipettor and fresh tips for each row. Dispense the volume of Probe into each hybridization well, preventing back splashing. Avoid touching the sides of the wells. Inspect the rack from underneath to verify that all wells have received the appropriate amount of Probe Mix.

   Note: For this step, an eight-channel pipettor capable of delivering 25 µl – 75 µl and equipped with 25 µl – 200 µl tips must be used.

6. Cover the Hybridization Microplate with a plate lid. Shake the Hybridization Microplate on the Hybrid Capture System 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, specimens should be retested.

7. Incubate in a preheated and equilibrated to 65 ± 2°C Microplate Heater I for 60 ± 5 minutes.

Note: When placing the Hybridization Microplate in the Microplate Heater I, care should be taken not to cause splashing.

Hybrid Capture

1. Remove all but the required number of Capture Microplate wells from the plate frame. Return the unused microwells to the original bag and reseal. With a marker, number each column 1, 2, 3. . . and label microplate with an appropriate identifier. The specimens will be added to the wells according to the example layout shown below and/or the template previously prepared under *Setup*:

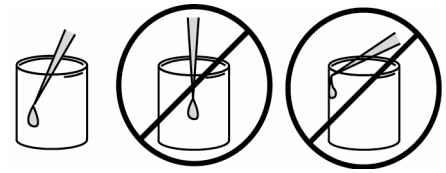
EXAMPLE LAYOUT FOR A RUN OF 24 MICROWELLS:

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

2. Carefully remove Hybridization Microplate containing Calibrators, Quality Controls and specimens from 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 Hybridization Microplate wells to the bottom of the corresponding Capture Microwell using an eight-channel pipettor. Use new pipette tips on the eight-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



CORRECT | Do not pipette Vertically. Avoid backsplash. | Do not allow tip to touch side of well.

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 plate over the 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 eight-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 pad 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 eight-channel pipettor and the reverse pipetting technique described below.

   Reverse Pipetting Technique:
   a) Insert tips into eight-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 reimmersed 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 the plate with a plate lid and incubate at 20-25°C for 30 minutes up to a maximum of 45 minutes if needed to process multiple plates.

Washing

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

Automated Plate Washer Method:

Note: Always keep the Automated Plate Washer powered 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.

BEFORE EACH USE:

- Verify that the wash reservoir is filled at least to the 1 L mark. 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 the plate lid and the place plate on the 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 a 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

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 six times. Each well is washed to overflowing to remove conjugate from the tops of the wells. Washing begins at well A1 and continues in a serpentine fashion to the right and downward. After all wells have been filled, decant liquid into sink with a strong downward motion. The second wash is started at well H12 moving in a serpentine motion to the left and upward. This sequence of two washes is repeated twice more for a total of six 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 five 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 eight-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 the luminometer after 15 minutes of incubation (and no later than 30 minutes of incubation). Consult the applicable Digene assay analysis software user guide for plate measurement and data analysis instructions.
4. The Digene assay analysis software will evaluate both Calibrator and Quality Control results based on their location in the plate to verify the assay run. Proper placement of Calibrators and Quality Controls and selection of the proper software protocol are essential for valid results.

5. If a full microplate was not used, remove used microwells from the microplate holder, rinse the holder thoroughly with distilled or 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 hc2 CT-ID DNA Test requires calibration with each run. Therefore, it is necessary to verify each run 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 run in triplicate with each test run. The mean RLU value of the Negative Calibrator must be ≥ 10 and ≤ 150 RLUs in order to proceed. The coefficient of variation (%CV) for the Negative Calibrator replicates should be ≤ 25%. If the %CV is > 25%, the software will discard the replicate with the RLU value farthest from the mean as an outlier and recalculate the mean and %CV using the remaining two replicates. The recalculated %CV should be ≤ 25%; otherwise, **the assay calibration verification is invalid and the test run must be repeated for all patient specimens. Accordingly, patient specimen results should not be reported.**

2. Positive Calibrator
   The Positive Calibrator must be run in triplicate with each test run. 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 test run must be repeated for all patient specimens. Accordingly, patient specimen results should not be reported.**

3. Mean PC/Mean NC Ratio
   The mean of the Positive Calibrator replicates (mean PC) and the mean of the Negative Calibrator replicates (mean NC) are used to calculate the mean PC/mean NC ratio. The software will calculate the mean PC/mean NC ratio. This ratio must meet the following criteria to verify the assay calibration **before the specimen results can be interpreted**. If the ratio is ≥ 2.0 and ≤ 20, the software will proceed to the cutoff calculation. If the ratio is < 2.0 or > 20, **the assay calibration is invalid and must be repeated. All patient specimens should be repeated within the run.**

Note: To determine the reproducibility of the Calibrator and Quality Control for the hc2 CT-ID DNA Test, the results generated with the Digene Microplate Luminometer 2000 (DML 2000™) Instrument during internal studies involving 63 test runs using the Rapid Capture System Application and 43 test runs using the manual method were compiled (see *Table 1*). The results showed that the average %CV for the Positive Calibrator for these 106 runs was equal to or lower than 5.8%, and the average %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 test runs, 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. This is based on a statistical calculation of ±3SD of the mean RLU value for the Negative Calibrator observed for the 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 between ≥ 10 and ≤ 150 and the CV ≤ 25%. Each laboratory should monitor Quality Control and Calibration performance according to the Clinical and Laboratory Standards Institute document CLSI/NCCLS 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 1* has been shown to yield an average value upon calibration of 7.11. In this case, results are acceptable provided the NC RLU remains less than or equal to 250 and the PC/NC ratio is greater than or equal to 2.0 and ≤ 20. Should the NC RLU exceed 250 or the PC/NC fall below 2.0 or above 20, the assay is invalid.

Table 1. Statistical Summary of Negative and Positive Calibrator Values for the RCS Application and Manual Method Runs.

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 Values for determining positive specimens. The Cutoff values are calculated as follows:

Cutoff Value = mean Positive Calibrator

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 Value is (mean PC) = 318

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

Note: RLU/CO values and positive/negative results for all specimens tested are reported in the Digene assay analysis software test result reports.

QUALITY CONTROL

Quality Control specimens are supplied with the hc2 CT-ID DNA Test. Consult the applicable Digene assay analysis software user guide for instructions on how to input the Lot Numbers and Expiration Dates of the Quality Controls. These controls must be included in each test run, and the RLU/CO of each Quality Control must fall within the following acceptable ranges for the test run to be considered valid. **If the Quality Controls do not fall within these ranges, the assay is invalid and must be repeated.** No patient results can be reported.

	QC CT	QC GC	Negative Calibrator
Minimum RLU/CO	1.00	0	0
Maximum RLU/CO	20.00	0.9999	0.9999
Maximum %CV	20.00	20.00	25.00

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 this test kit. As recommended by the Clinical and Laboratory Standards Institute/NCCLS (CLSI/NCCLS Document C24-A2) the Positive Calibrator contains a different concentration (five times lower) of target DNA to ensure that the quality control procedure provides an independent assessment of performance.

2. This control material is not the same as CT organism in the specimen matrix and will not act as an appropriate control for the processing of Specimen Transport Medium.
3. The Positive Calibrator is used to normalize specimen results by establishing the cutoff for each run of the assay. The specimens provided with this test kit may be used for internal quality control or users may develop their own internal quality control material, as defined by CLSI/NCCLS C24-A2. Additional controls that are processed as patient specimens may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. Please refer to CLSI/NCCLS C24-A2 for additional guidance on appropriate internal quality control testing practices.
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 VR866) to a fresh tube of STM. Incubate for at least one hour prior to testing in the same manner as a normal clinical specimen. An RLU/CO > 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 and Positive Calibrators have been established only for Digene-approved luminometers. The Negative and Positive controls monitor for substantial reagent failure and will not ensure precision of the assay cutoff.

INTERPRETATION OF SPECIMEN RESULTS

By the criteria of the hc2 CT-ID DNA Test:

1. Specimens with RLU/Cutoff Value 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/Cutoff Value ratios < 1.00 do not contain *Chlamydia trachomatis* DNA or contain DNA below the detection limit of the assay. 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/Cutoff Value ratios ≥ 1.00 and < 2.50 are considered equivocal. Results may be considered presumptively positive for *C. 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.
5. In the case when the hc2 CT-ID DNA Test is used as a follow-up to identify organism(s) present in hc2 CT/GC DNA Test-positive specimens, if, after follow-up testing with the hc2 CT-ID DNA Test the specimen is negative, report result as "No *Chlamydia trachomatis* DNA detected." (See the *hc2 CT/GC DNA Test Package Insert*.)

* During the clinical evaluation of the 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 seven were apparent false positive. However, these seven false-positive specimens were among a total of only 11 specimens not found negative by the 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 seven initial positive specimens had an initial RLU/CO of 1.00-2.50 with three of these specimens negative by all other testing done (all three of these specimens were negative when repeated twice with the hc2 CT-ID DNA Test). For the remaining four specimens, all were culture/DFA/PCR positive and both repeat hc2 CT-ID DNA Test replicates were ≥ 1.00 RLU/CO.

LIMITATIONS OF THE PROCEDURE

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

1. For *in vitro* diagnostic use only.
2. The hc2 CT-ID DNA Test *Procedure*, *Quality Control* and the *Interpretation of Results* must be followed closely to obtain reliable test results.

3. 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 help confirm that these conditions have been met.
4. A negative result does not exclude the possibility of *Chlamydia trachomatis* infection because very low levels of infections or sampling error may cause a false-negative result.
5. In addition to human whole blood, only one commercial douche, antifungal cream, and contraceptive jelly were tested for assay interference. All substances were tested at 5% v/v. The effects of other exogenous substances have not been determined. In general, presence of exogeneous substances should be avoided as they may interfere with accurate test results.
6. The hc2 CT-ID DNA Test can only be used with cervical specimens collected using the hc2 DNA Collection Device and placed in STM, or with cervical specimens collected with the Female Swab Specimen Collection Kit and placed in STM.
7. The hc2 DNA Collection Device should not be used for collection of specimens from pregnant women. For pregnant women, cervical specimens should be collected using the Female Swab Specimen Collection Kit (Dacron swab and STM).
8. Results of this test should be interpreted only in conjunction with information available from clinical evaluation of the patient and from other procedures.
9. The 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.
10. Reliable results are dependent on adequate specimen collection. Because the transport system used for this assay does not permit microscopic assessment of specimen adequacy, training of clinicians in proper specimen collection techniques is necessary. To ensure optimal recovery of the columnar epithelial cells lining the endocervix, excess mucus should be removed prior to specimen collection as described in the hc2 DNA Collection Device and Female Swab Specimen Collection Kit procedures.
11. The hc2 CT-ID DNA Test is not intended for the evaluation of suspected sexual abuse or for other medico-legal indications. Additional testing is recommended in any circumstance when false-positive or false-negative results could lead to adverse medical, social or psychological consequences.
12. As is true for all non-culture methods, a positive specimen cannot be interpreted as indicating the presence of viable *Chlamydia trachomatis*.
13. Parameters necessary to determine therapeutic success or failure have not yet been established for the hc2 CT-ID DNA Test.
14. The 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 Digene. Other plate washers or other plate washer settings are not acceptable for use with the hc2 CT-ID DNA Test.
15. The ability of the hc2 CT-ID DNA Test to verify hc2 CT/GC DNA Test-positive specimens has not been demonstrated with a sufficient number of specimens collected with a Dacron swab, as compared to specimens collected with the hc2 DNA Collection Device. Because the use of the hc2 DNA Collection Device is contraindicated in the collection of cervical specimens from pregnant women, predictive value may be reduced in this subpopulation of patients.
16. In low prevalence populations, the false-positive rate of any single diagnostic test can exceed the true positive rate so that the predictive value of a positive result with that test is very low. Because some patients who are truly infected will not be identified by the testing of a single specimen, the true rate of false positives can not necessarily be determined or presumed from the clinical data.
17. In order to minimize variability of the results obtained with the 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 at a frequency consistent with CLIA requirements.

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 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 (see *Table 2*). Seven of these 14 specimens were verified positive by CT culture/DFA or CT PCR.

Table 2. Prevalence of 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, respectively) for different prevalence rates using the 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 hc2 DNA Collection Device (cervical brush) and for specimens collected with the HC Female Swab Specimen Collection Kit (Dacron swab). *Table 3* represents the hypothetical PPV and NPV for brush specimens (overall sensitivity 97.71% and specificity 98.2%) and *Table 4* represents the hypothetical PPV and NPV for swab specimens (overall sensitivity 92.31% and specificity 98.6%).

Table 3. 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 4. hc2 CT-ID Test Hypothetical Predictive Values at Different Prevalence Rates (Swab).

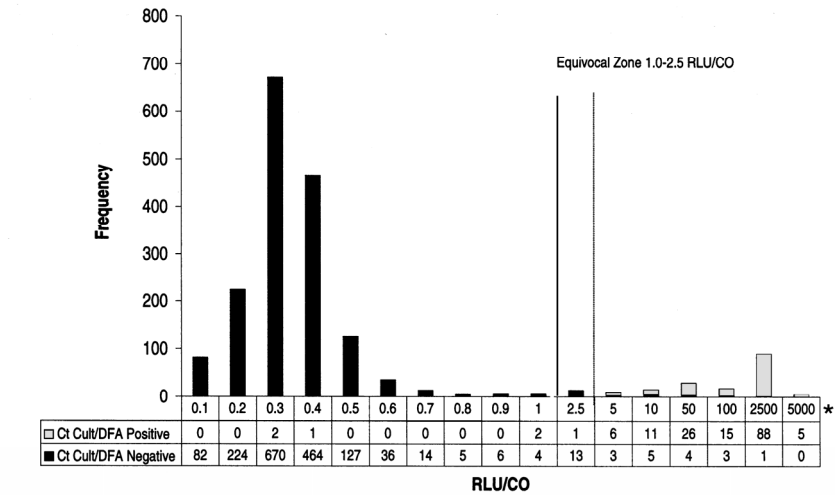
Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
5	92.3	98.6	77.4	99.6
10	92.3	98.6	87.8	99.1
15	92.3	98.6	92.0	98.6
20	92.3	98.6	94.2	98.1

Frequency Distribution: hc2 CT-ID DNA Test RLU/CO Results

The distribution of the hc2 CT-ID DNA Test RLU/CO ratios observed during the multicenter clinical study are indicated below (see *Figure 1*). These data include specimens for which the 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.

Distinct separation of the RLU/CO ratios is observed between hc2 CT-ID DNA Test-positive results and hc2 CT-ID DNA Test-negative results. Ninety-nine percent (99%, 1620/1637) of the 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 six of which (46%) were positive by CT PCR. Eighty- five percent (85%, 142/167) of the hc2 CT-ID DNA Test-positive results have RLU/CO Values between 10 and 5000.

Figure 1. Frequency Distribution of the 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

PERFORMANCE CHARACTERISTICS

Clinical Trial Results by Specimen

hc2 CT-ID DNA Test performance characteristics were determined by comparing the assay results to results of *Chlamydia* culture and DFA. From five different sites (including STD, Family Planning, and OB/GYN clinics), 1818 specimens were collected from patients and later tested. DFA testing was performed on the sediment of the CT culture transport medium after centrifugation for specimens that were hc2 CT-ID DNA Test-positive/culture-negative. PCR testing was then performed on hc2 CT-ID DNA Test-positive/culture-negative/DFA-negative specimens. hc2 CT-ID DNA Test results were NOT resolved by PCR test results, and therefore PCR had no impact on the calculations of the 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 hc2 CT-ID DNA Test. Results from the clinical trial for specimens collected with the hc2 DNA Collection Device (cervical brush) are shown in *Table 5* and specimens collected with the HC Female Swab Specimen Collection Kit (Dacron swab) in *Table 6*.

The performance characteristics of the 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 this package insert. Therefore, the performance of the 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 this package insert (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 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 this insert for additional information.

Sufficient data have not been generated to estimate equivalent sensitivity and positive predictive value of the hc2 CT-ID DNA Test using the HC Female Swab Specimen Collection Kit compared with specimens collected using the hc2 DNA Collection Device. Because the use of the 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 Dacron swab is used for specimen collection.

The clinical sensitivity and specificity of the 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 hc2 CT-ID DNA Test-positive and six presumptive positive specimens obtained from 24 CT culture - DFA negative patients; however, the 1637 hc2 CT-ID DNA Test-negative specimens in that study and five of the 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 hc2 CT-ID DNA Test-positive results found in patients who were culture or DFA-positive for *Chlamydia trachomatis*. Therefore, the hc2 CT-ID DNA Test sensitivity can only be deduced relative to culture-DFA positivity that may have a sensitivity of 60-85%. In addition, several studies have been conducted by various independent research groups showing the performance of the hc2 CT-ID DNA Test compared to commercially available and research NAA tests.[28]

Retesting of hc2 CT/GC DNA Test-Positive Specimens using the hc2 CT-ID DNA Test

A summary of the performance of the hc2 CT-ID DNA Test when used for the retesting of specimens initially positive with the hc2 CT/GC DNA Test is presented below. The results have been stratified by the collection device used to collect the specimen, the hc2 DNA Collection Device (designated in *Table 7* as "Brush") and the HC Female Swab Specimen Collection Kit (designated in Table 7 as "Swab"). A total of only four specimens (three using Brush and one using Swab) that were positive by CT culture/DFA initially tested negative with the hc2 CT/GC DNA Tests (2.5%, 4/157). Overall, the sensitivity of the hc2 CT/GC DNA Test used in combination with the hc2 CT-ID DNA Test, estimated versus CT Culture/DFA, is 96.2% (151/157). This sensitivity was determined relative to CT culture/DFA, which may have a sensitivity of 60-85%. Furthermore, PCR testing was performed on 21 CT culture/DFA-negative specimens that were initially hc2 CT/GC DNA Test positive and verified as positive or presumptive positive by the hc2 CT-ID DNA Test. Sixteen of these 21 specimens (76%) were positive by PCR.

The effective ability of the combined hc2 CT/GC and CT-ID DNA Tests to verify the absence of CT DNA was demonstrated by the 1646 specimens that either initially tested negative with the hc2 CT/GC DNA Test (1549) or were verified not to contain CT DNA upon subsequent retesting with the hc2 CT-ID DNA Test (97). Only six of these 1646 specimens were determined to contain CT organism by culture/DFA, for a true negative rate relative to culture/DFA of 99.6%.

In addition, 12 of the 97 hc2 CT/GC DNA Test-positive specimens referred to above that tested negative with the hc2 CT-ID DNA Test also tested negative with the hc2 GC-ID DNA Test. Only one of those 12 specimens was CT culture/DFA-positive and none were determined to be GC culture positive. No additional NAA testing was performed on these specimens. Of the remaining 85 specimens in this subgroup, two were CT Culture/DFA-positive as indicated in *Table 7* (one of which was also GC culture positive), 76 were GC Culture positive (73 of which were hc2 GC-ID DNA Test-positive and three of which were hc2 GC-ID DNA Test equivocal), and the remaining nine were GC culture negative (see *Table 8*). Five of these nine GC-culture-negative specimens were hc2 GC-ID DNA Test-positive and four were equivocal.

Regarding equivocal test results, six of the eight hc2 CT/GC DNA Test-positive specimens indicated in *Table 7* that fell into the equivocal zone upon retesting with the hc2 CT-ID DNA Test were verified positive by CT culture/DFA and PCR combined. GC was detected in one of the two remaining specimens by GC culture and the hc2 GC-ID DNA Test, whereas neither CT nor GC was detected in the last specimen in this subgroup by culture or PCR. This specimen did, however, yield an equivocal result with the hc2 GC-ID DNA Test and is discussed in the *Coinfected Specimens* section (specimen 1 in *Table 9* below). As suggested by these six specimens, the usefulness of the hc2 CT-ID DNA Test to verify the presence of CT DNA in specimens that test positive by the hc2 CT/GC DNA Test is not compromised by interpreting hc2 CT-ID DNA Test equivocal specimens as presumptive positive, as instructed in the *Interpretation of Results* section of this package insert.

Table 5. 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 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 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 6. 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	7	2	0	0	1	4	66.67	100.00	80.00	100.00	N/A
95% CI							94.3-99.2	39.8-100	28.4-99.5	15.8-100	
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	**258**	**20**	**1**	**5 (2*)**	**2**	**230 (233)**	**91.30**	**97.87 (99.15)**	**99.14 (99.15)**	**80.77 (91.30)**	**2/3[4] (1/1[3])**
95% CI							**72.0-98.9**	**95.1-99.9**	**95.7-99.9**	**60.7-98.9**	
Asymptomatic											
1	1	1	0	0	0	0	100.00	N/A	N/A	100.00	N/A
95% CI							2.5-100		2	2.5-100	
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	**190**	**3**	**0**	**1***	**0**	**186**	**100.00**	**99.47**	**100.00**	**75.00**	**N/A[3]**
95% CI							**29.2-100**	**97.1-100**	**98.0-100**	**19.4-99.4**	
Total Patient Population											
1	8	3	0	0	1	4	75.00	100.00	80.00	100.00	N/A
95% CI							19.4-99.4	39.8-100	28.4-99.5	29.2-100	
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	**448**	**23**	**1**	**6 (3)**	**2**	**416 (419)**	**92.31**	**98.58 (99.29)**	**99.52**	**80.00 (88.89)**	**2/3[5] (1/1[4])**
95% CI							**67.3-99.1**	**96.9-99.9**	**97.6-99.9**	**61.4-97.7**	

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

Table 7. Summary of hc2 CT-ID DNA Test Results Obtained for Specimens Tested with the hc2 CT/GC DNA Test (n=1818). Performance Estimates - Sensitivity and Specificity of the hc2 CT-ID and GC-ID DNA Test Verification System.

hc2 DNA Test Results			Combined CT Culture and DFA					
			Brush		Swab		Total	
CT/GC	CT-ID	n	POS	NEG	POS	NEG	POS	NEG
POS	POS	164	126	11	24	3	150	14
	EQUIV	8	1	5	0	2	1	7
	NEG	97	1	75	1	20	2	95
	TOTAL	**269**	**128**	**91**	**25**	**25**	**153**	**116**
NEG	POS	3	1	2	0	0	1	2
	EQUIV	6	0	5	0	1	0	6
	NEG	1540	2	1141	1	396	3	1537
	TOTAL	**1549**	**3**	**1148**	**1**	**397**	**4**	1545

Table 8. Summary of hc2 GC-ID DNA Test and Culture Results Obtained for Specimens that tested hc2 CT/GC DNA Test-Positive and hc2 CT-ID DNA Test-Negative (n=97).

		POS	GC-ID EQUIV	NEG
GC Culture	POS	73	3	0
	NEG	5	4	12
CT Culture/DFA	POS	1	0	1
	NEG	77	7	11

Coinfected Specimens

Although not evident from *Table 7*, of particular interest are the 32 specimens determined positive by CT culture/DFA and GC culture to be coinfected with these organisms. CT or GC DNA was detected in 31 of these 32 (97%) coinfected specimens with the hc2 CT/GC DNA Test. It was only with this one hc2 CT/GC DNA Test-negative specimen that the three hc2 DNA Tests (CT/GC, CT-ID and GC-ID) were unable to detect CT or GC DNA. Of the 31 hc2 CT/GC DNA Test-positive specimens, 28 (90%) were verified by the hc2 CT-ID and GC-ID DNA Tests to contain CT and GC DNA, leaving only three specimens (10%) that were not detected from patients with dual infections. The hc2 CT-ID DNA Test detected two of these three coinfected specimens, and the hc2 GC-ID DNA Test detected one of the coinfected specimens. An additional three specimens determined to be coinfected utilizing available CT/GC culture and PCR test results were verified as positive by the hc2 CT/GC, CT-ID and GC-ID DNA Tests combined. No equivocal hc2 DNA Test results were observed when testing coinfected specimens. This information is summarized in *Table 9*.

Conversely, only five specimens identified as positive or presumptive positive by all three hc2 DNA Tests were not verified by culture, DFA, or PCR to contain both organisms. This included only one specimen that was negative by all of these methods (see *Table 10*). Specimens 1 and 2 from *Table 10* were presumptive positive (equivocal) by both the hc2 CT-ID and GC-ID DNA Tests and only the hc2 CT-ID DNA Test result for Specimen 3 was equivocal.

Table 9. hc2 DNA Test Results for Specimens determined to be Coinfected with CT and GC by CT Culture/DFA, GC Culture, and PCR (n=35).

	hc2 DNA Test Results		
	CT/GC	**CT-ID**	**GC-ID**
POS	34	33	32
EQUIV	N/A	0	0
NEG	1	2	3

Table 10. Specimens Positive or Presumptive Positive by All Three hc2 DNA Tests and Unconfirmed as Coinfected with CT and GC by CT Culture/DFA, GC Culture and PCR (n=5).

			PCR	
No.	CT Culture	DFA GC Culture	CT	GC
1	NEG	NEG	NEG	NEG
2	NEG	NEG	POS	NEG
3	NEG	POS	NEG	ND
4	POS	NEG	POS	NEG
5	POS	NEG	ND	ND

Reproducibility

As part of the Multicenter Clinical Trial, a reproducibility study was performed to determine the run-to-run, day-to-day, site-to-site and total reproducibility of the hc2 CT-ID DNA Test using a panel composed of *Chlamydia trachomatis* DNA targets and hc2 CT-ID DNA Test-positive and hc2 CT-ID DNA Test-negative clinical specimens.

A 10-member panel of masked, denatured clinical and non-clinical specimens, consisting of eight positive specimens and two negative specimens, was tested in replicates of six, twice per day, over a three-day period, at each of four sites (three external sites and Digene). Each site generated 36 data points for every target tested. All specimens were denatured and stored frozen prior to testing. A 99.9% agreement was observed for the 1152 expected positive results (1151/1152), and a 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 run-to-run, day-to-day or site-to-site variability observed. Therefore, the data from all runs at each site were combined and are presented below (see *Table 11*).

Table 11. Reproducibility of the hc2 CT-ID DNA Test in a Multicenter Trial.

	Site 1		Site 2		Site 3		Site 4		Total		
Target Number	$\bar{X}$RLU /CO	% Agree	$\bar{X}$RLU /CO	% Agree	$\bar{X}$RLU /CO	% Agree	$\bar{X}$RLU /CO	% Agree	$\bar{X}$RLU /CO	Observed/ Expected	% Agree
1	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* 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-positives (at or near the limit of detection) and medium-positives with two CT serovars, mixed infections with *Neisseria gonorrhoeae* and specimens that contained blood. Twelve specimens were expected to be positive and 13 specimens were expected to be negative. The percent agreement between observed and expected results of the hc2 CT-ID DNA Test at the three individual test sites and for all sites combined are shown in *Table 12*. Sensitivity, specificity, agreement and kappa values for each site are included in *Table 13*.

Table 12. 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 13. Results of the 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 12*, all of which contained low concentrations of CT organism (~$5x10^4$ organisms/ml), would be interpreted according to the *Interpretation of Results* section of this package insert 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 Digene 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 14* below).

Table 14. CT and GC Specimen Panel Results.

	Specimen	**hc2 CT/GC DNA Test Result**		
Site	**ID**	**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-run and total precision of the hc2 CT-ID DNA Test using a panel of positive and negative masked, simulated clinical specimens. In addition, the intra- and inter-instrument precision observed with two separate luminometers was assessed using the same panel. The two luminometer models included the DML 2000 Instrument, which is one of the Digene-approved luminometers recommended for use with the 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 technologist who performed the testing at this site had been trained in proper assay technique. However, had not performed any hc2 CT-ID DNA Test testing for over six months.

Table 15 shows the performance of the hc2 CT-ID DNA Test, including the site that experienced technical problems. The technologist 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 16*. 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 technologists performing the hc2 CT-ID DNA Test.

Table 15. Within Instrument, Between Instrument, Within Run, Total Precision Estimates For RLU/CO by Target Prior to Technologist Retraining.

			Within Instrument		Between Instrument		Within Run		Total	
Panel Member	n	Mean	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 16*. 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 technologists performing the hc2 CT-ID DNA Test.

Table 16. Within Instrument, Between Instrument, Within Run, Total Precision Estimates For RLU/CO by Target After Technologist Retraining.

			Within Instrument		Between Instrument		Within Run		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 Digene to determine the total precision of the hc2 CT-ID DNA Test using the DML 2000 Instrument. A six-member precision panel was prepared using a simulated clinical specimen matrix consisting of cultured epithelial cells suspended in 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 five days. A freshly denatured panel was used per plate. The total precision results for the hc2 CT-ID DNA Test compiled for all five days of testing are presented in *Table 17*. 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 1.0.

Table 17. Total Precision for 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

Analytical Sensitivity
The analytical sensitivity (limits of detection) of the 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 hc2 CT-ID DNA Test to determine the organism load estimate of the highest dilution yielding a positive result with the hc2 CT-ID DNA Test. Each concentration of each target type was tested in triplicate following the hc2 CT-ID DNA Test package insert.

The limit of detection for each *Chlamydia* serovar is summarized in *Table 18*. 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 fewer than 30 years old are E, I, and D (in decreasing order).[29] 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 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 hc2 CT-ID DNA Test demonstrated a lower limit of detection of 50 EBs/assay.

Table 18. 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

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 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 hc2 CT-ID DNA Test are presented in *Table 19*. All bacteria, except *Chlamydia psittaci*, tested negative with the 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.[30] 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 Probes in the hc2 CT-ID DNA Test do not cross-react with targets of the GC Probe.

Table 19. 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 hc2 CT-ID DNA Test are presented in *Table 20*. Presumptive cross-reactivity was observed with plasmid vectors pBR322, pGEM® 3Z 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 hc2 CT-ID DNA Test amongst 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 pGEM3Zf(-) 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 20. 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 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 hc2 CT-ID DNA Test. This effect is not of concern as it is the opposite of an inhibitory effect.

Precision at the Cutoff of the hc2 CT-ID DNA Test with Clinical Specimens Collected in STM

The reproducibility of the 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. The mean RLU/CO value, 95% confidence intervals about the mean (CIs) and percent positive results were calculated for each specimen over five days and are shown in *Table 21*.

Table 21. Mean RLU/CO with Confidence Intervals and Percent 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 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 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 Instrument to generate data and determine the performance characteristics of the hc2 CT-ID DNA Test. The MLX luminometer is no longer available for use, and only

Digene-approved luminometers (including the DML 2000 Instrument) are used to generate results. The following data were generated from the Multicenter Clinical Trial to determine the reproducibility of the Positive and Negative Calibrators and are provided below as historical information.

To determine Positive and Negative Calibrator reproducibility, the results from the clinical evaluations involving 81 assay runs performed with the hc2 CT-ID DNA Test were compiled (see *Table 22*). The results showed that the average %CV for these 81 runs was 6.4%, and no runs had Negative Calibrator Mean values in excess of 150 RLUs. Positive Calibrator reproducibility in excess of 25% CV was observed for only two out of the 81 runs (2.5%). None of the test runs' %CV remained greater than 25%, indicating that all of the test runs were valid.

Table 22. Performance of the Positive and Negative Calibrator. Combined Data from the Multicenter Clinical Trial and the Precision Study (n = 81 runs).

				Mean of Calculated Mean (RLU)		Mean of the Calculated %CVs	
Instrument	No. of runs	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
Instrument			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.

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TROUBLESHOOTING GUIDE

hc2 CT-ID DNA TEST		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTIONS**
1. QC-CT and QC-GC give incorrect result.	Incorrect software protocol chosen for test (i.e., ran CT protocol on GC run). Reversed placement of CT and GC QC on plate.	If the software protocol is incorrect for the test being run, the plate should be read again as soon as possible with the correct protocol and original data discarded. Rerun specimens.
2. Improper 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.
3. Improper 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 two minutes. If there are wells that still remain purple or gray, add an additional 25 µl of Probe 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 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.
4. 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 the package insert. 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 and Probe Diluent.	After adding Probe to Probe Diluent, mix very thoroughly by vortexing at high speed for at least five seconds. A visible vortex must be produced.
	Inadequate mixing of diluted Probe and denatured specimen.	After adding denatured specimen to Probe Mix, 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 3* of this package insert. Check for color change from purple to yellow in every well. If no color change, specimens should be retested.
	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 4* of this package insert. Check temperature of Microplate Heater I. Ensure that the heater is set to heat specimens to correct temperature and was preheated for one 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 this package insert. Verify Rotary Shaker I speed by calibration as outlined in the *Shaker Speed Calibration* section of the *Rotary Shaker I Operator's 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 eight-channel pipettor. Incubate at 20-25°C for 30-45 minutes.

hc2 CT-ID DNA TEST		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTIONS**
	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 eight-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 instruction, or call your local Digene representative.
5. Elevated RLU values in manual assay controls and/or specimens (≥ 150 RLUs in many or all wells). Assay may fail validation criteria. **OR** **Elevated RLU values in RCS assay controls and/or specimens (≥ 250 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). Calibrators, Quality Controls, and specimens should turn purple after addition of Denaturation Reagent. Check speed calibration of Multi-Specimen Tube Vortexer. 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 empty microwells. 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 your local Digene representative.
	Contamination of Detection Reagent 2 or Capture Microplate wells by Detection Reagent 1 or exogenous alkaline phosphatase.	Reference the *Contamination Check* in this *Troubleshooting* section.
	Contaminated Wash Buffer.	Reference the *Contamination Check* in this *Troubleshooting* section.
	Contaminated Automated Plate Washer.	Reference the *Contamination Check* in this *Troubleshooting* section.
	Inadequate washing of Capture Microplate wells after Detection Reagent 1 incubation.	Wash Microplate wells thoroughly with Wash Buffer six times, filling wells to overflowing 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 Operator's Manual* for instructions on testing for contamination or malfunctions.
	Detection Reagent 1 contamination of Microplate wells.	Ensure all work surfaces are clean and dry. Use care when using Detection Reagent 1. Avoid aerosols.
	Blotting hybridization solution on same area of Kimtowels Wipers or equivalent low-lint paper towels. Use of wrong blotting towels.	Do not reblot on same area of the Kimtowels Wipers or equivalent low-lint paper towels. Use Kimtowels Wipers or equivalent low-lint paper towels for blotting.
6. 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 method for at least five seconds (for the manual vortexer method, vortex for at least five 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.
	Probe inadequately mixed or insufficient Probe added to assays.	Prepare Probe Mix as described. Mix thoroughly by vortexing, ensuring that a visible vortex is produced. Probe Mix must be added to tubes with a multichannel or repeating pipettor to ensure accurate delivery.
	Inadequate volume of Probe Mix added to each hybridization microplate well.	Verify that the eight-channel pipettor is delivering accurately prior to adding Probe Mix to Hybridization Microplate. Add 25 µl of Probe Mix to each microwell containing denatured Calibrators, Quality Controls, and clinical specimens. Color change should be from dark purple to yellow upon addition and thorough mixing of Probe Mix.

hc2 CT-ID DNA TEST		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTIONS**
	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 Operator's Manual*.
	Inadequate washing.	Wash Microplate wells thoroughly with Wash Buffer six times, filling the wells to overflowing each time or using Automated Plate Washer.
	Contaminated Wash Buffer.	Check the Wash Buffer for contamination. Pipette 10 µl of Wash Buffer into 75 µl of Detection Reagent 2 in a blank Capture Microplate well. Cover and incubate 15 minutes at 20-25°C. Read the microwell on the luminometer. Readings above 150 RLUs indicate Wash Buffer contamination. See *Reagent Preparation and Storage* section of this package insert for instructions on cleaning and maintenance of Wash Apparatus. See the *Troubleshootin*g section of the *Automated Plate Washer Operator's Manual* for instructions on decontamination or malfunctions.
7. 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.	See the *Troubleshooting* section *Automated Plate Washer Operator's Manual* for instructions on testing for contamination or malfunctions.
8. 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 and after adding Probe Mix. 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 six times, filling the wells to overflowing 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.
	Blotting on same area of Kimtowels Wipers over several rows.	Do not reblot on the same area of the Kimtowels Wipers.
9. 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 six times, filling to overflowing each time or using Automated Plate Washer. There should be no residual pink liquid visible in the microplate wells after washing.
	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 method for at least five 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.

hc2 CT-ID DNA TEST		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTIONS**
	Improper washing conditions.	Wash Microplate wells thoroughly with Wash Buffer six times, filling the wells to overflowing each time or using Automated Plate Washer and proper Automated Plate Washer protocols.
10. Elevated manual assay Negative Calibrator RLU values (> 150 RLUs). Remainder of assay performs as expected. **OR** **Elevated RCS Negative Calibrator RLU values (> 250 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. Rerun 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.	Check Detection Reagent 2 for contamination by pipetting 75 µl into a blank Capture Microplate well. Incubate at 20-25°C for 15 minutes and read on the luminometer. Readings above 150 RLUs indicate Detection Reagent 2 contamination. Take care when pipetting Detection Reagent 2. Wear powder-free gloves and avoid touching tips to any work surfaces. Repeat troubleshooting procedure on the master vial of Detection Reagent 2, and if not contaminated, repeat assay using this material. If contaminated, obtain a new hc2 CT-ID DNA Test and repeat assay. If Detection Reagent 2 is not contaminated, check the Wash Buffer for contamination. Pipette 10 µl of Wash Buffer into 75 µl of Detection Reagent 2 in a blank Capture Microwell. Cover and incubate 15 minutes at 20-25°C. Read the microwell on the luminometer. Readings above 150 RLUs indicate Wash Buffer contamination. See *Reagent Preparation and Storage* section in the package insert for instructions on cleaning and maintenance of Wash Apparatus. See *Automated Plate Washer Operator's Manual* for instructions on testing for contamination or malfunctions.

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 three 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 Buffer Apparatus and/or Water Source	• Pipette 75 µl of Detection Reagent 2 into three 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* section for instructions on cleaning and maintenance of Wash Apparatus.
Automated Plate Washer	• Pipette 75 µl of Detection Reagent 2 into five 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 Operator's Manual*, *Decontamination Procedure*.

DIGENE CONTACT INFORMATION

Use the Digene Contact Information Sheet provided with this product to contact your local Digene Representative.

U.S. Hybrid Capture Patent Numbers
4,732,847
4,865,980
6,228,578B1

Use of CDP-Star is covered by U.S. patent numbers 4,931,569; 4,978,614; 5,145,772; 5,326, 882; 5,538,847; 5,582,980; 5,851,771 licensed from Tropix, Inc.

SUMMARY OF hc2 CT-ID DNA TEST

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

	PROCEDURE	
Denaturation	**Manual 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. Vortex each Calibrator, Quality Control, and specimen individually for five seconds at high speed and invert (see package insert 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	Vortex denatured Calibrators, Quality Controls, and specimens well, then transfer 75 µl into appropriate Microplate well. ↓ **Cover Microplate and incubate for 10 minutes at 20-25°C. Remove Cover.** ↓ 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*. ↓ **Incubate at 65 ±2°C for 60 ±5 minutes. Prepare Capture Microplate.** ↓	
Hybrid Capture	Transfer contents from each Hybridization Plate Well or microtube to corresponding well of Capture Microplate using an eight-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 package insert for details). ↓	
Hybrid Detection	Pipette 75 µl Detection Reagent 1 into each well of Capture Microplate. Cover Capture Microplate with Plate Lid, 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 six times. ↓ Blot on low-lint paper towels. ↓ ↓	**Automated Plate Washer Method** Place plate on washer and press “START/STOP” to begin. ↓ Go to next step. ↓ ↓ ↓ ↓
Signal Amplification	Pipette 75 µl Detection Reagent 2 into each well of Capture Microplate. Incubate at 20-25°C for 15-30 minutes. ↓	
Reading	Read Capture Microplate on luminometer. ↓ **Validate assay and interpret specimen results.**	