hc2

Hybrid Capture® 2 High-Risk HPV DNA Test®

Digene HPVtest™

An In Vitro Nucleic Acid Hybridization Assay with Signal Amplification using Microplate Chemiluminescence for the Qualitative Detection of Human Papillomavirus (HPV) Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 in Cervical Specimens.

For use with:
hc2 DNA Collection Device
Specimen Transport Medium™
Cytyc ThinPrep® Pap Test PreservCyt® Solution

KEY CHANGES FROM PREVIOUS PACKAGE INSERT REVISION

1. Added references for an additional catalog number, REF 5199-00018, of the hc2 High-Risk HPV DNA Test®. REF 5199-00018 contains four microplates and reagent volumes sufficient to test 384 specimens using the Rapid Capture® System.
2. Added optional vortex method for processing of PreservCyt® Solution Specimens.

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

REF 5199-1220 (1-plate kit)
5199-00018 (4-plate kit)

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DIGENE

L00665 Rev. 2

IMPORTANT!

When using the Rapid Capture System, refer to the *Rapid Capture System User Guide* for preparation of hc2 High-Risk HPV DNA Test reagents for the 1-plate or 4-plate kits, as appropriate.

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Table of Contents

NAME AND INTENDED USE

The hc2 High-Risk HPV DNA Test® using Hybrid Capture® 2 (hc2) technology is an in vitro nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of thirteen high-risk types of human papillomavirus (HPV) DNA in cervical specimens. The HPV types detected by the assay are the high-risk HPV types 16/18/31/33/35/39/45/51/52/56/58/59/68. The hc2 High-Risk HPV DNA Test cannot determine the specific HPV type present.

Caution: Federal law restricts this device to sale by or on the order of a physician.

Cervical specimens that may be tested with the hc2 High-Risk HPV DNA Test include the following:

- Specimens collected with the hc2 DNA Collection Device
- Biopsies collected in Specimen Transport Medium™ (STM)
- Specimens collected using a broom type collection device and placed in Cytyc ThinPrep® Pap Test PreservCyt® Solution (refer to the hc2 Sample Conversion Kit package insert for complete details).

The use of this test is indicated:

1. To screen patients with ASC-US (atypical squamous cells of undetermined significance) Pap smear results to determine the need for referral to colposcopy. The results of this test are not intended to prevent women from proceeding to colposcopy.

2. In women 30 years and older the hc2 High-Risk HPV DNA Test can be used with Pap to adjunctively screen to assess the presence or absence of high-risk HPV types. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.

WARNING

- The hc2 High-Risk HPV DNA Test is not intended for use as a screening device for Pap normal women under age 30 and is not intended to substitute for regular Pap screening.
- There is insufficient evidence to indicate whether a single WNL Pap result with concurrent negative high-risk HPV result confers low risk similar to consecutive annual, technically adequate WNL Pap results.
- Detection of HPV using the hc2 High-Risk HPV DNA Test does not differentiate HPV types or infection with more than one type, and cannot evaluate persistence of any one type.
- The use of this test has not been evaluated for the management of women with prior cytologic or histologic abnormalities, hysterectomy, who are postmenopausal, or who have other risk factors (e.g., HIV+, immunocompromised, DES exposure, history of STI).

The hc2 High-Risk HPV DNA Test is designed to augment existing methods for the detection of cervical disease and should be used in conjunction with clinical information derived from other diagnostic and screening tests, physical examinations and full medical history in accordance with appropriate patient management procedures.

hc2 High-Risk HPV DNA Test results **should not** be used as the sole basis for clinical assessment and treatment of patients.

Another Digene HPV kit, the hc2 HPV DNA Test (REF 5198-1220), which detects both high-risk and some low-risk HPV types **should not** be used as an adjunct for screening because low-risk types are not associated with risk of cervical cancer. Only the hc2 High-Risk HPV DNA Test should be used as an adjunct for screening.

For high-volume sample-throughput testing, the hc2 High-Risk HPV DNA Test [REF 5199-1220 (1-plate kit)] may be performed using the Rapid Capture® System (RCS) Instrument Application. The hc2 High-Risk HPV DNA Test® [REF 5199-00018 (4-plate kit)] is for use with high-volume testing on the Rapid Capture® System and cannot be used for manual testing.

A Pap test and associated testing materials are not included in the test kit and must be obtained separately.

SUMMARY AND EXPLANATION

In women, human papillomaviruses (HPVs) can infect the cervix, vagina, vulva, urethra, or the area around the anus. More than 70 types of HPV have been identified, and are generally classified as high-risk or low-risk depending on their known association or lack of association with cancer and its precursor lesion, high-grade cervical intraepithelial neoplasia (CIN 2-3). The presence of certain HPV types in the female genital tract is associated with a number of diseases, including condyloma, Bowenoid papulosis, cervical, vaginal, and vulvar intraepithelial neoplasia and cancer.[1,2] It is generally accepted that these viruses are predominantly sexually transmitted and that high-risk HPV types are a major recognized risk factor for development of cervical cancer.[2-6]Infection of the cervix with high-risk

HPV types can be associated with cytological and histological changes that are detected by Pap screening, colposcopy, or biopsy. The natural history of how HPV infection progresses to cancer, however, is not completely understood. Low-risk HPV types 6 and 11 have been associated with the presence of genital warts, or condylomas, but have been linked infrequently with precancerous or cancerous cervical changes. There are many other low-risk HPV types that are not associated with genital warts or cervical cancer.[7,8]

Human papillomaviruses are composed of an icosahedral viral particle (virion) containing an 8000 base pair double-stranded circular DNA molecule surrounded by a protein capsid. Following infection of epithelial cells, the viral DNA becomes established throughout the entire thickness of the epithelium, but intact virions are found only in the upper layers of the tissue. Thus, viral DNA can be found either in virions or as episomal or integrated HPV sequences, depending upon the type and grade of lesion.

To date, HPV cannot be cultured *in vitro*, and immunological tests are inadequate to determine the presence of HPV cervical infection. Indirect evidence of anogenital HPV infection can be obtained through physical examination and by the presence of characteristic cellular changes associated with viral replication in Pap smear or biopsy specimens. Alternatively, biopsies can be analyzed by nucleic acid hybridization to directly detect the presence of HPV DNA.

Historically, HPV 16 and HPV 18 have been regarded as high-risk cancer associated HPV types.[2,9,10] HPV types 31, 33, and 35 have been demonstrated to have an intermediate association with cancer.[2,11] This intermediate association is due to the fact that these types are more frequently detected in CIN 2-3 rather than in cancers. Therefore, cancers associated with the presence of these types are less common than cancers that are associated with high-risk HPV DNA types 16 and 18.[2,12] These five HPV types together account for about 80% of cervical cancers.[2,13,14] Additional high- and intermediate-risk HPV DNA types, including types 39, 45, 51, 52, 56, 58, 59 and 68, have been identified as the principal HPVs detectable in the remaining cancers.[2,14-20]

HPV infection is common in adults who have had more than one sexual partner (or a single partner who has had multiple partners) and can persist for years with no symptoms. Infection with some HPV types is an important risk factor for cervical cancer; however, most women with HPV infection do not develop cervical cancer or CIN 2-3, and infections regress. Most infections cause mild cytologic changes that resolve. HPV DNA has been shown to be present in approximately 10% of women with normal cervical epithelium but the actual prevalence in specific groups of women is strongly influenced by age and other demographic variables.[2,13,21] Prospective studies (age 16-60 years) have shown that 15-28% of HPV DNA positive women developed squamous intraepithelial lesions (SIL) suggestive of CIN 1-3 or cancer within 2 years compared to only 1-3% of HPV DNA negative women.[4,22,23] In particular, the risk of progression for HPV types 16 and 18 was greater (approximately 40%) than for other HPV types.[4,6,10,23,24] Most SIL was low-grade.

Very few HPV DNA positive women develop cytologic high-grade SIL (HSIL) indicating underlying CIN 2-3 or cancer.[25] The absolute risk of developing an incident cytologic abnormality following an HPV infection with types detected by hc2 has not been adequately described, and is known to vary in different populations.[6]

Although current scientific literature suggests that persistent infection with high-risk HPV is the main risk factor for development of high-grade cervical neoplasia and cancer[2,4,5,10,24,26-31], apparent persistence may represent continuous infection with a single HPV type, with multiple HPV types, or reinfection. Nonetheless, women who are repeatedly Pap negative and High-Risk HPV negative appear to be at low risk for having or developing cervical precancerous lesions.[5,24,32,33]

A negative hc2 High-Risk HPV DNA Test result with a concurrent normal Pap result implies low risk at a single point in time for the development of cervical neoplasia and is therefore clinically meaningful for assessing risk; however there are insufficient data to establish a definitive time period over which this lower risk is clinically relevant.

PRINCIPLE OF THE PROCEDURE

The hc2 High-Risk HPV 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 HPV 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 the 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 that 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 the Cutoff Value (CO) indicates the presence of high-risk HPV DNA sequences in the specimen. An RLU measurement less than the Cutoff Value indicates the absence of the specific high-risk HPV DNA sequences tested or HPV DNA levels below the detection limit of the assay.

REAGENTS AND MATERIALS PROVIDED

	hc2 High-Risk HPV DNA Test® **REF 5199-1220 (1-plate kit)**	**hc2 High-Risk HPV DNA Test®** **REF 5199-00018 (4-plate kit)**
Number of Tests	96 tests in one kit. The number of patient results will vary, depending on the number of uses per kit: 1 use = 88 patient results 2 uses = 80 patient results 3 uses = 72 patient results 4 uses = 64 patient results	384 tests in one kit. This kit is designed for high-volume use of the Rapid Capture system only and **must be consumed in ≤ 2 Rapid Capture System runs** to obtain the full 384 tests.
Indicator Dye **INDIC** Contains 0.05% (w/v) sodium azide.	1 x 0.35 ml	1 x 2.0 ml
Denaturation Reagent* **REAG DENAT** Dilute sodium hydroxide (NaOH) solution.	1 x 50 ml	2 x 100 ml
Probe Diluent **DIL PROBE** Buffered solution with 0.05% (w/v) sodium azide.	1 x 5 ml	1 x 20 ml
High-Risk HPV Probe **PROBE HPV HIGH-RISK** HPV 16/18/31/33/35/39/45/51/52/56/58/59/68 RNA probe cocktail in buffered solution (red cap).	1 x 200 µl	3 x 200 µl
Low-Risk HPV Quality Control **QC HPV LOW-RISK** 5 pg/ml (500,000 copies/ml) cloned HPV 6 DNA and carrier DNA in STM with 0.05% (w/v) sodium azide. The Low-Risk HPV Quality Control functions as an additional negative calibrator in this assay.	1 x 1 ml	1 x 1 ml
High-Risk HPV Quality Control **QC HPV HIGH-RISK** 5 pg/ml (500,000 copies/ml) cloned HPV 16 DNA and carrier DNA in STM with 0.05% (w/v) sodium azide.	1 x 1 ml	1 x 1 ml
Negative Calibrator **CAL -** Carrier DNA in STM with 0.05% (w/v) sodium azide.	1 x 2 ml	1 x 2 ml
High-Risk HPV Calibrator **CAL HPV HIGH-RISK** 1 pg/ml cloned HPV 16 DNA and carrier DNA in STM with 0.05% (w/v) sodium azide.	1 x 1 ml	1 x 2 ml
Capture Microplate **PLATE CAPTURE** Coated with Goat polyclonal anti-RNA:DNA hybrid antibodies.	1 each	4 each
Detection Reagent 1 **REAG DET 1** Alkaline phosphatase-conjugated antibodies to RNA:DNA hybrids in buffered solution with 0.05% (w/v) sodium azide.	1 x 12 ml	1 x 40 ml
Detection Reagent 2 **REAG DET 2** CDP-Star® with Emerald II (chemiluminescent substrate).	1 x 12 ml	1 x 40 ml
Wash Buffer Concentrate* **BUF WASH X30** Contains 1.5% (w/v) sodium azide.	1 x 100 ml	2 x 100 ml

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

MATERIALS REQUIRED BUT NOT SUPPLIED

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

Digene Hybrid Capture® 2 System ("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 HPV, 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
Hybrid Capture System Multi-Specimen Tube (MST) Vortexer 2
Conversion Rack and Rack lid (required when using the Rapid Capture System with the hc2 High-Risk HPV DNA Test® and PreservCyt specimens)
Digene Specimen Rack and Rack lid (required when using the Rapid Capture System with the hc2 High-Risk HPV DNA Test® and hc2 specimens collected with the hc2 DNA Collection Device)
hc2 DNA Collection Device
Tube Sealer Dispenser and cutting device (used with the MST Vortexer 2)
Rapid Capture® System - optional for high-volume sample-throughput testing with the hc2 High-Risk HPV DNA Test® [REF 5199-1220 (1-plate kit)] required for use with the hc2 High-Risk HPV DNA Test® [REF 5199-00018 (4-plate kit)][B]
Wash Apparatus
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 Tubes
Specimen Collection Tube Rack
Specimen Tube Rack
Specimen Collection Tube Screw Caps
Disposable Reagent Reservoirs
DuraSeal™ Tube Sealer Film
Hybridization Microtubes[C]
Microtube Racks[C]
Plate Sealers[C]

General Laboratory Use Equipment and Accessories (Manual System)

65±2°C water bath of sufficient size to hold either one MST Vortexer 2 Rack (36 x 21 x 9 cm) or two specimen racks (each 31.7 x 15.2 x 6.4 cm)
Microcentrifuge (optional for centrifuging probe vials to obtain maximum probe volume)
Vortex mixer with cup attachment
Single-channel Micropipettor; variable settings for 20-200 µl volumes
Repeating positive displacement Pipettor, such as Eppendorf® Repeater™ Pipette or equivalent
8-channel Pipettor: variable settings for 25-200 µl volumes
Timer
Sodium hypochlorite solution, 0.5% final concentration
Parafilm® or equivalent[D]
Disposable aerosol-barrier Pipette Tips for single-channel pipettor (20 to 200 µl)
Disposable Tips for Eppendorf® Repeater™ Pipette (25 and 500 µl)
Disposable Tips for 8-channel pipettor (25 to 200 µl)
Kimtowels® Wipers or equivalent low-lint paper towels
Power Surge Protector
Disposable bench cover
Powder-free gloves
5-ml and/or 15-ml snap-cap, round-bottom Polypropylene Tubes (for Probe dilution)
Disposable serological pipette or single-channel pipettor and tips capable of 1000-µl volume (for probe Diluent and PreservCyt Solution Specimen processing)

PreservCyt Solution Specimen Processing Equipment and Accessories

hc2 Sample Conversion Kit[A] (for PreservCyt Solution specimen processing)
Swinging Bucket Centrifuge capable of reaching 2900 ± 150 x g and holding 10-ml or 15-ml conical polypropylene centrifuge tubes
hc2 Sample Conversion tubes (15 ml conical)[D] or Sarstedt 10-ml Conical tubes with Caps (Cat. # 62 9924-283) or VWR or Corning® brand 15-ml conical-bottom polypropylene centrifuge tubes with caps
Disposable tips for Eppendorf® Repeater™ Pipette (50 and 100 µl)

[A] Only equipment and accessories validated with hc2 HPV DNA Tests are available from Digene.
[B] 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.
[C] These items are used for the water bath method only and are not required when the Microplate Heater I Hybridization method is used.
[D] The hc2 Sample Conversion Tubes (VWR or Corning® brand) available from Digene must be used to assure proper assay performance when using the Multi-Specimen Tube Vortexer 2 procedure.

WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

SAFETY PRECAUTIONS

1. HANDLE ALL ASSAY SPECIMENS AND DISPOSED MATERIALS AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS. Patient specimens should be handled at the BSL 2 level as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories*, 3rd Edition, 1993, pp. 10 – 13 and Clinical and Laboratory Standards Institute/NCCLS Approved Guideline M29-A, *Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue.*

2. Do not pipette by mouth.
3. Do not smoke, eat, or drink in areas where reagents or specimens are handled.
4. Wear disposable powder-free gloves while handling reagents or specimens. Wash hands thoroughly after performing the test.
5. All materials used in this assay, including reagents and specimens, should be disposed of in a manner that will inactivate infectious agents.
   Solid Wastes: Autoclave.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 0.5% (1:10 dilution of household bleach). Allow 30 minutes for decontamination before disposal.[34,35]
6. SPILLS: Clean and disinfect all spills of specimens using a tuberculocidal disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant. Base-containing spills should be neutralized, wiped dry, and then the spill areas should be wiped with a 0.5% sodium hypochlorite solution.
7. The wiped area should be covered with absorbent material, saturated with a 0.5% sodium hypochlorite solution and allowed to stand for at least 10 minutes. A glass or plastic cover or tray can be used to reduce exposure to fumes.
8. Treat all wiping materials as hazardous waste.

SAFETY AND HEALTH RISK INFORMATION

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

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.
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.

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. Performing the assay outside the established time and temperature ranges may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
3. Do not use the reagents beyond the expiration date on the outer box label.
4. The hc2 High-Risk HPV DNA Test Procedure, Quality Controls, assay Calibration and Verification criteria, and the Interpretation of Specimen Results must be followed closely to obtain reliable test results.
5. 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.
6. With the exception of Wash Buffer, these components are lot specific and have been tested as a unit. **Do not** interchange components from other sources or from different lots.
7. 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.**
8. 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 the skin. **Covering the Capture Microplate after the wash step and during Detection Reagent 2 incubation is especially important, because exogenous alkaline phosphatase may react with Detection Reagent 2 producing false-positive results.**
9. Protect Detection Reagent 2 from prolonged exposure to direct light. Use reagent immediately after aliquoting and avoid direct sunlight.
10. 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 the liquid, and refilling. See pipettor instruction manuals for specific directions for use.
11. 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. Refer to the manufacturer's multichannel pipettor operator's manual.
12. Care should be taken during washing to ensure that each microwell is washed thoroughly, as indicated in the Manual Washing instructions. Inadequate washing will result in increased background and may cause false-positive results. Residual Wash Buffer in wells may result in reduced signal or poor reproducibility.
13. The cervical brush is for use with non-pregnant women only.
14. Allow 60 minutes for the Microplate Heater I to equilibrate to 65°C ± 2°C from a cold start. Not allowing for this warm-up period could result in melting of the Hybridization Microplate. Consult the *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 15-30°C, if desired.
2. Do not use after the expiration date printed 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, High-Risk HPV Probe, and Wash Buffer.

When using the Rapid Capture System, refer to the *Rapid Capture System User Guide* for preparation of hc2 High-Risk HPV DNA Test reagents for the 1-plate or 4-plate kits, as appropriate.

REF 5199-00018 (4-plate kit) must be used with the Rapid Capture System; reagent volumes for this kit are insufficient to run the manual procedure.

Reagent	Preparation Method
Denaturation Reagent	**PREPARE FIRST:** • Add 5 drops of Indicator Dye to the bottle of Denaturation Reagent and mix thoroughly. The Denaturation Reagent should be a uniform, dark purple color. Once prepared, the Denaturation Reagent is stable for 3 months when stored at 2-8°C. Label it with the new expiration date. If the color fades, add 3 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 removing cap from bottle and when handling.

<table>
<tr><th>Reagent</th><th>Preparation Method</th></tr>
<tr><td>High-Risk HPV Probe Mix

(Prepared from High-Risk HPV Probe and Probe Diluent reagents)</td><td>
PREPARE DURING SPECIMEN DENATURATION INCUBATION:

IMPORTANT: SOMETIMES PROBE GETS TRAPPED IN THE VIAL LID.

Note: Probe Mix must be made fresh prior to performing each assay run. 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 High-Risk HPV Probe. A visible vortex must form in the liquid during the mixing step. Incomplete mixing may result in reduced signal.

• Centrifuge the vial of High-Risk HPV Probe briefly to bring liquid to bottom of vial. Tap 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 the pipette tips or on the side of the vial. Refer to suggested volumes listed below. The smallest number of wells recommended for each use is 24. If fewer than 24 wells per assay are desired, the total number of tests per kit may be reduced due to limited Probe and Probe Diluent volumes.
• Transfer the required amount of Probe Diluent to a new disposable container. Depending on the number of tests, either a 5-ml or 15-ml snap-cap, round bottom, polypropylene tube is recommended. Make a 1:25 dilution of High-Risk HPV 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 µl</td></tr>
<tr><td>72/9</td><td>3.0 ml</td><td>120 µl</td></tr>
<tr><td>48/6</td><td>2.0 ml</td><td>80 µl</td></tr>
<tr><td>24/3</td><td>1.0 ml</td><td>40 µl</td></tr>
<tr><td>1 test</td><td>0.045 ml</td><td>1.8 µl</td></tr>
</table>
*These values include the recommended extra volume.

• Pipette High-Risk HPV Probe into Probe Diluent by placing the pipette tip against the inner wall of the tube just above the meniscus and expelling the contents. Do not immerse the tip into Probe Diluent.
• Vortex for at least 5 seconds at maximum speed to mix thoroughly. A visible vortex must be produced. Label as “High-Risk HPV Probe Cocktail.” Unused Probe Mix should be discarded.
</td></tr>
</table>

Reagent	Preparation Method
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 covered container, or prepared 1 L at a time and placed in the Automated Plate Washer Reservoirs. See the table below for mixing volumes: **Warning: Wash Buffer Concentrate is toxic by ingestion. Wear suitable protective clothing, gloves, eye/face protection. To minimize exposure, add water to the Wash Buffer Concentrate when preparing.** Amount of Wash Buffer Concentrate | Amount of Distilled or Deionized Water | Final Volume of Wash Buffer 33.3 ml | 966.7 ml | 1 L 66.7 ml | 1,933.3 ml | 2 L 100.0 ml | 2,900.0 ml | 3 L **It is very important to always leave the power to the unit on at all times. This allows the maintenance rinse to be performed after eight hours of nonuse.** **Prior to each assay, make sure the waste reservoir is empty and the rinse reservoir is filled with deionized water.** See *Automated Plate Washer Operator's Manual* for additional *Care and Maintenance* instructions. **Alternative manual plate washing method:** • Mix Wash Buffer Concentrate well. • Dilute 100 ml Wash Buffer Concentrate with 2.9 L of distilled or deionized water in Wash Apparatus and mix well (final volume should be 3 L). • Seal the container to prevent contamination or evaporation. Once prepared, the Wash Buffer is stable for three months at 2-25°C. Label it with the new expiration date. If Wash Buffer has been refrigerated, equilibrate to 20-25°C before using. It is recommended that the Wash Apparatus and tubing be cleaned with bleach and rinsed thoroughly with distilled or deionized water once every three months to prevent possible contamination from alkaline phosphatase present in bacteria and molds.

Volumes for Ready-to-Use Reagents

Detection Reagent 1 **&** **Detection Reagent 2**	**IMMEDIATELY PRIOR TO USE:** Mix reagent thoroughly, then carefully measure the appropriate volume of Detection Reagent 1 (or Detection Reagent 2) into a clean reagent reservoir following the guidelines shown below. To avoid contamination, these reagents MUST NOT be returned to the original bottles: **Discard unused material after use**. If an 8-channel pipettor is not being used, an appropriate repeating pipettor may be substituted. In this case, aliquots of the reagent should be made into a polypropylene tube of sufficient size to hold the required volume as indicated below. No. of Tests/Strips | Volume Detection Reagent 1 and 2 96/12 | contents of bottle 72/9 | 7.0 ml 48/6 | 5.0 ml 24/3 | 3.0 ml 1 test | 0.125 ml

SPECIMEN COLLECTION AND HANDLING

The types of cervical specimens recommended for use in the hc2 High-Risk HPV DNA Test are listed below. Specimens taken with other sampling devices or transported in other transport media have not been qualified for use with this assay. **The hc2 High-Risk HPV DNA Test's performance characteristics with other specimen types and collection devices are unknown**.

Cervical specimens must be collected prior to the application of acetic acid or iodine if colposcopic examination is being performed. See the hc2 DNA Collection Device package insert for additional specimen collection and handling procedures.

CERVICAL BRUSHES*

The hc2 High-Risk HPV DNA Test is designed for use with specimens collected and transported using the hc2 DNA Collection Device (cervical brush and STM). Specimens may be held for up to two weeks at room temperature and shipped to the testing laboratory, after which specimens can be stored an additional week at 2-8°C. If the assay will be performed more than 3 weeks from collection, specimens can be placed at -20°C for up to three months prior to testing. A preservative has been added to the STM to retard bacterial growth and to retain the integrity of DNA. It is **not intended** to preserve viability of organisms or cells. The hc2 DNA Collection Device should not be used for collection of specimens from pregnant women.

Time Prior to Testing	***Storage Duration***	***Storage Temperature***
3 weeks	Up to 2 weeks	Room Temperature
	Up to an additional week	2-8°C
Greater than 3 weeks	Up to three months	-20°C

Specimens may be shipped without refrigeration to a testing laboratory; however, specimens should be shipped in an insulated container using either an overnight or 2-day delivery vendor.

CERVICAL BIOPSIES*

Freshly collected cervical biopsies, 2-5 mm in cross-section, may also be analyzed with the hc2 High-Risk HPV DNA Test. The biopsy specimen must be placed immediately into 1.0 ml of STM and stored frozen at -20°C. Biopsy specimens may be shipped at 2-30°C for overnight delivery to the testing laboratory and stored at -20°C until processed. Biopsies less than 2 mm in diameter should not be used.

SPECIMENS IN CYTYC PRESERVCYT SOLUTION

Specimens collected with a broom-type collection device and placed in Cytyc PreservCyt Solution for use in making Cytyc ThinPrep® Pap Test slides can be used in the hc2 High-Risk HPV DNA Test. Specimens should be collected in the routine manner, and the ThinPrep Pap Test slides should be prepared according to Cytyc instructions.

There must be at least 4 ml of PreservCyt Solution remaining for the hc2 High-Risk HPV DNA Test. Specimens with less than 4 ml after the ThinPrep Pap Test has been prepared may contain insufficient material and could be falsely negative with the hc2 High-Risk HPV DNA Test.

PreservCyt Solution specimens may be held for up to three months at temperatures between 2°C and 30°C, following collection and prior to processing for the hc2 High-Risk HPV DNA Test. PreservCyt Solution specimens cannot be frozen. To process these specimens, refer to the hc2 Sample Conversion Kit package insert. For convenience, the specimen processing steps have also been included in the *Test Procedure* section below.

***Note:** *To prevent caps from popping off specimens that are shipped or stored frozen (for STM specimens or converted PreservCyt Solution specimens):*

1. *Cover caps with Parafilm® or equivalent prior to shipping specimens previously frozen. Specimens may be shipped frozen or 20-25°C.*
2. *When removing specimens from the freezer for testing, replace caps immediately with specimen collection tube screw caps.*

TEST PROCEDURE

Specimens may contain infectious agents and should be handled accordingly.

The hc2 High-Risk HPV 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 Instrument Application

The Rapid Capture System is a general-use automated pipetting and dilution system that can be used with the hc2 High-Risk HPV 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 performed for the manual method of the hc2 High-Risk HPV 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 High-Risk HPV DNA Test's procedural steps is performed in the exact sequence as the manual test procedure. The RCS Application allows for the staggered processing of up to 4 microplates, each plate containing specimens and the required assay calibrators and quality controls.

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

SETUP

1. Allow 60 minutes for the HCS Microplate Heater I to equilibrate to 65 ± 2°C from a cold start. Consult 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 specimen 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 at least 15 to 30 minutes.

> **Note:** Prepare PreservCyt Solution specimens prior to equilibrating any previously denatured specimens and kit reagents to room temperature.

3. Create a plate layout using the Digene assay analysis software with Digene assay protocols for HPV. See the applicable software user guide for details.
4. Place Calibrators, Quality Controls, and specimens to be tested in a test tube rack, in the same order in which they will be tested. **The Negative Calibrator and High-Risk HPV Calibrator must be tested FIRST**. Negative Calibrator (NC), High-Risk HPV Calibrator (HRC), Low-Risk HPV Quality Control (QC1-LR), High-Risk HPV Quality Control (QC2-HR), and specimens should be tested in an 8-microwell column configuration. See *Example Layout* below.

EXAMPLE LAYOUT FOR A TEST USING 24 MICROWELLS:

Row	Column		
	1	2	3
A	NC	Spec. 1	Spec. 9
B	NC	Spec. 2	Spec. 10
C	NC	Spec. 3	Spec. 11
D	HRC	Spec. 4	Spec. 12
E	HRC	Spec. 5	Spec. 13
F	HRC	Spec. 6	Spec. 14
G	QC1-LR	Spec. 7	Spec. 15
H	QC2-HR	Spec. 8	Spec. 16

5. NC and HRC are tested in triplicate with High-Risk HPV Probe Cocktail. Digene assay analysis software determines the calibrator and quality control positions in the microplate. See the applicable Digene assay analysis software user guide for proper Calibrator/Quality Control/specimen setup in the software.

DENATURATION

Notes:

- **Caution:** Denaturation Reagent is corrosive. Use care and wear powder-free gloves when handling.
- **Important:** Some cervical specimens may contain blood or other biological material, which 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 this step. In this case, failure to exhibit the proper color change will not affect the results of the assay.
- Do not remove hc2 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.
- Calibrators, Quality Controls, and specimens may be prepared up through the denaturation step and stored at 2-8°C overnight, or at -20°C for up to 3 months. A maximum of 3 freeze/thaw cycles may be performed with a maximum of 2 hours at room temperature during each thaw cycle. Mix well before using.
- 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; **if using the Hybrid Capture System Multi-Specimen Tube (MST) Vortexer 2, make sure it is set to 100 (maximum speed) and a visible vortex of liquid is observed during mixing such that the liquid washes the entire inner surface of the tube. If performing manual vortexing, make sure that each Calibrator, Quality Control, and specimen is mixed individually by vortexing each for at least 5 seconds at full speed such that the liquid vortex washes the entire inner surface of the tube, followed by inverting the tube once.**
- Following denaturation and incubation, the specimens are no longer considered infectious.[36] However, laboratory personnel should still adhere to practical universal precautions.

Calibrators, Quality Controls, and STM specimens (including biopsy hc2 DNA Collection Device specimens)

Note: This procedure is not for preparation and denaturation of PreservCyt Solution specimens.

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

2. 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.

Refer to the *Rapid Capture System User Guide* when using the hc2 High-Risk HPV DNA Test [REF 5199-00018 (4-plate kit)]

Calibrator, Quality Control or Specimen	Vol. Of Denaturation Reagent Required
Negative Calibrator	1000 µl
High-Risk HPV Calibrator	500 µl**
Low-Risk or High-Risk Quality Controls	500 µl*
Cervical Specimen	500 µl*

*If using an Eppendorf® Repeater™ Pipette, use a 12.5-ml tip and a pipettor setting of 2.

**1000 µl for the hc2 High-Risk HPV DNA Test® [REF 5199-00018 (4-plate kit)].

- Dilute remaining Denaturation Reagent in bottle prior to disposing. Dispose of in accordance with local, state and federal regulations.

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

Manual/Individual Tube Vortexing Method

a. Recap the Calibrators, Quality Controls, and specimen tubes with clean specimen collection tube screw caps.
b. Mix each tube thoroughly by vortexing individually, at high speed, for 5 seconds.
c. Invert specimen tube one time to wash the inside of the tube, cap and rim.
d. Return tube to rack.
e. Incubate 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 the High-Risk HPV Probe cocktail during this incubation. See *Reagent Preparation and Storage* section.
f. Remove rack from the water bath.
g. Proceed to the *Hybridization* step below or see *Optional Stop Point* for storage and treatment of denatured specimens.

Multi-Specimen Tube (MST) Vortexer 2 Method

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

a. Cover the Calibrator/Quality Control/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 the lid into place with the two side clamps. Cut the film with the cutter device after the lid is securely fastened.
c. Move the red-handled lever up so that it is in a horizontal position.
d. Place the MST or Digene Specimen Rack and Lid on the MST Vortexer 2 so that the largest notched corner of the rack is located in the right front corner. Position the rack and lid on the MST Vortexer 2 platform so that it fits securely within the guides. Secure the rack in place by moving the red-handled lever down to the vertical position. This will lock the rack in place.
e. Verify that the speed setting is at 100 (maximum speed) and the Pulser button is in the OFF position.
f. Turn the Vortexer power switch to the ON position. **Vortex the tubes for 10 seconds.**
g. Turn the Vortexer power switch to the OFF position.
h. Remove the Rack from the Vortexer by lifting up on the red-handled lever.
i. Incubate 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 the High-Risk HPV Probe cocktail during this incubation. See *Reagent Preparation and Storage* section.
j. Remove rack from water bath, dry the rack, and secure on the vortexer.
k. Turn the Vortexer power switch to the ON position. **Vortex the tubes for 5 seconds.**
l. Turn the Vortexer power switch to the OFF position. Remove the rack.
m. Immediately remove the rack lid and DuraSeal tube sealer film from the specimens.
n. Proceed to the *Hybridization* step below or see *Optional Stop Point* for storage and treatment of denatured specimens.

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.

PreservCyt Solution Specimen Preparation and Denaturation Procedure

Notes:

- Consult the hc2 Sample Conversion Kit package insert for complete details.
- Processing a 4-ml aliquot of PreservCyt Solution produces enough for 2 tests, when tested manually. The minimum volume that can be processed is 4 ml.
- Prepare PreservCyt Solution specimens in batches of 36 or fewer; otherwise, pellets may become dislodged when decanting the supernatant. This is important for maintaining the integrity of the cell pellet during the decanting step. If preparing additional PreservCyt Solution vials, do not start to prepare them until after completing the preparation of the first batch (see the table below).

Reagent Preparation

If not performed previously, prepare the Denaturation Reagent (DNR) from the hc2 Sample Conversion Kit by adding 3 drops of Indicator Dye to the bottle of DNR and mix well. The solution should be a uniform, dark purple color.

Volume requirements are determined based on the number of replicates to be tested per specimen. Use the table below.

Volume Requirements: Reagent Preparation

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

1. Label the 10-ml conical Sarstedt tube or a 15-ml VWR or Corning brand conical tube (required for MST Vortexer 2 method) with the appropriate specimen identification number.
2. Handling one specimen at a time:
   a. For 5-10 seconds, vigorously shake the PreservCyt vial by hand or vortex each vial individually using a vortex mixer at maximum speed setting. Do this to resuspend cells and ensure homogeneity.
   b. Immediately, as cells settle very quickly, pipette the appropriate volume of the PreservCyt specimen into the labeled tube. Deliver the PreservCyt solution to the bottom of the conical tube to minimize cellular material adhering to the inside of the tube.
3. Add the appropriate volume of Sample Conversion Buffer to each tube (see the Table above, *Volume Requirements: Reagent Preparation*).
4. Recap and mix the contents of each tube thoroughly by using a vortex mixer with cup attachment.
5. Centrifuge the tubes in a swinging bucket rotor at 2,900 ± 150 x *g* for 15 ± 2 minutes.
6. During centrifugation, prepare the Specimen Transport Medium (STM) + Denaturation Reagent DNR) mixture in a 2:1 ratio, according to the table below (*Volume Requirements, STM/DNR*).

Note: Solution must be prepared fresh each day the test is being performed.

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

Volume Requirements: STM/DNR

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

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

b. Mix the solution thoroughly by vortexing.

7. Remove tubes from the centrifuge one tube at a time and place into a Rack or MST Vortexer 2 Conversion Rack. A pink/orange pellet should be present in the bottom of each tube.
8. Handling each tube individually:
   a. Remove the cap and set aside on a clean low-lint paper towel.
   b. Carefully decant supernatant.
   c. Maintain the inverted tube position and gently blot (approximately 6 times) on absorbent low-lint paper towels to remove the excess liquid. Use a clean area of the towel each time. **Do not** allow the cell pellet to slide down the tube during blotting.
   d. Place the tube in a Rack or the MST Vortexer 2 Conversion Rack.

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

Manual/Individual Tube Vortexing Method

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

Note: Make sure that all pellets are completely resuspended at this point. Specimens that still have visible pellets are not acceptable for testing.

4. Return the rack to the 65 ± 2°C water bath and continue denaturation for another 30 ± 3 minutes.
5. Proceed to the *Hybridization* step below or see *Optional Stop Point* for storage and treatment of denatured specimens.

Multi-Specimen Tube (MST) Vortexer 2 Method

Notes:

- The Hybrid Capture System Multi-Specimen Tube (MST) Vortexer 2 Method is validated for the processing of PreservCyt Solution specimens following centrifugation and decanting of the supernatant.
- The MST Vortexer 2 procedure has not been validated for vortexing PreservCyt Solution specimens with Sample Conversion Buffer prior to centrifugation.
- Only the MST Vortexer 2 is designed for PreservCyt Solution specimen processing.
- The Conversion Rack and Lid are specifically designed to accommodate VWR or Corning brand 15-ml conical tubes. The

user should use only one tube type on the Conversion Rack at a time. Other brands are not validated for use.

- Strict adherence to the specified vortexing times of the Conversion Rack and Lid is required.
- The Conversion Rack and Lid cannot be used to vortex the hc2 DNA Test kit Calibrators or Quality Controls. The height of the STM tubes prevents adequate Vortexing using the Conversion Rack.

1. After blotting each labeled 15-ml conical tube, place each in its proper position in the Conversion Rack.
2. Add the appropriate volume of Specimen Transport Medium + Denaturation Reagent mixture to each pellet.
3. Cover the 15-ml conical tubes with DuraSeal tube sealer film by pulling the film over the tubes in the rack.
4. Place the rack lid over the film-covered tubes and lock the lid into place with the two side clamps. Cut the film with the cutter device after the lid is securely fastened.
5. Move the red-handled lever up so that it is in a horizontal position.
6. Place the Conversion Rack and Lid on the MST Vortexer 2 so that the largest notched corner of the Conversion Rack is located in the right front corner. Position the rack and lid on the MST Vortexer 2 platform so that it fits securely within the guides. Secure the rack in place by moving the red-handled lever down to the vertical position. This will lock the rack in place.
7. Verify that the speed setting is at 100 (maximum speed) and the Pulser button is in the OFF position.
8. Turn the Vortexer power switch to the ON position. **Vortex the tubes for 30 seconds.**
9. Turn the Vortexer power switch to the OFF position.
10. Remove the Conversion Rack from the Vortexer by lifting up on the red-handled lever.
11. Place the rack in the 65 ± 2°C water bath for 15 ± 2 minutes. Be sure the water level completely covers all liquid in all of the tubes.
12. After the 15-minute incubation, remove the rack with specimens from the water bath.
13. To prevent splashing, dry the rack of excess water prior to placing it on the MST Vortexer 2.
14. Secure the Conversion Rack on the MST Vortexer 2 as described in *Step 6*.
15. Verify speed setting is at 100, and turn the vortexer power switch to the ON position. **Vortex the tubes for 1 minute.**
16. Turn the power switch to the OFF position.
17. Return the rack to the 65 ± 2°C water bath, and continue denaturation for 30 ± 3 minutes.
18. Remove rack from water bath, dry the rack, and secure on the vortexer.
19. Turn the Vortexer power switch to the ON position. **Vortex for 10 seconds at the maximum setting.**
20. Turn the Vortexer power switch to the OFF position. Remove the rack.
21. Immediately remove the rack lid and DuraSeal tube sealer film from the specimens.
22. Proceed to the *Hybridization* step below or see *Optional Stop Point* for storage and treatment of denatured specimens.

Optional Stop Point

After denaturation, all specimens may be stored at 2 - 8°C overnight or at -20°C for up to 3 months. For overnight refrigeration, specimens may be left in the MST, Digene Specimen, or Conversion Rack with new DuraSeal film and Rack Lid replaced. Prior to storage at -20°C, the Rack Lid and DuraSeal film must be removed, and caps placed on the tubes. If the manual vortex procedure was used, place the rack of capped tubes in the desired storage temperature. In either case, the specimens must be equilibrated to room temperature (20 - 25°C) and thoroughly vortexed before proceeding to the Hybridization step.

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

A maximum of 3 freeze/thaw cycles may be performed with a maximum of 2 hours at room temperature during each thaw cycle. For specimens processed using the MST Vortexer 2, remove the Conversion Rack Lid and DuraSeal tube sealer film from the 15-ml conical tubes and cap each tube before storing specimens at -20°C.

HYBRIDIZATION

Notes:

High-Risk HPV Probe mix is viscous. Care should be taken to ensure thorough mixing and that the required amount is completely dispensed into each microplate well. See *Reagent Preparation and Storage* section.

Important: Some cervical 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 change at this step. 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 change of the Calibrators and Quality Controls.

Hybridization Method Using Hybridization Plate and Microplate Heater I

Notes:

- Specimens collected with the hc2 DNA Collection Device in STM and processed using the MST Vortexer method can be hybridized utilizing the Microplate Heater I method **only**.
- If the denatured specimen has been frozen or refrigerated, equilibrate to 20-25°C and vortex on the maximum speed setting for 5 seconds if Vortexing manually, or 10 seconds if using the MST Vortexer 2 with PreservCyt specimens.
- 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. Pipette 75 µl of each Calibrator, Quality Control, or specimen into the **bottom** of an empty hybridization microplate well following the plate layout created under *Setup*. Avoid touching the sides of the wells and limit formation of air bubbles. Use a clean extra-long pipette tip for each transfer to avoid cross-contamination of Calibrators, Quality Controls, or specimens. 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.

   Note: 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.

3. After transferring the last specimen, cover with Parafilm or a plastic lid and **incubate the hybridization microplate for 10 minutes at 20-25°C**.
4. Aliquot the prepared and thoroughly vortexed High-Risk HPV Probe Mix into a disposable reagent reservoir. Carefully pipette 25 µl of the High-Risk HPV Probe mix into each well of the Hybridization Microplate using an 8-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.

   Note: For the above step, use an 8-channel pipettor that is equipped with 25-200 µl tips and that is capable of delivering 25-75 µl. For a small number of wells, use a single-channel pipettor (equipped with 25-200 µl tips) in place of an 8-channel pipettor.

5. Cover the Hybridization Microplate with a plate lid and shake on 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.

Notes:

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

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

Note: Create a plate layout file using the Digene assay analysis software with Digene assay protocols for HPV if this has not been completed earlier.

Hybridization Method using Microtubes and Water Bath

Notes:

- The processing of specimens collected with the hc2 DNA Collection Device in Specimen Transport Medium (STM) using the MST Vortexer 2 method for mixing and the water bath method for hybridization **has not been validated**. Specimens collected with the hc2 DNA Collection Device in STM and processed using the MST Vortexer 2 method can be hybridized using the Microplate Heater I method **only**.
- If the denatured specimen has been stored at -20°C, allow the specimen to thaw to 20-25°C, and thoroughly vortex the specimen before proceeding with hybridization.

1. Label and place the required number of clean hybridization microtubes into the microtube rack.
2. Remove Calibrators, Quality Controls, and specimens from the water bath after incubation. Vortex each tube individually for at least 5 seconds just prior to removing aliquots.
3. Pipette 75 µl of each Calibrator, Quality Control, or specimen into the bottom of empty hybridization microtubes following the plate layout created under *Setup*. 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 stored with the specimen collection devices remaining in the tubes.

Note: 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.

4. After transferring the last specimen, **incubate the hybridization microtubes 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 microtube containing Calibrators, Quality Controls, and specimens using an 8-channel pipettor and fresh tips for each row. Dispense the volume of probe into each hybridization microtube, preventing back splashing.
6. Cover the microtubes with a plate sealer. Place rack cover on top of rack. Shake the microtube rack on Rotary Shaker I set at 1100 ± 100 rpm for 3 ± 2 minutes. *The Calibrators, Quality Controls, and specimens should turn yellow after shaking.* Tubes 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 tubes remain purple after following this procedure, specimens should be retested.

Note: After shaking PreservCyt Solution specimens should turn pink instead of yellow.

7. Incubate in a 65 ± 2°C water bath for 60 ± 5 minutes. Ensure that the water level in the water bath is sufficient to cover the entire volume of hybridization mixture. The microtube rack may be allowed to float in the water bath.

Note: Create a plate layout file using the Digene assay analysis software with Digene assay protocols for HPV if this has not been completed earlier.

HYBRID CAPTURE

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

2. Carefully remove Hybridization Microplate containing Calibrators, Quality Controls, and specimens from the Microplate Heater I. Immediately remove Plate Lid and place it on clean surface. Alternatively, remove microtube rack from the water bath. Immediately remove the rack lid and slowly pull the plate sealer up and across the rack.
3. Transfer the entire contents (approximately 100 µl) of each Calibrator, Quality Control, and specimen from Hybridization Microplate wells or microtubes to the bottom of the corresponding capture microwell using an 8-channel pipettor. Use new pipette tips on the 8-channel pipettor for each column transferred and allow each pipette tip to drain well to ensure complete specimen transfer. If desired, the pipettor may be steadied by resting the middle of the pipette tips on the top edge of the capture microwells (see *Diagram 1*).

DIAGRAM 1: CORRECT PIPETTING

4. Cover microplate with the plate lid or new plate sealer and shake on the Rotary Shaker I at 1100 ± 100 rpm, at 20-25°C for 60 ± 5 minutes.
5. Prepare Wash Buffer. If using the Automated Plate Washer, check Rinse and Waste reservoirs 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 or plate sealer. Remove the liquid from the wells by discarding into a sink; fully invert plate over sink and shake hard with a downward motion being careful not to cause a backsplash by decanting too closely to the bottom of the sink. Do not reinvert plate; blot by tapping firmly 2-3 times on clean Kimtowels® Wipers or equivalent low-lint paper towels. Ensure that all liquid is removed from the wells and the top of the plate is dry.

HYBRID DETECTION

Notes:
- Make additions across the plate in a left-to-right direction using an 8-channel pipettor.
- It is recommended that the reverse pipetting technique be utilized to improve consistency of reagent delivery. With this technique, the pipette tips are initially over-filled by using the second stop on the pipettor's aspirate/dispense control (plunger). See procedure below. Wipe tips on disposable 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 above.

1. Aliquot the appropriate volume of Detection Reagent 1 into a reagent reservoir (see *Reagent Preparation and Storage* section for instructions). Carefully pipette 75 µl of Detection Reagent 1 into each well of the Capture Microplate using an 8-channel pipettor and the reverse pipetting technique. *Verify that all wells have been filled accurately by observing the intensity of the pink color. All wells should have similar intensity.*

   Reverse Pipetting Procedure:

   a. Insert tips into 8-channel pipettor; ensure all tips are firmly seated.
   b. Push the plunger of the pipettor past the first stop to the second stop.
   c. Immerse tips into the Detection Reagent 1 solution.
   d. Release plunger slowly and allow solution to fill the tips.
   e. Dispense 75 µl of solution into microwells by pressing 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 plates with plate lid or clean Parafilm or equivalent and incubate at 20-25°C for 30-45 minutes.

WASHING

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

Automated Plate Washer Method

Note:
- **Always keep the Automated Plate Washer on.** Ensure that the Rinse Reservoir is filled and the Waste Reservoir is empty. The Automated Plate Washer will routinely rinse the system for cleaning. See the Automated Plate Washer Operator's Manual for further instructions, as needed.

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 deionized water.
- Verify that the Waste Reservoir is empty and the cap is securely fastened.
- The Automated Plate Washer will automatically prime itself before each wash, and rinse after each wash.

1. Remove plate lid and place plate on Automated Plate Washer platform.

2. Verify that the power is on, and that the display reads "Digene Wash Ready" or "P1".

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

3. Select the number of strips to be washed by pressing the **ROWS** key and then **+** or **-** to adjust. Press **ROWS** key to return to "Digene Wash Ready" or "P1".

4. Press **START/STOP** to begin.

5. The Automated Plate Washer will perform six fill and aspirate cycles taking approximately 10 minutes. There will be a brief pause during the program so be sure not to remove the plate prematurely. When the Automated Plate Washer is finished washing, it will read "Digene Wash Ready" or "P1".

6. Remove the microplate from the Automated Plate 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 Kimtowels Wipers or equivalent low-lint paper towels to avoid alkaline phosphatase contamination of later steps.

2. Using the Wash Apparatus, hand wash the plate 6 times. Each well must be washed to overflowing to remove Detection Reagent 1 from the top of the wells. Washing begins at well A1 and continues in a serpentine fashion to the right and downward. After all wells have been filled, decant liquid into sink with a strong downward motion. The second wash is started at well H12 moving in a serpentine motion to the left and upward. This sequence of 2 washes is repeated 2 more times for a total of 6 washes per well.

3. After washing, blot the plate by inverting on clean Kimtowels Wipers or equivalent low-lint paper towels and tapping firmly 3-4 times. Replace the toweling and blot again. Leave plate inverted and allow to drain for 5 minutes. Blot the plate one more time.

4. Plate should appear white, and no pink residual liquid should remain in the microwells.

SIGNAL AMPLIFICATION

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

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

ASSAY CALIBRATION VERIFICATION CRITERIA

Assay Calibration Verification is performed to ensure that the reagents and furnished Calibrator material are functioning properly, permitting accurate determination of the assay cut-off value. The hc2 High-Risk HPV DNA Test requires calibration with each assay, therefore it is necessary to verify each assay using the following criteria. This verification procedure is not intended as a substitute for internal quality control testing. **The Digene assay analysis software with version 4.01 or higher Digene assay protocols for HPV automatically verify the criteria below.**

1. Negative Calibrator

   The Negative Calibrator must be tested in triplicate with each assay. The Negative Calibrator mean must be ≥ 10 and ≤ 250 RLUs in order to proceed. The Negative Calibrator results should show a coefficient of variation (%CV) of ≤ 25%. If the %CV is > 25%, discard the Calibrator value with a RLU value farthest from the mean as an outlier, and recalculate the mean using the remaining two values. If the difference between the mean and each of the two values is ≤ 25%, proceed to step 2; otherwise, **the assay calibration verification is invalid and the test must be repeated for all patient specimens. Accordingly, patient specimen results should not be reported.**

2. High-Risk Calibrator

   The High-Risk HPV Calibrator (HRC) must be tested in triplicate with each assay. The Calibrator results should show a coefficient of variation (%CV) of ≤ 15%. If the %CV is > 15%, discard the Calibrator value with a RLU value farthest from the mean as an outlier, and recalculate the mean using the remaining two Calibrator values. If the difference between the mean and each of the two values is ≤ 15%, proceed to step 3; otherwise, **the assay calibration verification is invalid and the test must be repeated for all patient specimens. Accordingly, patient specimen results should not be reported.**

   The assay calibration verification described above for the Calibrators is performed automatically by the Digene assay analysis software and printed in test result reports. **The Digene assay analysis software with version 4.01 or higher Digene assay protocols for HPV automatically verify that the High-Risk Calibrator %CV is ≤ 15%.** However, previous versions (1.0.2 and 1.0.3) of the Digene Qualitative Software will **NOT** invalidate the assay unless the %CV is >25% for the High-Risk HPV Calibrator. **Therefore, the user of version 1.0.2 or 1.0.3 Digene Qualitative Software assay protocols for HPV must manually verify that the %CV calculated by the Digene Qualitative Software is ≤15% and proceed as indicated for Situation 1 in the table below. If the %CV of the Calibrator replicates falls between 15 and 25%, refer to the instructions in Situation 2 or 3 in the table below and proceed with the indicated "User Action."**

Situation	*Reported %CV for the Calibrator Replicates*	*Action Taken by Digene Qualitative Software*	*User Action*
1	≤ 15%	Assay reported as "Valid"	Results may be reported; no further action required.
2	Between 15% and 25%	No outliers removed and assay reported as "Valid"	Remove the Calibrator RLU value farthest from the mean. Recalculate the %CV of the Calibrator with the two remaining values. **If the %CV of the two remaining RLU values is >15%, the assay is invalid. The results must not be reported.** If the %CV of the two remaining RLU values is ≤15%, recalculate the assay cutoff, then recalculate the RLU/cutoff ratio for each specimen using this cutoff. These recalculated values may be reported.
3	Between 15% and 25%	One outlier removed and assay reported as "Valid"	Assay is invalid, results must not be reported. Assay must be repeated.
4	> 25%	One outlier removed and assay reported as "Invalid"	Assay is invalid, results must not be reported. Assay must be repeated.

In order to manually calculate the %CV as required in Situation 2 above, the user should divide the standard deviation (n-1) of the two remaining replicate RLU values by the mean of the two remaining replicate RLU values (HRC) and multiply that result by 100.

To calculate the %CV using Microsoft® Excel® (supplied with the Digene Qualitative Software), the user can calculate the standard deviation of the Calibrator replicates using the formula "STDEV" and determine the mean RLU of the Calibrator using the formula "AVERAGE". Once these two values are obtained, divide the STDEV by the AVERAGE and multiply the result by 100 to obtain the %CV.

(STDEV/AVERAGE) * 100 = %CV

If there are any questions related to calculating %CV's, recalculating the assay cutoff, or recalculating the RLU/cutoff of the specimens, please call Digene Technical Services.

To determine High-Risk Calibrator reproducibility and estimate the frequency in which manual recalculations may be necessary, the results from three clinical evaluations involving 152 assays performed with the hc2 High-Risk HPV DNA Test were compiled. The results showed that the average %CV for these 152 assays was 8.1%. Considering all 3 replicates of the Calibrator per assay, Calibrator reproducibility of greater than 15%CV was observed for only 17 out of 152 (11.2%), with 10 out of these 17 resulting in %CV between 15-25% (Situation 2). For the 17 assays that yielded a %CV greater than 15, a single outlier was removed and the %CV recalculated. Following the User Action for Situation 2, only one of the %CV's remained greater than 15%, invalidating the assay. The %CVs of the remaining 151 assays were calculated for an average %CV of 6.0.

3. The Calibrator mean (HRC$\bar{\chi}$) and Negative Calibrator mean (NC$\bar{\chi}$) results are used to calculate the HRC$\bar{\chi}$ /NC$\bar{\chi}$ ratio. Version 4.01 or higher Digene assay protocols for HPV automatically verify the HRC$\bar{\chi}$ /NC$\bar{\chi}$ ratio acceptable range within 2-15. However, previous versions (v1.0.2 and v1.0.3) of the Digene Qualitative Software assay protocols do not verify the upper limit of this range. This ratio must meet the following criteria to verify the assay calibration **before the specimen results can be interpreted**:

Assay Calibration Verification Acceptable Ranges
$2.0 \leq HRC\bar{\chi} / NC\bar{\chi} \leq 15.0$

4. Calculate the HRC$\bar{\chi}$ /NC$\bar{\chi}$ ratio. If the ratio is ≥2.0 and ≤15.0, proceed to the next step. If the ratio is <2.0 or >15.0, **the assay calibration is invalid and must be repeated.** All patient specimens should be repeated within the assay.

Note: Acceptable ranges for the Calibrators have been established only for Digene-approved luminometers.

CUTOFF CALCULATION

Once an assay has been validated according to the criteria stated above, the Cutoff Value for determining positive specimens is the HRC$\bar{x}$.

Example Cutoff Calculation:

	NC RLU Values	HRC RLU Values
	97 101 91	312 335 307
Mean Value	96	318
%CV	4.9	4.7
HRC$\bar{x}$ /NC$\bar{x}$	NA	3.31

Therefore, the Cutoff Value is (HRC$\bar{x}$) = 318

Notes:

- RLU/CO values and positive/negative results for all specimens tested are calculated and reported in the Digene assay analysis software test result reports.
- For the Rapid Capture System, the RCS HPV software protocol has been programmed to apply a Calibration Adjustment Factor (CAF) of 0.8 to the mean RLU value of the valid Positive Calibrator replicates. This CAF is necessary so that the performance characteristics of the assay remain equivalent to the manual test procedure. This change only applies to assays performed using the Rapid Capture System. Therefore, it is critical to select the correct software protocol for use with each specific test method in order to generate accurate test results. All specimen RLU values should be converted into a ratio to the appropriate Cutoff (CO) Value. For example, all assays should be expressed as Specimen RLU/Cutoff Value.

QUALITY CONTROL

Quality control specimens are supplied with the hc2 High-Risk HPV 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 assay, and the RLU/CO of each control must fall within the following acceptable ranges for it to be considered valid. Version 4.01 and higher Digene assay protocols for HPV will automatically invalidate an assay if the controls are not within the specified limits. Earlier protocol versions 1.0.2 and 1.0.3 will not automatically invalidate an assay if the quality controls are not within the specified limits. **If the quality controls do not fall within these ranges, the assay is invalid and must be repeated.** Accordingly, no patient results should be reported for any invalid assay.

Control	**HPV Type**	**Expected Result** (RLU/Cutoff Value) High-Risk HPV Probe		
		Minimum	Maximum	Average
QC1-LR	Low-Risk (HPV 6)	0.001	0.999	0.5
QC2-HR	High-Risk (HPV 16)	2	8	5.0

Digene Hybrid Capture 2 System users: if predefined controls (QC1, QC2, QC3, and QC4) are in the Controls list, delete them within the software and define the controls (QC1-LR and QC2-HR) listed in the table above. If the laboratory chooses to run replicates of QC1 and QC2 on a plate, the Digene Hybrid Capture 2 System software will not report out patient results if the CV of QC1 or QC2 exceeds 25%.

1. The High-Risk HPV Quality Control provided in the kit is cloned HPV DNA target and is not derived from wild-type HPV. The QC2-HR is 5 pg/ml HPV 16 DNA while the Calibrator contains 1 pg/ml of this same material.

2. This control material will not act as an appropriate processing control for the PreserveCyt specimen.

3. The controls provided with this test kit must be used for internal quality control. Alternatively, external controls may be tested according to guidelines or requirements of local, state, and/or country regulations or accrediting organizations.

Users may develop these external quality control material, as defined by NCCLS C24-A (currently known as CLSI).[37] Please refer to NCCLS C24-A for additional guidance on appropriate internal quality control testing practices.

INTERPRETATION OF SPECIMEN RESULTS

1. Specimens with RLU/CO ratios ≥ 1.0 are considered positive.

2. Specimens with RLU/CO ratios < 1.0 are considered negative or non-detected for the 13 HPV types tested. High-risk HPV DNA sequences are either absent or the HPV Solution DNA levels are below the detection limit of the assay.

3. When testing PreservCyt Solution specimens, if the RLU/CO ratio of a specimen is ≥ 1.0 and < 2.5, the specimen must be retested. If the initial retest result is positive (≥ 1.0 RLU/CO), the specimen can be reported as positive and no further retesting needs to be completed. However, if the first retest result is negative (< 1.0), then a second retest (third result) needs to be completed to generate a final result. The result of the second retest is considered the final result and is to be reported (see Table 1, below).

4. Because this assay only detects high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68, other low-risk HPV types may be present in the specimen.

Table 1
Interpretation of hc2 High-Risk HPV DNA Test Results

RLU/CO ratio	hc2 High-Risk HPV DNA Test Result	Result Report	Interpretation
<1.0	Negative	HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 not detected.	PAP WNL: Very low likelihood of underlying CIN 2-3 or cancer; results do not preclude future HPV infection or cytologic abnormalities with underlying CIN 2-3 or cancer.
			PAP ASC-US: Low likelihood of underlying CIN 2-3 or cancer; results are not intended to prevent women from proceeding to colposcopy.
			PAP LSIL: Reduced likelihood that CIN 2-3 or cancer will be found at colposcopy compared with hc2 High-Risk HPV DNA Test positive LSIL.
			PAP HSIL: Expected to be uncommon result, representing possible error in hc2 High-Risk HPV DNA Test or cytology.
≥ 1.0	Positive	HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, or 68 detected.	PAP WNL: Low likelihood of underlying high-grade CIN; HPV infection may be transient, resolving or persistent.
			PAP ASC-US/LSIL: Low but increased likelihood that underlying high-grade CIN will be detected at colposcopy. Medical literature suggests that progression to high-grade disease is possible.[3,10]
			PAP HSIL: High likelihood that CIN 2-3 or cancer will be detected at colposcopy

The magnitude of the measured result (RLU) above the cutoff is indicative of the total amount of high-risk HPV DNA present but this measurement has no established clinical utility.

Negative assay results do not completely rule out the presence of HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, or 68, particularly at very low concentrations.

The effects of age and HPV positivity are not fully known. It has been demonstrated in studies that HPV prevalence will decrease with age.[38] For information on the age-specific performance of the hc2 High-Risk HPV DNA Test versus a histological diagnosis of high-grade neoplasia, please refer to Table 8 of this package insert.

Additional testing is recommended in any circumstance when false-positive or false-negative results could lead to adverse medical, social or psychological consequences.

Results of this test should be interpreted only in conjunction with information available from clinical evaluation of the patient and from other procedures.

Results of this test are not intended to prevent women from proceeding to colposcopy or from continuing regular cervical cancer screening. This test is not intended for use in women with normal cytology who are under age 30.

DIAGNOSTIC ALGORITHM

This algorithm is used to interpret the results of the hc2 High-Risk HPV DNA Test in conjunction with Pap test results as an aid in determining appropriate patient management. Results should be interpreted only in conjunction with information available from clinical evaluation of the patient including other procedures, patient history and demographics.

Table 2

Diagnostic Algorithm

	High-Risk HPV	
Cytology	**Positive**	**Negative**
Normal (Age 30 and over)	Follow up in accordance with accepted screening guidelines for cytologically normal women with risk factors for cervical cancer.[a,b]	Follow up according to routine screening guidelines[a,c]
ASC-US	Refer to ACS, ASCCP, CDC, US Public Health Service or ACOG current guidelines	
LSIL or HSIL	Refer to ACS, ASCCP, CDC, US Public Health Service or ACOG current guidelines	

[a] At the discretion of the physician, in accordance with ACS, ASCCP, CDC, US Public Health Service and ACOG current guidelines.

[b] The medical literature indicates that although the risk of developing CIN 2-3 and cancer is increased when high-risk HPV is present, most infections are transient and are not indicative of underlying CIN 2-3 or cancer.

[c] A negative hc2 High-Risk HPV DNA Test result with a concurrent normal Pap result implies low risk at a single point in time for the development of CIN 2-3 or cancer and is therefore clinically meaningful for assessing risk; however, there are insufficient data to establish a definitive time period over which this lower risk is clinically relevant.

LIMITATIONS OF THE PROCEDURE

- **Refer to *Rapid Capture System User Guide* for additional Limitations of the Procedure specific to the use of that system for high-volume sample-throughput.**
- The hc2 High-Risk HPV DNA Test for human papillomavirus types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 is not recommended for evaluation of suspected sexual abuse.
- Prevalence of HPV infection in a population may affect performance. Positive predictive values decrease when testing populations with low prevalence or individuals with no risk of infection.
- A negative result does not exclude the possibility of HPV infection because very low levels of infection or sampling error may cause a false-negative result. Also, this test does not detect DNA of HPV low-risk types (6, 11, 42, 43, 44 and many other low-risk types).
- The hc2 High-Risk HPV DNA Test should only be used with cervical specimens collected using the hc2 DNA Collection Device with Specimen Transport Medium (STM) or cervical cytologic specimens collected using a broom-type collection device and placed in PreservCyt Solution. Biopsy specimens may be assayed only if they are placed immediately in STM and stored at -20°C until assayed.
- The hc2 DNA Collection Device should not be used for collection of specimens from pregnant women.
- Infection with HPV is not an indicator of cytologic HSIL or underlying high-grade CIN, nor does it imply that CIN 2-3 or cancer will develop. Most women infected with one or more high-risk HPV types do not develop CIN 2-3 or cancer.
- A negative High-Risk HPV result does not exclude the possibility of future cytologic HSIL or underlying CIN 2-3 or cancer. A small proportion of high-grade lesions occur in women who are High-Risk HPV negative by existing technologies.[6]
- A small amount of cross-hybridization between HPV types 6 and 42 (low-risk HPV types) and the High-Risk HPV Probe exists. Specimens with high levels (4 ng/ml or higher) of HPV 6 or HPV 42 DNA may be positive. It has also been reported in the literature that complex probe cocktails similar to that used in this test may cause false-positive results due to cross-hybridization with HPV types 11, 40, 53, 54, 55, 66, MM4, MM7, MM8, or MM9.[39] Although several of these HPV types are rare or novel types not often encountered with high-grade disease, patients whose specimens contain high levels of these HPV DNA types may incorrectly be reported as positive in the hc2 High-Risk HPV DNA Test.[12,40]
- The hc2 High-Risk HPV DNA Test is designed to detect high-risk HPV types including 39, 58, 59, and 68. Analytical studies conducted by Digene, using cloned HPV plasmid DNA, demonstrate that the assay detects these types at levels ranging from 0.62 pg/ml to 1.39 pg/ml. This is equivalent to the detection characteristics of the other HPV types targeted by the hc2 High-Risk HPV DNA Test. Digene was able to validate the detection of these HPV types in only a limited number of clinical specimens. Due to the low prevalence of these types in the general population (as demonstrated by Bosch et. al.), the performance characteristics of the hc2 High-Risk HPV DNA Test for the detection of HPV types 39, 58, 59, and 68 has not been statistically confirmed.
- If high concentrations of anti-fungal cream, contraceptive jelly, or douche are present at the time a specimen is collected for HPV testing, there is a likelihood of obtaining a false-negative result should these specimens contain HPV DNA levels that yield RLU/CO values near the assay cutoff.

- Cross-reactivity between the hc2 high-risk HPV DNA Test probe and the plasmid pBR322 is possible. The presence of pBR322 homologous sequences has been reported in human genital specimens and false-positive results could occur in the presence of high levels of bacterial plasmid.
- There is no known utility for HPV testing in Pap AGUS results.
- When processing PreservCyt Solution specimens, false-negative results could occur if the cell pellet is not visible after centrifugation. This observation is indicative of insufficient cellular material available to obtain a reliable test result.
- PreservCyt Solution specimens containing volumes less than 4 ml after the ThinPrep Pap Test slides are prepared are considered inadequate for the hc2 DNA Tests.
- Prepare PreservCyt Solution specimens in batches of 36 or fewer. If processing more than 36 specimens at the same time, the additional pellets formed after centrifugation may loosen and be inadvertently discarded during the decanting step.
- The denaturation step of the specimen processing procedure must be performed as directed in this package insert. Improper execution of the denaturation step of the hc2 High-Risk HPV DNA Test Procedure may lead to false-positive results. Improper specimen vortexing, tube inversion and agitation could result in incomplete denaturation of non-specific RNA/DNA hybrids endogenous to cervical specimens. False-positive results could occur due to contamination of the hc2 High-Risk HPV DNA Test specimen with these non-specific RNA/DNA hybrids. In order to prevent possible carryover of this non-denatured cellular material, it is important that the micro-pipette tip not touch the sides of the specimen denaturation tube during transfer of the denatured specimen to the microtube or microplate well used for HPV probe hybridization.

EXPECTED RESULTS

HIGH-RISK HPV PREVALENCE

The prevalence of infection by HPV type, as measured by the detection of an HPV DNA risk group, varies with the patient population. Important variables include age at first intercourse, number of sexual partners, concurrent sexually transmitted diseases and history of abnormal Pap smears.[2,24,31,41] Also, it has been reported that the prevalence of HPV infection decreases dramatically with age.[2,38] Hence, it is not possible to define a single typical pattern of prevalence for HPV infection. Table 3 shows the prevalence in the United States of each high-risk HPV type detected by the hc2 High-Risk HPV DNA Test as reported by two independent researchers. These prevalence values are representative only of the populations tested and may vary in specific areas of the country.

Table 3
Prevalence of Specific High-Risk HPV types in the United States
(Restricted to High-Risk HPV-Positive Specimens)

HPV Type	Prevalence (%)
16	54.5[14]
18	9.1[14]
31	9.1[14]
33	0.2[13]
35	0.2[13]
39	*
45	27.3[14]
51	0.4[13]
52	0.5[13]
56	0.2[13]
58	*
59	*
68	*

*Bosch, et al. reported that HPV types 39, 58, 59 and 68 showed worldwide prevalence of 1.6%, 2.1%, 1.7%, and 1.2% respectively, however prevalence in the U.S. was not determined independently.[14]

Table 4 shows high-risk HPV prevalence results compiled from several groups of women referred to three gynecology clinics within metropolitan medical centers (high prevalence for HPV infection) for cervical abnormality and tested using the hc2 High-Risk HPV DNA Test. These results demonstrate a fairly consistent pattern of HPV positivity across sites.

Table 4
Prevalence of High-Risk HPV Types Across Sites
ASC-US or More Severe Pap Population

Site	Number of Patients	Percent of HPV Positive (# pos/total #) High-Risk Types
1	200	62.0% (124/200)
2	140	63.6% (89/140)
3	184	52.7% (97/184)
Total	524	59.2% (310/524)

Table 5 shows the prevalence of single or combined high-risk HPV types as detected by the hc2 High-Risk HPV DNA Test as reported by six independent researchers. These prevalence values are representative only of the populations tested and may vary from prevalence found in specific areas of the United States.

Table 5
Prevalence of High-Risk HPV* in Various Populations
Women Age 30 years and Older

Location	Study Time Frame	Study Size	Prevalence (%)
USA Portland, OR[6,25,42]	1989-1999	13,493	9.0
Costa Rica[40,43]	1993-1995	6991	8.7
South Africa[44]	1998-1999	2925	23.4
China[45]	1999	1940	18.8
France[46]	1998-2002	2115	4.8
Germany[47]	1999-2000	7592	4.2

*Any combination of the 13 HR types detected by the hc2 High-Risk HPV DNA Test

PERFORMANCE CHARACTERISTICS

CLINICAL SENSITIVITY AND SPECIFICITY FOR SCREENING PATIENTS WITH ASC-US PAP SMEAR RESULTS TO DETERMINE THE NEED FOR REFERRAL TO COLPOSCOPY

A study entitled “Utility of HPV DNA Testing for Triage of Women with Borderline Pap Smears” was conducted in 1996 under the direction of the Kaiser Foundation Research Institute and the Kaiser Permanente Medical Group. Cervical specimens for routine Pap smear and for the hc2 High-Risk HPV DNA Test were obtained from women attending several Kaiser clinic facilities. Initial Pap smears were evaluated according to the Bethesda Classification. Women (15 years or older) with Pap smear results of ASC-US returned for colposcopy and biopsy. Colposcopically directed histological specimens were examined by pathologists and an initial diagnosis was made. Each histologic specimen was also reviewed by an independent pathologist and discrepancies between the initial review and the independent review were adjudicated by a third pathologist.

The hc2 High-Risk HPV DNA Test was performed on the initial specimen. HPV DNA testing was performed with a prototype of the hc2 High-Risk HPV DNA Test that contained probes to 11 of the 13 HPV types included in the hc2 High-Risk HPV DNA Test, but did not contain probes to HPV types 59 and 68. This difference would not be expected to result in significantly different performance profiles for the two assays.

hc2 High-Risk HPV DNA Test results and histological diagnoses were available from 885 women with ASC-US Pap smears. Testing on the majority of patients was performed with specimens collected in both STM and PreservCyt Solution. Due to the similarities between the hc2 High-Risk HPV DNA Test’s performance characteristics for STM and PreservCyt Solution, assay performance is presented for only the PreservCyt Solution.

Table 6 shows that among those presenting with an ASC-US referral Pap smear, the negative predictive value of the hc2 High-Risk HPV DNA Test for having HSIL or greater disease at colposcopy is 99.0%.

Table 6
Comparison of hc2 High-Risk HPV DNA Test Versus Consensus Histology
ASC-US Referral Pap Population
Kaiser Study
PreservCyt Solution Specimens

	CIN 2-3 or cancer at the time of colposcopy			
hc2 High-Risk HPV DNA Test		**+**	**-**	**Total**
	+	**66**	**317**	**383**
	-	**5**	**497**	**502**
	Total	**71**	**814**	**885**

Sensitivity [TP/(TP+FN)] = 93.0% (66/71)
95% CI = 84.3 to 97.7
Specificity [TN/(TN+FP)] = 61.1% (497/814)
95% CI = 57.7 to 64.4
Disease Prevalence = 8.0% (71/885)
Assay Positive Predictive Value = 17.2% (66/383)
Assay Negative Predictive Value = 99.0% (497/502)

Table 7 shows theoretical positive and negative predictive values based on various prevalence results for an initial ASC-US being found to be CIN 2-3 or cancer based on hc2 High-Risk HPV DNA Test results.

Table 7
Theoretical Positive and Negative Predictive Values
hc2 High-Risk HPV DNA Test
ASC-US Pap Smear Results

	Initial ASC-US Pap Smear Result	
Theoretical Prevalence for CIN 2-3 or Cancer	Assay Positive Predictive Value	Assay Negative Predictive Value
5	11.2	99.4
10	21.0	98.7
15	29.7	98.0
20	37.4	97.2
25	44.3	96.3
30	50.6	95.3

Table 8 illustrates the variation between the various age groups contained in this study:

Table 8
Kaiser Study Data
hc2 High-Risk HPV DNA Test Performance versus Consensus Histology Results (CIN 2-3)
Age-Specific Characteristics

	Age < 30	***Age 30 - 39***	***Age >39***
N	287	233	365
Prevalence of Disease (%)	12.2	11.2	2.7
Sensitivity (%)	100.00 (35/35)	88.46 (23/26)	80.00 (8/10)
95% Confidence Interval	90.0-100	69.9-97.6	44.4-97.5
Specificity (%)	31.4 (79/252)	66.2 (137/207)	79.15 (281/355)
95% Confidence Interval	25.7-37.5	59.3-72.6	74.6-83.3
Negative Predictive Value (%)	100 (79/79)	97.86 (137/140)	99.29 (281/283)
Positive Predictive Value (%)	16.83 (35/208)	24.73 (23/93)	9.76 (8/82)

IN WOMEN 30 YEARS AND OLDER, SCREENING PERFORMANCE OF THE hc2 HIGH-RISK HPV DNA TEST AS AN ADJUNCT TO THE PAP TEST TO HELP GUIDE PATIENT MANAGEMENT

Test Performance in Clinical Specimens

Although no clinical trial was performed specifically to support the use of hc2 High-Risk HPV DNA Test as an adjunct to the Pap test, compared with Pap test alone, consistent data obtained from multiple cross-sectional and prospective cohort studies conducted with a variety of cell sampling methods and utilizing the hc2 HPV DNA Tests and several research-use testing methods provide strong evidence that a negative HPV DNA test implies very low risk of prevalent or incipient CIN 2-3 or cancer when Pap results are normal (WNL).[6,25,42-49]

ANALYTICAL SENSITIVITY

Internal Study using Plasmid DNA

A non-clinical panel of cloned HPV plasmid DNA was tested to determine if each of the 13 HPV types are detectable by the hc2 High-Risk HPV DNA Test and to determine the analytical sensitivity of the assay for each of the HPV types. Each HPV target concentration (100 pg/ml, 10 pg/ml, 2.5 pg/ml, 1.0 pg/ml, 0.5 pg/ml, and 0.2 pg/ml targets of each of the 13 HPV DNA types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) was run in triplicate. The mean signal (in Relative Light Units, RLU) for each concentration of each HPV type was calculated and compared to the HRC$\bar{x}$.

The detectable limit of each HPV type is shown in Table 9. The detectable limits varied from 0.62 pg/ml to 1.39 pg/ml depending on the HPV type tested. All HPV types were detectable at an estimated level of 1.08 pg of HPV DNA target per 1 ml of specimen. The mean detectable limit of all 13 HPV DNA types was 1.08 pg/ml with a standard deviation of 0.05 pg/ml.

Table 9
Summary of hc2 High-Risk HPV DNA Test Detectable Limits of
Sensitivity for Each Detectable HPV DNA Type

HPV DNA Type	Detectable HPV DNA Concentration (pg/ml)	Standard Deviation	95% Confidence Range
16	1.09	0.06	0.94 - 1.29
18	1.05	0.05	0.88 - 1.29
31	1.01	0.05	0.91 - 1.15
33	1.35	0.02	1.26 - 1.45
35	1.11	0.05	0.95 - 1.31
39	1.39	0.09	1.16 - 1.71
45	1.14	0.04	0.99 - 1.35
51	0.78	0.10	0.70 - 0.88
52	1.37	0.06	1.21 - 1.58
56	0.62	0.04	0.58 - 0.67
58	0.82	0.04	0.73 - 0.94
59	1.10	0.06	1.00 - 1.21
68	1.19	0.04	1.03 -1.39
Mean (all types)	1.08	0.05	0.95 - 1.25

Note: These analytical detection levels have also been clinically validated for the hc2 HPV DNA Test; however, analytical performance can be poorly correlated to clinical performance if there is inadequate attention devoted to setting a correct threshold for positive results, particularly for molecular test methods with high analytical sensitivity. It has recently been stated in the published literature that a low threshold appears to exist under which levels of HPV infection are not associated with cervical disease, rendering detection at such levels clinically irrelevant. This study concluded further that it is important to make a clear distinction between clinically relevant and irrelevant high-risk HPV infections when considering HPV tests for cervical cancer screening programs.[50]

External Study Using Clinical Specimens

Note: The following information is provided for analytical purposes only to demonstrate that the hc2 assay detects the thirteen HPV types for which it was designed and does not infer any correlation to clinical performance.

In addition to the constructed HPV plasmid data shown above, the ability of the hc2 HPV DNA test to detect High-Risk HPV DNA from archived clinical specimens characterized by type-specific PCR was evaluated. In a study conducted by Digene and the National Cancer Institute (NCI) involving 209 PreservCyt specimens, a research use type-specific HPV polymerase chain reaction (PCR) test method was utilized by NCI to determine concordance with the hc2 High-Risk HPV DNA Test. Specimens were selected specifically to demonstrate detection of the thirteen high-risk types of HPV recognized by the hc2 assay. The NCI PCR result was used as the sole determinant for the presence of HPV DNA. Of the 209 specimens, the proportion of PCR negative results that were positive by hc2 High-Risk HPV DNA Test was 31/56. Conversely, the proportion of PCR positive results that were negative by hc2 was 5/153 (see Table 10 below). When analyzed in this manner, an overall 82.8% agreement (173/209; 95% CI = 77.0-87.6) was observed between the methods and a positive and negative agreement of 96.7% and 44.6%, respectively (95% CI =92.5-98.9 and 31.3-58.5).

Table 10

Analytical Detection of HPV DNA Comparing the hc2 High-Risk HPV DNA Test to HPV Type-specific PCR

		PCR		
		Positive	*Negative*	*Total*
hc2 High-Risk HPV DNA Test	*Positive*	148	31	179
	Negative	5	25	30
	Total	153	56	209

When comparing the test methods described above, PCR was used as an indicator of HPV DNA detection. However, analytical PCR test performance can vary greatly due to a lack of test method standardization and inherent issues known to affect PCR method performance. In addition, this study was conducted prior to the introduction of a procedural modification to the hc2 test that demonstrated improved assay reproducibility around the test cut-off and a reduction in apparent false positive HPV results due to possible technique-related variability when performing the test with specimens collected in PreservCyt.

EQUIVALENCE BETWEEN STM AND PRESERVCYT SOLUTION SPECIMENS

Equivalence between STM and PreservCyt Solution specimens was examined for equal recovery of HPV 18 DNA from approximately 10^6 positive HeLa cells containing integrated HPV 18 genomes spiked into STM and into a negative cell pool in PreservCyt Solution. Each specimen type was processed according to its respective processing/denaturation procedures described in this package insert and tested with the hc2 High-Risk HPV DNA Test. The results demonstrated that recovery of HPV 18 DNA from human carcinoma cells is equivalent for the two media and that the PreservCyt Solution preparation procedure does not affect the analytical sensitivity of the hc2 High-Risk HPV DNA Test.

REPRODUCIBILITY

A multicenter reproducibility study was performed to determine the between days, between sites, and overall reproducibility of the hc2 High-Risk HPV DNA Test using a panel of HPV DNA targets and HPV-positive and HPV-negative clinical specimens.

Three external laboratories performed the testing with the same lot of hc2 High-Risk HPV DNA Test kits on three different days with an identical reproducibility panel. The reproducibility panel included the following specimens: 12 denatured clinical STM specimen pools; three undenatured clinical PreservCyt Solution specimen pools; Negative Calibrator; and Positive High-Risk HPV Calibrator at concentrations of 0.5 pg/ml, 1 pg/ml, 2.5 pg/ml, 5 pg/ml, and 10 pg/ml. All panel members were tested each day in triplicate. The results are shown in Table 11.

Table 11

Summary of Overall Statistics for Multicenter Reproducibility of the hc2 High-Risk HPV DNA Test

Statistical Measure	High-Risk HPV Probe[a]
Proportion of expected positives with an observed positive result	100% (99.0-100.0)
Proportion of expected negatives with an observed negative result	99.0% (97.49-99.73)
Agreement	99.5% (98.70-99.86)
Kappa	0.990

[a] Numbers in parentheses indicate 95% confidence intervals. Overall data are a combination of all assays at all sites.

This indicates that the reproducibility of the hc2 High-Risk HPV DNA Test with clinical specimens is very good.

A second study was performed using simulated PreservCyt Solution specimens and conducted at two external laboratories and Digene. Each testing laboratory performed two hc2 HPV DNA Test assays per day on five different days. For each assay, a reproducibility panel of six simulated PreservCyt Solution specimens was individually processed and tested in quadruplicate. Each panel member was formulated by spiking cultured cells into PreservCyt solution to yield an approximate RLU/CO value simulating two negatives (1N, 2N), two low positives (3P, 4P), one mid positive (5P) and one high positive (6P). Results are shown in Table 12.

Table 12
Summary of Overall Statistics for Multicenter Reproducibility for
PreservCyt Solution Specimens using the hc2 High-Risk HPV DNA Test

Specimen	N	Mean RLU/CO	95% Confidence Interval	HPV Positive n (%)	HPV Negative n (%)
1N	120	0.17	0.01 - 0.33	0 (0.0)	120 (100.0)
2N	120	0.18	0.03 - 0.33	0 (0.0)	120 (100.0)
Total Negative	240			0 (0.0)	240 (100.0)
3P	120	4.97	3.46 - 6.48	120 (100.0)	0 (0.0)
4P	120	5.14	3.43 - 6.85	120 (100.0)	0 (0.0)
5P	120	33.1	19.47 - 46.73	120 (100.0)	0 (0.0)
6P	120	239.6	175.42 - 303.78	120 (100.0)	0 (0.0)
Total Positive	480			480 (100.0)	0 (0.0)

An additional in-house reproducibility study was performed using clinical PreservCyt specimens obtained predominately from 252 women with cytology of ASC-US or greater (HPV prevalence 57%). Specimens were divided into two aliquots; each aliquot was then processed individually using the hc2 Sample Conversion Kit and then tested in duplicate with the hc2 High-Risk HPV DNA Test. As with other qualitative IVDs, variability of hc2 High-Risk HPV DNA Test results obtained from clinical specimens is associated primarily with one or a combination of the following: 1) Specimen collection; 2) specimen processing prior to testing; and 3) the testing procedure. Since the test results under comparison were obtained from the same clinical specimen, the experimental design controlled for variability due to specimen collection. The reproducibility of results obtained from two individually processed specimen aliquots from the same clinical specimen (referred to as “Between Processed Aliquots”) reflects variation due to the combination of PC specimen conversion processing and the hc2 test procedure. In contrast, the reproducibility of replicate results obtained from the same processed specimen aliquot (referred to as “Within Processed Aliquot”) reflects variation from the hc2 test procedure alone. The results are shown in Table 13.

Table 13
Summary of hc2 HPV DNA Test Reproducibility
PreservCyt Specimens, ASC-US or Greater Cytology

Reproducibility	Positive Agreement (n/N) 95% CI	Negative Agreement (n/N) 95% CI	Overall Agreement (n/N) 95% CI
Within a Processed Aliquot	97.59 (283/290) 95.09 - 99.02	94.39 (202/214) 90.41 - 97.07	96.23 (485/504) 94.18 - 97.72
Between Processed Aliquots	98.62 (285/289) 96.49 - 99.62	94.88 (204/215) 91.03 - 97.42	97.02 (489/504) 95.14 - 98.32

Since each specimen in the study generated four test results, there was insufficient volume remaining to allow for retesting of specimens in the defined Cutoff Region Retest Area; therefore, Table 12 presents initial data only. In Table 13, the results from this study are tabulated where only results outside the 1.0 to 2.5 RLU/CO Cutoff Region are considered in the analysis. An assay user employing the Cutoff Region Retest Algorithm given in the **Interpretation of Specimen Results** section of this Package Insert would be expected to obtain results between those seen in Tables 13 and 14.

Table 14
Summary of hc2 High-Risk HPV DNA Test Reproducibility of Test Results <1.0 or ≥2.5 RLU/CO PreservCyt Specimens, ASC-US or Greater Cytology

Reproducibility	Positive Agreement (n/N) 95% CI	Negative Agreement (n/N) 95% CI	Overall Agreement (n/N) 95% CI
Within a Processed Aliquot	99.26 (268/270) 97.35 - 99.91	99.51 (202/203) 97.29 - 99.99	99.37 (470/473) 98.16 - 99.87
Between Processed Aliquots	99.62 (265/266) 97.92 - 99.99	99.03 (204/206) 96.54 - 99.88	99.36 (469/472) 98.15 - 99.87

A multi-center clinical study was also conducted to estimate the additional contribution of cervical specimen sampling to hc2 High-Risk HPV DNA Test result variability. These results are summarized in Table 15. Paired PreservCyt specimens were taken from each patient, processed separately using the hc2 Sample Conversion Kit, then tested separately. Paired STM specimens were also collected and tested separately. Specimens were collected from female patients attending an OB/GYN clinic, colposcopy clinic, STD clinic, hospital, or family planning center. Four geographically diverse sites within the United States collected the PreservCyt specimens, and the STM specimens were all collected from a separate population from multiple clinics in metropolitan San Diego. Testing was performed at four accredited U.S. laboratories. Results for each specimen type were interpreted as recommended, i.e., PC specimen testing employed a Cutoff Region Re-test Algorithm in the 1.0 to 2.50 RLU/CO range, while the initial test results were compared for STM specimens.

Note: These data do not equate to clinical false-positive or false-negative results due to the nature of the paired study design, which assesses duplicate specimen testing agreement.

Table 15
Clinical HPV DNA Paired Specimen Test Reproducibility

Specimen Type	HPV Prevalence	Positive Agreement (n/N) 95% CI	Negative Agreement (n/N) 95% CI	Overall Agreement (n/N) 95% CI
STM (initial)	29.2%	92.15 (270/293) 88.45 - 94.96	97.89 (695/710) 96.54 - 98.81	96.21 (965/1003) 94.84 - 97.31
PC (with Retest algorithm)	19.0%	88.37 (190/215) 83.31 - 92.33	97.53 (910/933) 96.20 - 98.35	95.82 (1099/1148) 94.40 - 96.83

These results reflect variability associated with specimen collection, in addition to the variability due to specimen processing and the assay procedure. Further inspection of these results revealed that result variability was concentrated in the assay cutoff region. When only specimens yielding results outside the cutoff region are included in the analysis, positive agreement for PC specimens increases to 94.0% while negative agreement increases to 99.4%; while similarly for STM specimens, the positive and negative agreement values increase to 97.5% and 99.6% respectively.

CROSS-REACTIVITY

CROSS-REACTIVITY PANEL

A battery of bacteria, viruses and plasmids commonly found in the female anogenital tract, as well as a collection of cutaneotropic HPV types for which clones were available, were assayed to determine if cross-reactivity would occur with the HPV probes used in the hc2 High-Risk HPV DNA Test. All microorganisms were assayed at concentrations of 10^5 and 10^7 organisms per ml. Purified DNA of viruses and plasmids were assayed at a concentration of 4 ng per ml.

Below is a list of the bacteria tested. All bacteria tested negative in the hc2 High-Risk HPV DNA Test.

Acinetobacter anitratus
Acinetobacter lwoffi (ATCC 17908)
Bacteroides fragilis (ATCC 25285)
Bacteroides melaninogenicus
Candida albicans (ATCC 14053 or 10231)
Chlamydia trachomatis
Enterobacter cloacae
Escherichia coli (HB101)*
Escherichia coli
Fusobacterium nucleatum
Gardnerella vaginalis
Haemophilus ducreyi
Klebsiella pneumoniae
Lactobacillus acidophilus
Mobiluncus curtisii
Mobiluncus mulieris
* Both the *E. coli* strain used to grow plasmids (HB101) and a clinical isolate of *E. coli* were assayed.

Below is a list of the viral or plasmid DNA or human serum tested:

Adenovirus 2
Cytomegalovirus
Epstein-Barr Virus
Hepatitis B surface antigen-positive serum
Herpes Simplex I
Herpes Simplex II
Human Immunodeficiency Virus (HIV, RT DNA)
Simian Virus type 40 (SV40)
Human Papillomavirus type 1
Human Papillomavirus type 2
Human Papillomavirus type 3
Human Papillomavirus type 4
Human Papillomavirus type 5
Human Papillomavirus type 8
Human Papillomavirus type 13
Human Papillomavirus type 30
pBR322

The only plasmid that showed cross-reactivity in the hc2 High-Risk HPV DNA Test was pBR322. Cross-reactivity between pBR322 and hc2 High-Risk HPV DNA Test Probe is not unexpected because it is difficult to remove all of the vector pBR322 DNA when isolating the HPV insert. The presence of pBR322 homologous sequences has been reported in human genital specimens, and false-positive results could occur in the presence of high levels of bacterial plasmid. However, 298 clinical specimens testing positive with the hc2 High-Risk HPV DNA Test, showed that no positive results were due to pBR322 when tested with a pBR322 probe. Thus, the likelihood of hc2 High-Risk HPV DNA Test false-positive result due to homologous pBR322 sequences in clinical specimens appears to be low.

CROSS-HYBRIDIZATION

Eighteen different HPV types (high- and low-risk) were tested with the hc2 High-Risk HPV DNA Test at concentrations of 4 ng/ml of HPV DNA. All of the high-risk HPV targets were positive with High-Risk HPV Probe. This study also showed that there is a small amount of crosshybridization between HPV types 6 and 42 and the High-Risk HPV Probe. Patient specimens with high levels (4 ng/ml or higher) of HPV 6 or HPV 42 DNA may be falsely positive with the High-Risk HPV DNA Test. The clinical significance of this is that patients with 4 ng/ml or higher of HPV 6 or HPV 42 DNA may be unnecessarily referred to colposcopy.

The hc2 High-Risk HPV DNA Test has also been shown to cross-react with HPV types 40, 53 and 66. These types are rare and there is insufficient evidence to establish the exact correlation between infection with these types and development of high-grade disease.

EFFECT OF BLOOD AND OTHER SUBSTANCES ON STM SPECIMENS

The effect of blood and other potentially interfering defined or undefined substances was evaluated in the hc2 High-Risk HPV DNA Test . Whole blood, douche, anti-fungal cream and contraceptive jelly (agents that may commonly be found in cervical specimens) were added to STM negative and positive specimens (clinical specimen pools and non-clinical specimens) at concentrations that may be found in cervical specimens. No false-positive results were observed with any of the four agents at any concentration. However, a false-negative result may be reported in clinical specimens with HPV DNA levels close to that of the positive cutoff for the assay (1 pg/ml) if high levels of anti-fungal cream or contraceptive jelly were present. However, it is very unlikely that a clinical specimen will consist almost entirely of one of these substances because the cervix is routinely cleared prior to obtaining specimens for Pap smear and for HPV testing.

EFFECT OF BLOOD AND OTHER SUBSTANCES ON PRESERVCYT SOLUTION SPECIMENS

The effect of blood and other potentially interfering defined or undefined substances potentially present in PreservCyt Solution clinical specimens was evaluated in the hc2 High-Risk HPV DNA Test. Whole blood, douche, anti-fungal cream and contraceptive jelly (agents that may commonly be found in cervical specimens) were added to PreservCyt Solution negative and positive clinical specimen pools at concentrations that may be found in cervical specimens. No false-positive or false-negative results were observed with any of the four agents at any concentration. Furthermore, substances inherent in some clinical specimens do not inhibit the detection of the HPV DNA by the hc2 High-Risk HPV DNA Test.

REPRODUCIBILITY OF hc2 HIGH-RISK HPV DNA TEST WITH CLINICAL SPECIMENS COLLECTED IN STM

The reproducibility of the hc2 High-Risk HPV DNA Test with clinical specimens collected in STM was determined in a study using 20 clinical pools (ten positive and ten negative) prepared by combining previously denatured and tested cervical brush specimens collected in STM. Specimens were tested in replicates of four on each of five days for a total of 20 replicates per specimen. Testing was performed using a combined probe cocktail consisting of the hc2 High-Risk HPV DNA Test probe and low-risk HPV type probes. Mean, standard deviation and 95% confidence interval about the mean (CI) were calculated for each specimen within day and over five days and results are shown in Table 16 below. The reproducibility of the assay would not be expected to differ when using only the high-risk HPV type probe in this kit.

Table 16
Mean RLU/CO with Confidence Intervals and Percent Positive
(Descending Order by Mean RLU/CO)

No.	Spec. ID	Mean RLU/CO	CI	% Positive
1	10	3.18	3.02 - 3.35	100 (20/20)
2	20	1.43	1.36 - 1.50	100 (20/20)
3	11	1.25	1.20 - 1.28	100 (20/20)
4	12	1.21	1.15 - 1.27	100 (20/20)
5	15	1.20	1.14 - 1.25	100 (20/20)
6	13	1.07	1.01 - 1.11	80 (16/20)
7	16	1.06	1.01 - 1.09	75 (15/20)
8	17	1.04	1.00 - 1.06	80 (16/20)
9	14	0.98	0.92 - 1.02	45 (9/20)
10	18	0.92	0.87 - 0.96	20 (4/20)
11	19	0.72	0.68 - 0.75	0 (0/20)
12	7	0.40	0.33 - 0.46	0 (0/20)
13	4	0.38	0.35 - 0.39	0 (0/20)
14	9	0.37	0.32 - 0.41	0 (0/20)
15	1	0.35	0.32 - 0.36	0 (0/20)
16	2	0.35	0.31 - 0.37	0 (0/20)
17	8	0.32	0.29 - 0.34	0 (0/20)
18	3	0.30	0.27 - 0.31	0 (0/20)
19	6	0.27	0.24 - 0.30	0 (0/20)
20	5	0.26	0.23 - 0.28	0 (0/20)

For the five specimens with a mean RLU/CO at 20% or more above the cutoff (Nos. 1-5), 100 of 100 replicates (100.0%) were positive. For the five specimens with a mean RLU/CO within 20% above or below the assay cutoff (Nos. 6-10), 60 of 100 (60%; 95% CI = 49.7-69.6) of the replicates were positive and 40 of 100 (40%) were negative. For the 10 specimens with the mean RLU/CO at more than 20% below the assay cutoff, 200 of 200 replicates (100%) were negative.

Thus, specimens with a mean RLU/CO of 20% or more above the cutoff were positive 100% of the time, while specimens with a mean RLU/CO of 20% or more below the cutoff were negative 100% of the time, indicating that specimens at 20% or more away from the

cutoff can be expected to yield consistent results. Specimens close to the cutoff yielded approximately equal numbers of positive and negative results. These data demonstrate that STM specimens yield reproducible results in the hc2 High-Risk HPV DNA Test.

REPRODUCIBILITY OF PRESERVCYT SOLUTION SPECIMENS IN THE hc2 HIGH-RISK HPV DNA TEST

The reproducibility of clinical specimens in PreservCyt Solution in the hc2 High-Risk HPV DNA Test was determined in a study using 24 mock specimens at a concentration spanning a range of HPV DNA concentrations. Specimens consisted of PreservCyt Solution and white blood cells, with and without HPV 16 plasmid-containing bacteria.

Specimens were tested in replicates of four on each of five days, for a total of 20 replicates per specimen. On each of the five days of the study, an 8-ml aliquot from each specimen was processed and tested according to the hc2 Sample Conversion Kit package insert instructions. Mean, standard deviation, and 95% confidence interval (CI) were calculated for each specimen within day and over all five days and replicates. The mean RLU/CO, confidence interval about the mean, and the percent of positive replicates are shown below in Table 17 for each specimen, in descending order based on the mean RLU/CO.

Table 17
Mean RLU/CO with Confidence Intervals and Percent Positive
(Descending Order by Mean RLU/CO)

No.	Spec #	Mean RLU/CO	CI	% Positive
1	21	3.51	3.19 - 3.83	100 (20/20)
2	12	1.58	1.48 - 1.69	100 (20/20)
3	13	1.42	1.32 - 1.52	100 (20/20)
4	17	1.38	1.23 - 1.53	90 (18/20)
5	18	1.36	1.23 - 1.48	95 (19/20)
6	15	1.32	1.16 - 1.49	85 (17/20)
7	23	1.17	1.06 - 1.27	75 (15/20)
8	16	1.14	1.07 - 1.20	75 (15/20)
9	20	1.10	0.96 - 1.21	85 (17/20)
10	19	1.06	0.95 - 1.17	45 (9/19)
11	22	1.05	0.99 - 1.10	70 (14/20)
12	11	1.04	0.96 - 1.11	65 (13/20)
13	14	0.94	0.86 - 1.01	25 (5/20)
14	24	0.77	0.73 - 0.81	0 (0/20)
15	3	0.28	0.25 - 0.30	0 (0/20)
16	1	0.27	0.24 - 0.30	0 (0/20)
17	7	0.27	0.25 - 0.30	0 (0/20)
18	2	0.27	0.25 - 0.28	0 (0/20)
19	5	0.26	0.24 - 0.28	0 (0/20)
20	4	0.24	0.22 - 0.25	0 (0/20)
21	9	0.23	0.21 - 0.25	0 (0/20)
22	8	0.22	0.18 - 0.27	0 (0/20)
23	10	0.22	0.20 - 0.25	0 (0/20)
24	6	0.19	0.17 - 0.21	0 (0/20)

For the six specimens with a mean RLU/CO at 20% or more above the cutoff (Nos. 1-6), 114 of 120 replicates (95.0%) were positive. For the seven specimens with a mean RLU/CO within 20% above or below the assay cutoff (Nos. 7-13), 88 of 139 (63.3%; 95% CI = 54.3 -70.9) of the replicates were positive and 51 of 139 (36.6%) were negative. For the 11 specimens with the mean RLU/CO at more than 20% below the assay cutoff, 220 of 220 replicates (100%) were negative.

Thus, specimens with a mean RLU/CO of 20% or more above the cutoff were positive greater than 95% of the time, while specimens with a mean RLU/CO of 20% or more below the cutoff were negative 100% of the time, indicating that specimens at 20% or more away from the cutoff can be expected to yield consistent results. Specimens close to the cutoff yielded approximately equal numbers of positive and negative results. These data demonstrate that PreservCyt Solution specimens yield reproducible results in the hc2 High-Risk HPV DNA Test.

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hc2 HIGH-RISK HPV DNA TEST TROUBLESHOOTING GUIDE

Observation	Probable Causes	Solutions
Improper or no color change observed during denaturation.	Denaturation Reagent not added, or Denaturation Reagent not prepared properly.	Verify that the Denaturation Reagent contains the Indicator Dye and is a dark purple color. 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.
	Specimen contains blood or other materials that 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.	If neither of the other causes apply, the specimen may be unusually acidic, and the expected color change will not occur. Collect a new specimen prior to the application of acetic acid to the cervix because improper specimen pH will adversely affect the test results.
Quality Controls give incorrect results	Incorrect software protocol chosen for test (e.g., used LR protocol for HR method)	If the software protocol is incorrect for the test being performed, the plate should be read again within 30 minutes after Detection Reagent 2 addition and with the correct protocol.
	Reverse placement of QC1-LR and QC2-HR	Retest Specimens.
Improper color change observed during hybridization.	Inadequate mixing of Probe Cocktail with denatured Quality Controls and/or specimens; or, Probe Cocktail not added; or, incorrect volume of reagent added.	Shake hybridization microplate or microtube rack for an additional 2 minutes. If there are wells that still remain purple, add an additional 25 µl of the appropriate Probe Cocktail and mix well. If upon probe addition and remixing, the proper color change does not occur, and the specimen did not contain blood or other materials, retest the specimen.
	Specimen contains blood or other materials that 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 STM.	Check the volume of the original specimen. Volume should be 1425 µl ± 20 µl (after removing 75 µl for High-Risk HPV Probe). If volume is < 1425 µl, original specimen contained < 1000 µl STM. Obtain a new specimen.
Assay fails validation criteria. No signal observed in Calibrator, Quality Controls, or in specimens.	No Probe added to Probe Diluent.	Prepare Probe Cocktail as described in the package insert. Label tubes carefully.
	Probe contaminated with RNase during preparation.	Use aerosol-barrier pipette tips when pipetting probe and wear gloves. Dilute probe in sterile containers. 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 5 seconds. A visible vortex must be produced.
	Inadequate mixing of diluted Probe and denatured specimen.	After adding Probe Cocktail and specimen to each Hybridization microwell or microtube, shake on Rotary Shaker I set at 1100 ± 100 rpm for 3 ± 2 minutes. Check for color change from purple to yellow in every tube or well.
	Incorrect time or temperature during hybridization step.	Hybridize for 60 ±5 minutes at 65 ±2°C. Check temperature of Microplate Heater I or hybridization water bath. Ensure the Microplate Heater I is set to heat specimens to correct temperature and is preheated for 60 minutes prior to use. Ensure that water level is adequate to heat specimens to correct temperature. Water baths should be calibrated periodically.
	Inadequate mixing during capture step.	Shake on a the Rotary Shaker I for 60 ± 5 minutes at 20-25°C as described in the 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 or to incubate for specified time.	Pipette 75 µl Detection Reagent 1 into each well using an 8-channel pipettor. Incubate 20-25°C for 30 to 45 minutes.
	Failure to add correct amount of Detection Reagent 2 or to incubate for specified time.	Pipette 75 µl Detection Reagent 2 into each well using an 8-channel pipettor. Incubate 20-25°C for 15 to 30 minutes.
	Luminometer malfunction or incorrect programming.	Refer to the maintenance/service and troubleshooting sections in the applicable Digene assay analysis software user guide for further instructions, or call Digene Technical Services.

Observation	Probable Causes	Solutions
Elevated RLU values in Calibrators, Quality Controls, and/or specimens (≥ 200 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.	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. Calibrators, Quality Controls, and specimens should turn purple after addition of Denaturation Reagent.
	Light leak in the luminometer. Door not sealed. Seal around door broken.	Perform a background reading (raw data measurement) of the luminometer by reading an empty microplate. A reading of greater than 50 RLUs indicates that a light leak may exist. Refer to the maintenance/service and troubleshooting sections in the applicable Digene assay analysis software user guide for further instructions, or call Digene Technical Services.
	Contamination of Detection Reagent 2 or capture microwells 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 microwells after Detection Reagent 1 incubation.	Wash microwells thoroughly with Wash Buffer 6 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 *Automated Plate Washer Operator's Manual* for instructions on testing for contamination or malfunctions.
	Detection Reagent 1 contamination of microwells.	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 Wiper or equivalent low-lint paper towels.	Do not reblot on previously used area of Kimtowels Wiper.
	Used incorrect blotting towels.	Use Kimtowels Wipers or equivalent low-lint paper towels for blotting.
	High-Risk HPV Quality Control material used as Positive Calibrator. Assay fails validation HRC$\bar{\chi}$ / NC$\bar{\chi}$ ratio > 15.	Ensure correct placement of Positive Calibration and Quality Control materials.
Low PC/NC ratios or high number of low positive specimens with ratios <2.0 (> 20%). 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 with both the manual and MST Vortexer methods (for the manual vortexer method, invert tube one time). For PreservCyt Solution specimens ensure proper mixing and resuspension of the cell pellet is completed prior to denaturation incubation. Consult hc2 Sample Conversion Kit package insert for protocol details. 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 Cocktail as described. Mix thoroughly by vortexing ensuring that a visible vortex is produced. Probe Cocktail must be added to tubes with a positive displacement pipettor or multichannel pipettor to ensure accurate delivery.
	Inadequate volume of diluted Probe added to each Hybridization microwell or microtubes.	Verify that the repeating pipettor is delivering accurately prior to adding Probe Cocktail to Hybridization Microplate or microtubes. 25 µl of diluted Probe should be added to the denatured specimen at the bottom of each microwell or microtube. Verify that the 8-channel pipettor is delivering accurately prior to adding probe cocktail to the hybridization wells. Color change should be from dark purple to yellow upon addition and thorough mixing of Probe Cocktail. PreservCyt Solution specimens should turn pink instead of yellow.
	Loss of Detection Reagent 1 activity.	Store Detection Reagent 1 at 2-8°C. Use before the expiration date on the kit outer box label.
	Insufficient capture.	The capture step should be performed using the Rotary Shaker I set at 1100 ± 100 rpm for 60 ± 2 minutes. Validate Rotary Shaker I speed by calibration.
	Inadequate washing.	Wash microwells thoroughly with Wash Buffer 6 times, filling the wells to overflowing each time or using the Automated Plate Washer.

Observation	Probable Causes	Solutions
Low PC/NC ratios or high number of low positive specimens with ratios <2.0 (> 20%). Assay may fail validation criteria. **(continued)**	Contaminated Wash Buffer. Contaminated Wash Buffer (continued).	If Detection Reagent 2 is not contaminated, check Wash Buffer for contamination. Pipette 10 µl Wash Buffer into 75 µl 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 200 RLUs indicate contamination. See Reagent Preparation and Storage section for instructions on cleaning and maintenance of Wash Apparatus. See the *Automated Plate Washer Operator's Manual* for instructions on testing for contamination or malfunctions.
Series of positive specimens with RLU values approximately the same.	Contamination of capture microwells during assay manipulation.	Cover Capture Microplate during all incubations. Avoid exposing tubes 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 microwells. Avoid contamination of Detection Reagent 2 by aerosols from Detection Reagent 1 or from laboratory dust, etc.
	Automated Plate Washer malfunction.	See the *Automated Plate Washer Operator's Manual* for instructions on testing for contamination or malfunctions.
Wide %CVs between replicates.	Inaccurate pipetting.	Check pipettor to ensure that reproducible volumes are being delivered. Calibrate pipettors routinely.
	Insufficient mixing.	Mix thoroughly at all steps. Vortex prior to denaturation incubation and after adding Probe Cocktail. Ensure that a visible vortex is produced.
	Incomplete transfer of liquid from hybridization microwells or microtubes to capture microwells.	Take care during transfer step from hybridization microwells or microtubes to capture microwells to ensure reproducible volumes are transferred.
	Improper washing conditions.	Wash microwells thoroughly with Wash Buffer 6 times, filling to overflowing each time or using Automated Plate Washer and proper Automated Plate Washer protocols.
	Detection Reagent 1 contamination of microwells.	Ensure all work surfaces are clean and dry. Use care when using Detection Reagent 1. Avoid aerosols.
False-positive results obtained from known negative specimens.	Detection Reagent 2 contaminated.	Be careful not to cross-contaminate specimens as you aliquot Detection Reagent 2 between specimens. If only using part of a kit, aliquot the volume needed for that assay into a clean disposable reagent reservoir prior to filling the pipettor.
	Detection Reagent 1 contamination of microwells.	Wash microwells thoroughly with Wash Buffer 6 times, filling to overflowing each time or using Automated Plate Washer. There should be no residual pink liquid visible in the microwells after washing.
	Contamination of pipette tip with undenatured material during transfer of denatured specimen to the microtube or microplate well used for HPV probe hybridization.	The denaturation step of the specimen processing procedure must be performed as directed in this package insert. Improper specimen vortexing, tube inversion and agitation can result in incomplete denaturation of non-specific RNA/DNA hybrids endogenous to cervical specimens. When using PreservCyt Solution specimens in particular, these hybrids are likely to be present on the inside walls of the specimen denaturation tube. In order to prevent possible carryover of this non-denatured cellular material, the micro-pipette tip must not touch the sides of the specimen denaturation tube during transfer of the denatured specimen to the microtube or microplate well used for HPV probe hybridization.
	During decanting and blotting of the capture plate, the plate was blotted on the same area of the Kimtowels Wipers or equivalent low-lint paper towels.	Do not blot on area that has been previously used as cross-contamination could occur.
	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 with both the manual and MST Vortexer methods (for the manual vortex method, invert tube one time). For PreservCyt Solution specimens, ensure proper mixing and resuspension of the cell pellet is completed prior to denaturation incubation. Consult hc2 Sample Conversion Kit package insert for protocol details. For all specimens, a distinct color change to dark purple should be seen. Incubate for 45 ± 5 minutes at 65 ± 2°C.
	Improper washing conditions.	Wash microwells thoroughly with Wash Buffer 6 times, filling the wells to overflowing each time or using the Automated Plate Washer and proper Automated Plate Washer protocols.

Observation	Probable Causes	Solutions
Elevated Negative Calibrator RLU values (> 200 RLUs). Remainder of assay performs as expected.	Detection Reagent 2 was incubated at a temperature greater than 20-25°C.	Repeat the test, and ensure that Capture and Detection steps are incubated at 20-25°C.
	Detection Reagent 2 was incubated longer than 30 minutes.	Read plate after 15 minutes of incubation (and no later 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 aliquoted Detection Reagent 2 for contamination by pipetting 75 µl into a blank capture microwell. Incubate 20-25°C for 15 minutes and read on the luminometer. Readings above 200 RLUs indicate Detection Reagent 2 contamination. Take care when pipetting Detection Reagent 2. Wear 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 kit 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 into a blank capture microwell. Cover and incubate 15 minutes at 20-25°C. Read the microwell on the luminometer. Readings above 200 RLUs indicate Wash Buffer contamination. See Reagent Preparation and Storage section for instructions on cleaning and maintenance of Wash Apparatus.

CONTAMINATION CHECK

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

hc2 HIGH-RISK HPV DNA TEST ORDERING INFORMATION REAGENTS, ACCESSORIES, AND EQUIPMENT

Reagents
hc2 High-Risk HPV DNA Test® [96 tests REF 5199-1220 (1-plate kit)]
hc2 High-Risk HPV DNA Test® [384 tests REF 5199-00018 (4-plate kit)]
hc2 DNA Collection Device
hc2 Sample Conversion Kit
Wash Buffer Concentrate
Cytyc ThinPrep® Pap Test PreservCyt® Solution

Accessories
Microtubes
Microtube Rack
Plate Sealers
Extra-long Pipette Tips
Specimen Collection Tube Rack
Specimen Collection Tube Screw Caps
Disposable Reagent Reservoirs
Specimen Collection Tubes
Microplate Lids
Multi-Specimen Tube Rack and Lid
Conversion Rack and Lid
Digene Specimen Rack and Lid
DuraSeal™ Film Tube Sealer
Tube Sealer Dispenser and Sealer Cutter
Hybridization Microplate
Microplate well strips
hc2 Sample Conversion Tubes

Equipment
Luminometer
PC System
Printer Cable
Printer
Digene Hybrid Capture 2 System Software CD (with Digene Hybrid Capture 2 System Software, Digene Hybrid Capture 2 System Assay Protocols for HPV, LumiCheck Plate Software)
Hybrid Capture System Rotary Shaker I
Hybrid Capture System Microplate Heater I
Hybrid Capture System Automated Plate Washer
Hybrid Capture System Multi-Specimen Tube (MST) Vortexer 2
Wash Apparatus
Rapid Capture System [optional, but required for use of the hc2 High-Risk HPV DNA Test® [REF 5199-00018 (4-plate kit)]

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CONTACT INFORMATION

USE THE DIGENE CONTACT INFORMATION SHEET PROVIDED WITH THIS PRODUCT TO CONTACT YOUR LOCAL DIGENE REPRESENTATIVE.

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This product and its method of use are covered by one or more of the following patents:

U.S. HPV Patent Nos.	Foreign HPV Patent Nos.	U.S. Hybrid Capture Patent Nos.	Other Patents
4,849,331	EP 294,659	4,732,847	CDP-Star® substrate is protected under one or more of U.S. Patent Nos.
4,849,332	JP 1047383	4,865,980	
4,849,334	EP 019200B1	6,228,578B1	4,931,569
4,908,306	EP 0591376B1		4,978,614
5,411,857	CA 1,339,729		5,145,772
5,643,715	EP 0370625B1		5,326,882
5,712,092	JP 3076578		5,538,847
5,876,922	JP 89/38944		5,582,980
5,952,487			5,851,771
5,958,674			
5,981,173			
6,107,086			

Registered trademark acknowledgments:
- Kimtowels Wipers: Kimberly-Clark Corporation
- Eppendorf: Eppendorf-Netheler-Hinz
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- PreservCyt and ThinPrep: Cytyc Corporation
- Windows: Microsoft
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- DuraSeal: Diversified Biotech, Inc.

hc2 HIGH-RISK HPV DNA TEST® SUMMARY

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

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