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digene® HC2 HPV DNA Test Instructions for Use

An in vitro nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of 18 low risk and high-risk types of human papillomavirus (HPV) DNA in cervical specimens

For use with:

digene HC2 DNA Collection Device

digene Specimen Transport Medium

Hologic PreservCyt® Solution

BD SurePath® Preservative Fluid

CE

REF 5196-1330

QIAGEN, 19300 Germantown Road, Germantown, MD 20874, USA

EC REP QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

L2126en Rev. 8

Sample to Insight

QIAGEN

Contents

Intended Use

For in vitro diagnostic use.

The *digene* HC2 HPV DNA Test using Hybrid Capture® 2 (HC2) technology is a nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of 18 low-risk and high-risk types of HPV DNA in cervical specimens.

Cervical specimens that may be tested with the *digene* HC2 HPV DNA Test include the following:

- Specimens collected with the *digene* HC2 DNA Collection Device
- Specimens collected using a broom-type collection device or brush/spatula combination and placed in PreservCyt Solution (refer to the *digene* HC2 Sample Conversion Kit instructions for use for complete details)
- Specimens collected in SurePath Preservative Fluid (ONLY for High-Risk HPV DNA testing)
- Biopsies collected in *digene* Specimen Transport Medium (STM)

When using the low-risk and high-risk HPV Probes, the use of this test is indicated:

- To aid in the diagnosis of sexually transmitted HPV infections with HPV types 6, 11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 56, 58, 59, and 68.
- To differentiate between two HPV DNA groups: low-risk HPV types 6, 11, 42, 43, and 44 and high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68; however, the specific HPV type present cannot be determined.

When using the High-Risk HPV Probe, the use of the test is indicated:

- For the detection of high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68, shown to be the primary causal factor in the development of cervical cancer.
- As an initial general population screening test, for use with or without Pap smear, to identify women at increased risk for the development of cervical cancer or presence of high-grade cervical disease. HPV diagnosis is increasingly indicative of cervical disease as age increases.
- As a follow-up test for patients after abnormal Pap smear results or cervical disease to determine the need for referral to colposcopy or other follow-up procedures.
- As a follow-up test for patients with Low-Grade Squamous Intraepithelial Lesion (LSIL) or High-Grade Squamous Intraepithelial Lesion (HSIL) Pap smear results prior to colposcopy. For these patients, a *digene* HC2 HPV DNA Test result will aid the physician in patient management by assisting with risk assessment of women to determine absence of high-grade disease.

The *digene* HC2 HPV DNA Test 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. Results from the *digene* HC2 HPV DNA Test should not be used as the sole basis for clinical assessment and treatment of patients.

Summary and Explanation

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 carcinoma.[1-3] It is generally accepted that these viruses are predominantly sexually transmitted and that high-risk HPV types are the major recognized risk factor for development of cervical cancer.[4-8]

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. Alternately, 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 HPVs and HPV types 6 and 11 as low-risk HPVs.[8-10] Subsequently HPV types 31, 33, and 35 have been demonstrated to have an intermediate association with cancer.[2,11-14] Despite this useful conceptual framework, these 7 HPV types account for only about 70% of cervical neoplasms.[9-11] Additional HPVs, including types 39, 42, 43, 44, 45, 51,52, 56, 58, 59, and 68 have been identified as the principal HPVs detectable in the remaining lesions[15-20,32-36]. These HPV types can also be categorized into low-, intermediate-, and high-risk groups based on their relative distribution in various histopathological diagnosis categories.[21, 32-37]

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.[2,10,21,31] Prospective studies have shown that 15–28% of HPV DNA positive women developed squamous intraepithelial neoplasia (SIL) within 2 years compared to only 1–3% of HPV DNA negative women.[22,23] In particular, the risk of progression for HPV types 16 and 18 was greater (approximately 40%) than for other HPV types.[22]

Principle of the Procedure

The *digene* HC2 HPV DNA Test using HC2 technology is a signal amplified hybridization antibody capture assay that utilizes microplate chemiluminescent detection. Specimens containing the target DNA hybridize with a specific HPV RNA probe. The resultant RNA:DNA hybrids are captured onto the surface of a microplate well coated with antibodies specific for RNA:DNA hybrids. Immobilized hybrids are then reacted with alkaline phosphatase conjugated antibodies specific for 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 which is measured as relative light units (RLUs) on a luminometer. The intensity of the light emitted denotes the presence or absence of target DNA in the specimen.

An RLU measurement equal to or greater than the Cutoff Value indicates the presence of HPV DNA sequences in the specimen. An RLU measurement less than the Cutoff Value indicates the absence of the specific HPV DNA sequences tested or HPV DNA levels below the detection limit of the assay.

High volume sample-throughput testing with the *digene* HC2 HPV DNA Test can be performed utilizing the Rapid Capture® System (RCS). This instrument processes up to 352 specimens in eight hours. To enable high volume sample-throughput testing, all the procedural steps of the assay are performed by the RCS, with the exception of specimen denaturation, chemiluminescent signal detection, and result reporting.

Kit Contents

There are 96 tests in one digene HC2 HPV DNA Test kit (cat. no. 5196-1330). The number of patient results will vary, depending on the number of uses per kit:

- 1 use = 40 patient results (low-risk and high-risk)
- 2 uses = 32 patient results (low-risk and high-risk)

1 x 0.35 ml	Indicator Dye Contains 0.05% w/v of sodium azide.
1 x 50 ml	Denaturation Reagent Dilute sodium hydroxide (NaOH) solution.
1 x 5 ml	Probe Diluent Buffered solution with 0.05% w/v sodium azide.
1 x 150 µl	Low-Risk HPV Probe HPV 6/11/42/43/44 RNA probe in buffered solution (green cap).
1 x 100 µl	High-Risk HPV Probe HPV 16/18/31/33/35/39/45/51/52/56/58/59/68 RNA probe in buffered solution (red cap).
1 x 1 ml	Low-Risk HPV Quality Control 5 pg/ml (500,000 copies/ml) cloned HPV 6 DNA and carrier DNA in STM with 0.05% w/v sodium azide.
1 x 1 ml	High-Risk HPV Quality Control 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 2.0 ml	Negative Calibrator Carrier DNA in Specimen Transport Medium with 0.05% w/v of sodium azide.
1 x 1.0 ml	Low-Risk HPV Calibrator 1 pg/ml cloned HPV 11 DNA and carrier DNA in STM with 0.05% w/v of sodium azide.

1 x 1.0 ml	High-Risk HPV Calibrator 1 pg/ml cloned HPV 16 DNA and carrier DNA in STM with 0.05% w/v of sodium azide.
1 x 1	Capture Microplate Coated with anti-RNA:DNA hybrid antibodies.
1 x 12 ml	Detection Reagent 1 Alkaline phosphatase-conjugated antibodies to RNA:DNA hybrids in buffered solution with 0.05% w/v of sodium azide.
1 x 12 ml	Detection Reagent 2 CDP-Star® with Emerald II (chemiluminescent substrate).
1 x 100 ml	Wash Buffer Concentrate Contains 1.5% w/v sodium azide.

Materials Required But Not Supplied

Hybrid Capture System In Vitro Diagnostic Equipment and Accessories*	
digene Hybrid Capture 2 System ("*digene* HC2 System"), consisting of a QIAGEN-approved luminometer ("DML instrument"), QIAGEN-approved personal computer and computer peripherals (monitor, keyboard, mouse, printer, and printer cable), *digene* HC2 System Software ("*digene* assay analysis software"), *digene* HC2 System Assay Protocols for HPV, LumiCheck Plate Software, and digene *HC2 System Software User Manual*	Rapid Capture System (optional for high volume sample-throughput testing)†
Hybrid Capture System Rotary Shaker I	Wash Apparatus
Hybrid Capture System Microplate Heater I	Hybridization Microplates
Hybrid Capture System Automated Plate Washer	Microplate Lids
Hybrid Capture System Multi-Specimen Tube (MST) Vortexer 2 (Optional)‡	Empty Microplate Strips (available from Costar, Model #2581); optional for use with the Automated Plate Washer
Conversion Rack and Rack lid (optional)	Extra-Long Pipette Tips for removal of specimen
digene Specimen Rack and Rack lid (optional)	Specimen Collection Tubes
EXPAND-4 Pipettor and Stand (optional)§	Specimen Collection Tube Rack
digene HC2 DNA Collection Device**	Specimen Collection Tube Screw Caps
Tube Sealer Dispenser and cutting device (optional, used with the MST Vortexer 2)	Disposable Reagent Reservoirs
DuraSeal™ Tube Sealer Film	Hybridization Microtubes
Microtube Rack	Plate Sealers

* Only equipment and accessories validated with *digene* HC2 HPV DNA Tests are available from QIAGEN.

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

‡ Also required for use when performing the Semi-automated RCS Application.

§ Custom item. Other custom expandable multi-channel pipettes can be used, provided tip spacing of 3.2 cm is achievable when expanded. Alternatively, a single-channel pipette capable of pipetting 75 µl may be used.

** The performance characteristics of the *digene* HC2 HPV DNA Test were established only with the collection kits indicated.

General Laboratory Use Equipment and Accessories	Additional Equipment and Accessories for PreservCyt Solution Specimen Processing
65 ± 2°C water bath of sufficient size to hold either 1 Conversion Rack (36 x 21 x 9 cm) or specimen racks	Swinging Bucket Centrifuge capable of reaching 2900 ± 150 x *g* and holding 10-ml or 15-ml conical polypropylene centrifuge tubes
Microcentrifuge (optional for centrifuging probe vials to obtain maximum probe volume)	5-ml serological pipettes or transfer pipettes
Vortex mixer with cup attachment	*digene* HC2 Sample Conversion Kit*
Single-channel Micropipettor; variable settings for 20-200 µl and 200-1000 µl volumes	Disposable tips for Eppendorf Repeater Pipette (50 and 100 µl)
Repeating positive displacement Pipettor, such as Eppendorf® Repeater® Pipette or equivalent	For Manual Vortex Procedure
8-channel Pipettor: variable settings for 25-200 µl volumes	*digene* HC2 Sample Conversion Tubes (15-ml conical)†, Sarstedt 10-ml Conical tubes with Caps or VWR® or Corning® brand 15-ml conical-bottom polypropylene centrifuge tubes with caps
Timer	Tube rack to hold 10-ml or 15-ml conical tubes
Sodium hypochlorite solution, 5% v/v (or household bleach)	For Multi-Specimen Tube Vortexer 2 Procedure
Parafilm® or equivalent	*digene* HC2 Sample Conversion Tubes (15-ml conical)†
Disposable aerosol-barrier Pipette Tips for single-channel pipettor (20 to 200 µl and 200-1000 µl)	Multi-Specimen Tube (MST) Vortexer 2
Disposable Tips for Eppendorf Repeater Pipette (25 and 500 µl)	Conversion Rack and Lid (specific for 15-ml conical tubes)
Disposable Tips for 8-channel pipettor (25 to 200 µl)	Tube Sealer dispenser and cutting device
Kimtowels® Wipers or equivalent lint-free paper towels	DuraSeal Tube Sealer Film (used with the MST Vortexer 2)
Disposable bench cover	
Powder-free gloves	
5-ml and/or 15-ml snap-cap, round-bottom Polypropylene Tubes (for Probe dilution)	
2.0-ml polypropylene microcentrifuge tubes with caps	

* Only equipment and accessories validated with *digene* HC2 HPV DNA Tests are available from QIAGEN.

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

Additional Equipment and Accessories for SurePath Preservative Fluid Specimen Processing
Swinging Bucket Centrifuge capable of reaching 800 ± 15 x *g* and holding 15 ml conical polypropylene centrifuge tubes
digene HC2 Sample Conversion Tubes (15-ml conical tubes) *****
7 ml standard tipped transfer pipettes or equivalent
QIAGEN Specimen Transport Media
Disposable tips for Eppendorf Repeater Pipette (100 µl)

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

Warnings and Precautions

READ ALL INSTRUCTIONS CAREFULLY BEFORE USING THE TEST.

Safety Precautions

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

- Do not pipette by mouth.
- Do not smoke, eat, or drink in areas where reagents or specimens are handled.
- Wear disposable powder-free gloves while handling reagents or specimens. Wash hands thoroughly after performing the test.
- Clean and disinfect all spills of specimens using a tuberculocidal disinfectant such as 0.5% v/v sodium hypochlorite, or other suitable disinfectant.[42,43]
- Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with national and local regulations.

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

RCS-Automated Testing

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

Safety and Risk Statements for Components

The following risk and safety phrases apply to components of the *digene* HC2 HPV DNA Test kit:

Denaturation Reagent

Contains: sodium hydroxide. Danger! May be corrosive to metals. Causes severe skin burns and eye damage. Wear protective gloves/ protective clothing/ eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician.

High-Risk HPV Calibrator

Warning! Causes mild skin irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

High-Risk HPV Quality Control

Warning! Causes mild skin irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

Low-Risk HPV Calibrator

Warning! Causes mild skin irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

Negative Calibrator

Warning! Causes mild skin irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

Probe Diluent

Contains: acetic acid; polyacrylic acid. Danger! Causes severe skin burns and eye damage. Wear protective gloves/ protective clothing/ eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician.

Wash Buffer Concentrate

Contains: sodium azide. Warning! Harmful if swallowed.. Harmful to aquatic life with long lasting effects. Wear protective gloves/ protective clothing/ eye protection/ face protection.

For further information, please see the Safety Data Sheets at www.qiagen.com/safety.

Handling Precautions

- For In Vitro Diagnostic Use.
- Cervical brush is for use with non-pregnant women only.
- Do not use the reagents beyond the expiration date indicated next to the symbol  on the outer box label.
- Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges are invalid and must be repeated.
- The *digene* HC2 HPV DNA Test Procedure, Assay Calibration Verification Criteria, Quality Control, and the Interpretation of Specimen Results must be followed closely to obtain reliable test results.
- 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. Making sure that the noted color changes occur will confirm that these conditions have been met.
- The kit components have been tested as a unit. Do not interchange components from other sources or from different lots.
- 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 bench cover and wear powder-free gloves when performing all assay steps.
- Make sure 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, since exogenous alkaline phosphatase may react with Detection Reagent 2 producing false-positive results.
- Protect Detection Reagent 2 from prolonged exposure to direct light. Use Detection Reagent 2 immediately after aliquoting and avoid direct sunlight.

- 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 vendor's instruction manuals for specific directions for use.
- Multi-channel pipetting should be performed using the reverse pipetting technique (see Hybrid Detection) for dispensing Detection Reagents 1 and 2. Check each pipette tip on the multi-channel pipettor for proper fit and filling.
- Make sure that each microplate well 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.
- Allow at least 60 minutes for the Hybrid Capture System Microplate Heater I to equilibrate to 65°C ± 2°C from a cold start. Not allowing for this warm-up period could result in melting of the Hybridization Microplate. See the *Microplate Heater I User Manual* for details.

Reagent Preparation and Storage

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

To test specimens for the presence of any of the 18 HPV types, a Combined-Probe Cocktail (CPC) Method has been provided. To test using this option, a Combined-Probe Cocktail must be prepared by mixing diluted Low-Risk HPV Probe Mix and diluted High-Risk HPV Probe Mix together in advance of performing the *digene* HC2 HPV DNA Test. The Dual Probe Method uses separate Low-Risk and High-Risk HPV Probe Mixes. See directions below.

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

<table>
<tr><th>Reagent</th><th>Preparation Method</th></tr>
<tr><td>Denaturation Reagent</td><td>Prepare First:
1. 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.
2. Once prepared, the Denaturation Reagent is stable for three months when stored at 2–8°C. Label it with the new expiration date. If the color fades, add 3 drops of Indicator Dye and mix thoroughly before using.
Warning: Denaturation Reagent is corrosive. Wear suitable protective clothing, glove, eye/face protection. Use care when handling.</td></tr>
<tr><td>Low-Risk HPV Probe Mix (Prepared from Low-Risk HPV Probe and Probe Diluent reagents)</td><td>Prepare During Specimen Denaturation Incubation:
Important: Sometimes Probe gets trapped in the vial lid.
Note: Make sure to prevent RNase contamination of Probe and Probe Mix. Use aerosol-barrier pipette tips for pipetting probe. Probe Diluent is viscous.
Make sure of thorough mixing when preparing HPV probes. A visible vortex must form in the liquid during the mixing step. Incomplete mixing may result in reduced signal.
● Centrifuge the vial of Low-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 Low-Risk HPV Probe in Probe Diluent to prepare Probe Mix.
<table><tr><th>No. of Test Strips</th><th>Volume Probe Diluent*</th><th>Volume Probe*</th></tr><tr><td>48/6</td><td>2.0 ml</td><td>80.0 µl</td></tr><tr><td>24/3</td><td>1.0 ml</td><td>40.0 µl</td></tr><tr><td>Per Well</td><td>0.045 ml</td><td>1.8 µl</td></tr></table>*These values include the recommended extra volume.
● Pipette Low-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 Low-Risk HPV Probe Mix and keep in a clean, closed container until ready for use. Unused Probe Mix should be discarded.</td></tr>
<tr><td>High-Risk HPV Probe Mix</td><td>Prepare as Low-Risk HPV Probe Mix above. Label as “High-Risk HPV Probe Mix.” Unused Probe Mix should be discarded.</td></tr>
</table>

Combined-Probe Cocktail	Prepare Low-Risk HPV Probe and High-Risk HPV Probe Mixes as described above. Add the entire contents of diluted Low-Risk HPV Probe Mix to the tube of diluted High-Risk HPV Probe Mix. Mix thoroughly by vortexing for at least 5 seconds at maximum speed. A visible vortex must be produced. Label as "Combined-Probe Cocktail." Unused Probe Mix should be discarded.
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 Wash Reservoirs. See the Table below for mixing volumes: See *Automated Plate Washer User Manual* for Care and Maintenance Instructions. Warning: Wash Buffer Concentrate is toxic by ingestion. Wear suitable protective clothing, gloves, eye/face protection. To minimize exposure, add water to Wash Buffer Concentrate when preparing.

Amount of Wash Buffer Concentrate	Amount of Distilled or Deionized Water	Final Volume of Wash Buffer
33.3 ml	966.7 ml	1L
66.6 ml	1,933.4 ml	2L
100 ml	2,900 ml	3L

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

Prior to each assay, make sure the Automated Plate Washer Waste Reservoir is empty and the Rinse Reservoir is filled with distilled or deionized water.

For the manual plate washing method:

- Mix wash buffer concentrate well.
- Dilute 100 ml Wash Buffer Concentrate with 2.9 L of distilled or deionized water 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 2-30°C. Label it with the new expiration date. If Wash Buffer has been refrigerated, equilibrate to 20-25°C before using.

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

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

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

Specimen Collection and Handling

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

Cervical Specimens in STM

STM specimens may be held for up to two weeks at room temperature and shipped without refrigeration to the testing laboratory. Specimens should be shipped in an insulated container using either an overnight or 2-day delivery vendor. At the testing laboratory, specimens should be stored at 2–8°C if the assay is to be performed within one week. If the assay will be performed later than one week, store specimens at –20°C for up to 3 months (see *Notes* under *Cervical Biopsies* prior to freezing). 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 *digene* HC2 DNA Collection Device should not be used for collection of specimens from pregnant women.

Cervical Biopsies

Freshly collected cervical biopsies up to 5 mm in cross section may also be analyzed with the *digene* HC2 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.

Notes: To prevent caps from popping off specimen tubes that are shipped or stored frozen:

- Cover caps with Parafilm prior to shipping specimen tubes previously frozen. Specimens may be shipped frozen or at 20–25°C.

- When removing specimens from the freezer for testing, replace caps immediately with Specimen Collection Tube Screw Caps.

Cervical Specimens in PreservCyt Solution

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

Note: There must be at least 4 ml of PreservCyt Solution remaining for the *digene* HC2 HPV DNA Test. Specimens with less than 4 ml after the Pap test has been prepared may contain insufficient material and could be falsely negative in the *digene* HC2 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 *digene* HC2 HPV DNA Test. PreservCyt Solution specimens cannot be frozen. To process these specimens, refer to the *PreservCyt Specimen Preparation Procedure*.

Cervical Specimens in SurePath Preservative Fluid

(ONLY for High-Risk HPV DNA testing)

Manual sample preparation of SurePath specimens is performed using the post-gradient cell pellet resulting from the preparation of SurePath Pap Test slides. Prepare the SurePath Pap Test slides according to the applicable instructions for the BD PrepStain® Slide Processor.

Important: Immediately after SurePath Pap slide preparation, 2.0 ml of SurePath Preservative Fluid must be pipetted into the centrifuge tube containing the residual cell pellet. This preserves the integrity of the post-gradient cell pellet for performance of the *digene* HC2 HPV DNA Test.

The post-gradient cell pellet with SurePath Preservative Fluid may be stored for up to 4 weeks at 2–30°C, prior to sample preparation for the *digene* HC2 HPV DNA Test.

Post-gradient cell pellet SurePath specimens are prepared as specified in these instructions for use. The result of manual sample preparation is a denatured sample ready to proceed to the hybridization step of the test.

Protocol

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

High Volume Sample-Throughput Testing Using the Rapid Capture System

The Rapid Capture System is a general use automated pipetting and dilution system that can be used with the *digene* HC2 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, prior to placing the specimens on the RCS platform. In addition, chemiluminescent signal detection and result reporting are performed using the offline DML instrument common to both the manual and RCS methods. The procedural steps of the *digene* HC2 HPV DNA Test are performed in the exact sequence as the manual test procedure. The RCS Application allows for the staggered processing of up to 4 microplates, each plate containing specimens and the required assay Calibrators and Quality Controls.

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

Manual Method

Setup

1. If using the Microplate Heater I, allow it at least 60 minutes to equilibrate to 65 ± 2°C from a cold start. See *Microplate Heater I User Manual* for details.
2. Confirm the temperature of the water bath is at 65°C and that there is sufficient water to cover the entire volume in the specimen tubes.
3. Remove the specimens and all required Reagents from the refrigerator prior to beginning the assay. Allow them to reach 20–25°C for 15 to 30 minutes.

   Note: Prepare PreservCyt Solution and SurePath specimens prior to equilibrating any previously denatured specimens and kit reagent to room temperature.
4. Use the *digene* assay analysis software to create the assay plate layout. Refer to the respective user manual for instructions on creating a plate layout.
5. 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, Low-Risk HPV Calibrator, and High-Risk HPV Calibrator must be tested FIRST. Negative Calibrator (NC), Low-Risk HPV Calibrator (LRC), or High-Risk HPV Calibrator (HRC), Low-Risk Quality Control (QC1-LR), High-Risk Quality Control (QC2-HR), and specimens are to be run in an 8-microplate well column configuration. See Example Layout below.

Example Layout for a Run of 24 Microplate wells			
Row	Column		
	1	2	3
A	NC	Spec. 1	Spec. 9
B	NC	Spec. 2	Spec. 10
C	NC	Spec. 3	Spec. 11
D	LRC or HRC	Spec. 4	Spec. 12
E	LRC or HRC	Spec. 5	Spec. 13
F	LRC or HRC	Spec. 6	Spec. 14
G	QC1-LR	Spec. 7	Spec. 15
H	QC1-LR	Spec. 8	Spec. 16

6. If using the Combined-Probe Cocktail Method (CPC), NC, LRC, and HRC are tested in triplicate with the Combined-Probe Cocktail in the same microplate. Use wells A1, B1, and C1 for the NC and wells D1, E1, F1, G1, H1, and A2 for LRC and HRC, respectively. Use wells B2 and C2 for the QC1-LR and QC2-HR Quality Controls, respectively, and the specimens beginning in D2. The CPC procedure has not been validated for use with the Rapid Capture System.

7. For the Dual Probe Method, perform Low-Risk HPV Probe Mix tests on the left side of the microplate and perform the High-Risk HPV Probe Mix tests on the right side of the microplate.

   FIRST, test the Negative Calibrator (NC) and Low-Risk Calibrator (LRC) in triplicate with the Low-Risk HPV Probe Mix. Then test the Quality Controls (QC1-LR and QC2-HR) and specimens singly, also with the Low-Risk HPV Probe Mix. Place the NC replicates in A1, B1, C1; the LRC replicates in D1, E1, F1; QC1-LR in G1; QC2-HR in H1; and the specimens beginning in A2.

   NEXT, test the NC and High-Risk Calibrator (HRC) in triplicate with the High-Risk HPV Probe Mix. Then test the QC1-LR and QC2-HR specimens singly, also with High-Risk HPV Probe Mix. Place the NC replicates in A7, B7, C7; the HRC replicates in D7, E7, F7;

QC1-LR in G7; QC2-HR in H7; and the specimens beginning in A8. See the example layout above.

Refer to the applicable user manual for proper Calibrator/Quality Control/specimen setup in the software.

8. Alternatively, two separate microplates can be used for Calibrators, Quality Controls, and specimens tested with Low-Risk and High-Risk HPV Probe. NC and LRC are tested in triplicate and QC1-LR and QC2-HR are tested singly with Low-Risk HPV Probe Mix in one microplate, and NC and HRC are tested in triplicate and QC1-LR and QC2-HR are tested singly with High-Risk HPV Probe Mix in a second microplate. Use wells A1, B1, and C1 for the NC and wells D1, E1, and F1 for LRC or HRC, respectively. Use wells G1 and H1 for the QC1-LR and QC2-HR Quality Controls, respectively.
9. Specimens may be tested singly with the Combined-Probe Cocktail if using the CPC Method or singly with Low-Risk HPV Probe Mix and singly with High-Risk HPV Probe Mix if using the Dual Probe Method.

Denaturation

Notes:

- Warning: 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 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.
- During the denaturation and hybridization steps, 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.
- Following denaturation and incubation, the specimens are no longer considered infectious.[26] However, lab personnel should still adhere to national/local precautions.
- Do not remove specimen collection device prior to Denaturation.
- To avoid false-positive results, it is critical that all Calibrator, Quality Control, and STM specimen material come into contact with Denaturation Reagent. Mixing after Denaturation Reagent addition is a critical step: Make sure the Multi-Specimen Tube Vortexer 2 is set to 100 (maximum speed) and a visible vortex of liquid is observed during mixing such that the liquid washes the entire inner surface of the tube. If performing manual vortexing, make sure that each Calibrator, Quality Control, and specimen is mixed individually by vortexing each for at least 5 seconds at full speed such that the liquid vortex washes the entire inner surface of the tube followed by inverting the tube one time.

Calibrators, Quality Controls, and STM Specimen Preparation Procedure

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

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

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

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

Calibrator, Quality Control, or Specimen	Volumes of Denaturation Reagent Required
Negative Calibrator	1000 µl
Low-Risk or High-Risk HPV Calibrator	500 µl
Low-Risk or High-Risk Quality Controls	500 µl
Cervical Specimen	500 µl

Mix the specimens using one of the two methods below.

Multi-Specimen Tube (MST) Vortexer 2 Method

Note: *digene* HC2 DNA Collection Device specimens mixed using the MST Vortexer 2 **must** be hybridized using the Hybridization Microplate and Microplate Heater I method.

2a. Cover the Calibrator, Quality Controls, and STM specimen tubes with DuraSeal Tube Sealer Film by pulling the film over the tubes in the rack.

2b. Place the rack lid over the film-covered tubes and lock into place with the two side clips. Cut the film with the cutting device.

2c. Place the rack on the Multi-Specimen Tube Vortexer 2 and secure the rack with the clamp. Make sure the speed setting is at 100 (maximum speed) and turn the vortexer power switch to the ON position. Vortex the tubes for 10 seconds.

Manual Individual Tube Vortexing Method

2a. Recap the Calibrator, Quality Controls, and STM specimen tubes with clean Specimen Collection Tube Screw Caps.
2b. Mix each tube thoroughly by vortexing individually, at high speed, for 5 seconds.
2c. Invert each specimen tube one time to wash the inside of the tube, cap and rim.
2d. Return the tube to the rack.

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.

3. Incubate the tubes in the rack in a 65 ± 2°C water bath for 45 ± 5 minutes (denatured Calibrators, Quality Controls, and specimens may be tested immediately, or stored as described in Notes above). Prepare HPV Probe Mix(es) during this incubation. See Reagent Preparation and Storage section.

PreservCyt Solution Specimen Preparation Procedure

Notes:

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

Reagent Preparation

Use either the Denaturation Reagent (DNR) provided with the *digene* HC2 HPV DNA Test (see Reagent Preparation and Storage) or the DNR provided with the *digene* HC2 Sample Conversion Kit. To prepare the DNR provided with the *digene* HC2 Sample Conversion Kit, add 3 drops of Indicator Dye to the bottle of DNR and mix well. The solution should be a uniform, dark purple color. To determine volume requirements, use Table 1.

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

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

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

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

   6a. To determine the total volume of STM/DNR mixture required, use the starting volume of the PreservCyt Solution specimen as a guide and then multiply the STM and DNR "per tube" volumes by the number of specimens to be processed (see Table 2).

Table 2. Volume Requirements: STM/DNR

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

6b. Mix the solution thoroughly by vortexing.

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

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

8. Handling each tube individually:

   8a. Remove the cap and set aside on a clean lint-free paper towel.

   8b. Carefully decant supernatant.

   8c. Maintain the inverted tube position and gently blot (approximately 6 times) on absorbent lint-free paper towels until liquid drips from the tube. Use a clean area of the towel each time. Do not allow the cell pallet to slide down the tube during blotting.

   Notes:

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

   8d. Place the tube in a rack or the Conversion Rack.

Vortexing and Denaturation

Manual Vortexing Procedure

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

   Note: Make sure that all pellets are completely resuspended at this point. Specimens that still have visible pellets are not acceptable for testing and should be discarded.
5. Return the rack to the 65 ± 2°C water bath and continue denaturation for another 30 ± 3 minutes.

Proceed to the Hybridization Step or see Optional Stop Point for storage and treatment of denatured specimens.

Multi-Specimen Tube (MST) Vortexer 2 Procedure

Notes:

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

1. After blotting each labeled 15-ml conical tube, place each in its proper position in the Conversion Rack.
2. Add the appropriate volume of STM/DNR mixture to each pellet (Table 2).
3. Cover the 15-ml conical tubes with DuraSeal tube sealer film by pulling the film over the tubes in the rack.
4. Place the rack lid over the film-covered tubes and lock the lid into place with the two side clamps. Cut the film with the cutting device after the lid is securely fastened.
5. Move the red-handled lever up so that it is in a horizontal position.
6. Place the Conversion Rack and Lid on the MST Vortexer 2 so that the largest 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. Make sure that the speed setting is at 100 (maximum speed) and the Pulser toggle switch is in the OFF position.
8. Turn the Vortexer power switch to the ON position. Vortex the tubes for 30 seconds.
9. Turn the Vortexer power switch to the OFF position.
10. Remove the Conversion Rack and Lid from the MST Vortexer 2 by lifting up on the red-handled lever.
11. Place the rack in the 65 ± 2°C water bath for 15 ± 2 minutes. Be sure the water level completely covers all liquid in all of the tubes.
12. After the 15-minute incubation, remove the rack with specimens from the water bath.
13. To prevent splashing, dry the rack of excess water prior to placing it on the MST Vortexer 2.
14. Secure the Conversion Rack and Lid on the MST Vortexer 2 as described in Step 6.
15. Make sure the speed setting is at 100, and turn the Vortexer power switch to the ON position. Vortex the tubes for 1 minute.
16. Turn the Vortexer power switch to the OFF position.

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

SurePath Specimen Preparation Procedure (ONLY for High-Risk HPV DNA Testing)

Following cytological processing, proceed as follows:

1. Make sure that the observed liquid volume equals 2.8 ml.

   CAUTION: If the residual cell pellet appears to contain less than 1 ml of fluid, it is possible that SurePath Preservative Fluid was not added post-cytology and the specimen is NOT suitable for high-risk HPV DNA testing.

2. Make sure that the specimens are equilibrated to room temperature.
3. Centrifuge the sample in a swinging bucket rotor at 800 ± 15 x *g* for 10 ± 1 minute.
4. Remove the tubes from the centrifuge.
5. Carefully decant supernatant immediately following centrifugation and gently blot each tube (~3 times) on absorbent paper towels to remove excess liquid. Observe pellet in each tube. Do not let cell pellets slide down the tube during blotting.
6. Place tubes into the rack.
7. Add 200 µl of STM to each pellet using a repeating or adjustable pipettor.

   Note: Tubes may be mixed without capping.

8. Resuspend each pellet by vortexing each tube individually for 15 seconds at high speed. If pellet is difficult to resuspend, vortex for an additional 5 to 30 seconds or until the pellet floats loose from the bottom of the tube and appears to dissolve.
9. Pipette 100 µl of prepared Denaturation Reagent (with Indicator Dye) to each specimen using a repeating or adjustable pipettor.

   CAUTION: Take care not to touch the sides of the tube or cross-contamination of specimens could occur.

   If disposing of remaining Denaturation Reagent, please adhere to applicable local, state and federal regulations for the disposal of corrosive substances.

10. Mix each tube thoroughly by vortexing individually, at high speed, for 5 seconds.

    Note: Tubes can be mixed without capping.

Label 15-ml conical tube with appropriate specimen identification and type (example "SP" for SurePath specimen type) and place them in a rack.

Note: If using the Rapid Capture System for semi-automated assay processing, VWR or Corning brand 15-ml conical tubes must be used for proper placement in the *digene* Conversion Rack (silver rack).

11. Transfer the entire tube volume to a 15-ml conical tube with screw cap using a disposable, 7-ml standard-tipped transfer pipette or equivalent*.
12. Cap the 15-ml conical tubes.
13. Incubate in a 65 ± 2°C water bath for 90 ± 5 minutes.

    CAUTION: This incubation time is longer than required for other approved specimen types.
14. If HPV testing will be completed on the same day, denature the *digene* HC2 DNA Test calibrators according to these instructions for use.
15. Remove sample rack from the water bath.

Optional Stop Point

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

* QIAGEN-verification tests utilized VWR-brand 15-ml conical tubes

Hybridization: Combined-Probe Cocktail (CPC) and Dual Probe Methods

Notes:

- HPV Probe Mixes are viscous. Make sure the Probe Mix is thoroughly mixed and that the required amount is completely dispensed into each microplate well. See Reagent Preparation and Storage section.
- 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.
- Preheat the Microplate Heater I to 65 ± 2°C for at least 60 minutes prior to use. See the *Microplate Heater I User Manual* for further instructions as needed.

Hybridization Method Using Hybridization Plate and Microplate Heater I

Note: Specimens collected with the *digene* HC2 DNA Collection Device in STM and processed using the MST Vortexer 2 method can be hybridized utilizing the Microplate Heater I method only.

1. Obtain and label a Hybridization Microplate.
2. Remove Calibrators, Quality Controls, and specimens from the water bath after the incubation. If the Multi-Specimen Tube Vortexer 2 is being used, vortex the entire rack of STM specimens for a minimum of 5 seconds on the maximum speed setting. For PreservCyt Solution or SurePath specimens, vortex the entire Conversion Rack for a minimum of 10 seconds on the maximum speed setting. Alternatively, vortex each tube individually for at least 5 seconds.
3. Pipette 75 µl of each Calibrator, Quality Control, or specimen into the bottom of 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. Do not remove the specimen collection device from the specimen transport tube. Denatured specimens may be capped with Specimen Collection Tube Screw Caps and stored with specimen collection devices remaining in the tubes. Denatured PreservCyt specimens may be recapped with their original caps.

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

4. After transferring the last specimen, cover the plate with a plate lid and incubate the hybridization microplate for 10 minutes at 20–25°C.
5. Aliquot the prepared and thoroughly vortexed Probe Mix into a disposable reagent reservoir. Carefully pipette 25 µl of the Probe Mix into each well containing Calibrators, Quality Controls, and specimens using an 8-channel pipettor and fresh tips for each row. Dispense the volume of probe into each hybridization well, avoiding back splashing. Avoid touching the sides of the wells. Place the plate lid on the microplate for the duration of the denaturation incubation.
6. 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, make sure not to cause splashing.

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

Hybridization Method Using MicroTubes and Water Bath

Notes:

- The processing of specimens collected with the *digene* HC2 DNA Collection Device in STM using the MST Vortexer 2 method for mixing and the water bath method for hybridization has not been validated. Specimens collected with the *digene* HC2 DNA Collection Device in STM and processed using the MST Vortexer 2 method can be Hybridized utilizing 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 microtube following the plate layout created under Setup. Avoid touching the sides of the microtubes 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.
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, avoiding back splashing. Avoid touching the sides of the tubes. Inspect the rack from underneath to make sure that all tubes have received the appropriate amount of Probe Mix.

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.

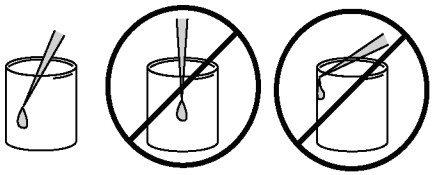
7. Incubate in a 65 ± 2°C water bath for 60 ± 5 minutes. Make sure that the water level in the water bath is sufficient to cover the entire volume of hybridization mixture. The microtube rack will float in the water bath.

   Note: Create a plate layout file using *digene* assay analysis software 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 microplate wells to the original bag and reseal. With a marker, number each column 1, 2, 3. . . . 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, Controls and specimens from 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 the Calibrators, Quality Controls, and specimens from Hybridization Microplate wells or Microtubes to the bottom of the corresponding Capture Microplate well 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 make sure of 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 microplate wells (see *Diagram 1*).

DIAGRAM 1: Correct Pipetting



4. Cover Capture microplate with the plate lid or 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 and check Automated Plate Washer 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 lint-free paper towels. Make sure 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 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 microplate wells. Take care not to touch the sides of the microplate wells or cross-contamination of specimens could occur. Refer to Diagram 1 shown earlier.

1. Aliquot the appropriate volume of Detection Reagent 1 into a disposable 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.

   Reverse Pipetting Procedure:

   1a. Attach tips onto an 8-channel pipettor; Make sure all tips are firmly seated.
   1b. Push the plunger of the pipettor past the first stop to the second stop.
   1c. Immerse tips into the Detection Reagent 1 solution.
   1d. Release plunger slowly and allow solution to fill the tips.
   1e. Dispense solution into microplate wells (75 µl) by depressing plunger to the first stop. Do not release plunger until pipette tips have been re-immersed into the Detection Reagent 1 solution.
   1f. Refill tips and repeal until all wells are filled. Fill wells of microplate from left to right. Make sure that all wells have been filled 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**. Make sure that the Rinse Reservoir is filled and the Waste Reservoir is empty. The Automated Plate Washer will routinely rinse the system for cleaning. See the *Automated Plate Washer User Manual* for further instructions as needed.

BEFORE EACH USE:

- Make sure that the Wash Reservoir is filled at least to the 1L mark with Wash Buffer Solution. If not, prepare the Wash Buffer solution. See Reagent Preparation and Storage section.
- Make sure the Rinse Reservoir is filled with deionized or distilled water.
- Make sure 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. Make sure 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.
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 washer will perform six fill and aspirate cycles taking approximately 10 minutes. There will be a brief pause during the program so be sure not to remove the plate prematurely. When the Automated Plate Washer is finished washing, it will read "Digene Wash Ready" or "P1".
6. Remove the microplate from the washer when the program is finished. Plate should appear white, and no residual pink liquid should remain in the microplate wells.

Manual Washing Method

1. Remove Detection Reagent 1 from the wells by placing clean Kimtowels Wipers or equivalent lint-free paper towels on top of the plate and carefully inverting. Before inverting, make sure 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 lint-free paper towels. Carefully discard the used paper towels to avoid alkaline phosphatase contamination of later steps.
2. Using the Wash Apparatus, hand wash the plate 6 times. Each well is washed to overflowing to remove Detection Reagent 1 from the tops of the wells. Washing begins at well A1 and continues in a serpentine fashion to the right and downward. After all wells have been filled, decant liquid into sink with a strong downward motion. The second wash is started at well H12 moving in a serpentine motion to the left and upward. This sequence of 2 washes is repeated 2 more times for a total of 6 washes per well.
3. After washing, blot the plate by inverting on clean Kimtowels Wipers or equivalent lint-free paper towels and tapping firmly 3-4 times. Replace the paper towels and blot again. Leave plate inverted and allow to drain for 5 minutes. Blot the plate one more time.
4. Plate should appear white, and no pink residual liquid should remain in the microplate wells.

Signal Amplification

Notes:

- Use a new pair of gloves for handling Detection Reagent 2.
- Aliquot only the amount of reagent required to perform the assay into the disposable reagent reservoir in order to avoid contamination of Detection Reagent 2. See Reagent Preparation 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 microplate well 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 as previously described. *All microplate wells should turn a yellow color*. Make sure that all wells have been filled 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 minutes. Avoid direct sunlight.
3. Read the microplate on the DML instrument after 15 minutes of incubation (and no later than 30 minutes of incubation).

   The assay-specific software protocol will allow the entry of pertinent assay information directly into the software.
4. If a full microplate was not used, remove used microplate wells from the microplate holder, rinse the holder thoroughly with distilled or deionized water, dry and reserve for next assay.

Assay Calibration Verification Criteria

Assay Calibration Verification is performed to make sure that the reagents and furnished Calibrator and Quality Control materials are functioning properly, permitting accurate determination of the assay cutoff value. The *digene* HC2 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 assay protocols automatically verify the criteria below.

- Negative Calibrator

  The Negative Calibrator must be tested in triplicate with each test 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 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 assay must be repeated for all patient specimens. Accordingly, patient specimen results should not be reported.

- Calibrators

  The calibrator(s) must be tested in triplicate with each assay. For CPC, both calibrators must be tested in triplicate. The Calibrator results should show a coefficient of variation (%CV) of ≤ 15%. For CPC, the %CV of LRC, HRC, and LRC-HRC combined must show a %CV ≤15%. If the %CV is >15, discard the calibrator value with an RLU value farthest from the mean as an outlier and recalculate the mean using the remaining calibrator values. Only 1 LRC and 1 HRC replicate may be deleted. If the %CV of the calibrators is ≤ 15%, proceed to step 3. Otherwise, the assay calibration verification is invalid and the assay must be repeated for all patient specimens. Accordingly, patient specimen results should not be reported.

The assay calibration verification described above for the Calibrators is performed automatically by the *digene* assay analysis software and printed on the data analysis report. The *digene* assay analysis protocols for HPV automatically verify that the Low-Risk and High-Risk HPV Calibrators %CV is ≤ 15%. However, versions v1.0.2 and v1.0.3 of the *digene* assay analysis software will NOT invalidate the assay unless the %CV is >25% for the calibrators. Therefore, the user must manually verify that the %CV calculated by the *digene* assay analysis 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	Report %CV for the LRC and/or HRC Replicates	Action Taken by *digene* assay analysis 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 remaining RLU values is > 15%, the assay is invalid. The results must not be reported. If the %CV of the remaining RLU values is ≤ 15%, recalculate the assay cutoff, then recalculate the RLU/cutoff ratio of each specimen using this cutoff. These recalculated values may be reported.
3	Between 15% and 25%	One outlier per calibrator 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 (STDEV) (n-1) of the remaining replicate RLU values by the mean of the remaining replicate RLU values (LRC or HRC or both) and multiply that result by 100.

To calculate the %CV using Microsoft® Excel® (supplied with the previous version of the *digene* assay analysis 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 %CVs, recalculating the assay cutoff, or recalculating the RLU/cutoff of the specimens, please call your local QIAGEN Representative.

To determine Calibrator reproducibility and estimate the frequency in which manual recalculations may be necessary, the results from three clinical evaluations involving 152 assay runs performed with the *digene* HC2 HPV DNA Test were compiled. The results showed that the average %CV for these 152 runs was 8.1. Considering all three replicates of the calibrator per test run, calibrator reproducibility of >15%CV was observed for only 17 out of 152 runs (11.2%), with 10 out of these 17 test runs resulting in %CV between 15-25 (Situation 2). For the 17 test runs that yielded a %CV >15, a single outlier was removed and the %CV recalculated. Following the User Action for Situation 2, only one of the test run's %CV remained >15, invalidating the test run. The %CVs of the remaining 151 test runs were calculated for an average %CV of 6.0.

- The Calibrator mean (LRC or HRC) and Negative Calibrator mean (NC) results are used to calculate the LRC/NC or HRC/NC ratio for each probe. Earlier versions (V1.0.2 and V1.0.3) of protocols of the *digene* assay analysis software do not correctly calculate the

acceptable ranges. These ratios must meet the following criteria to verify the assay calibration before the specimen results can be interpreted:

CPC Method	Dual Probe Method
Assay Calibration Verification	Assay Calibration Verification
Acceptable Ranges	Acceptable Ranges
2.0 ≤ LRC$\overline{\chi}$ / NC$\overline{\chi}$ ≤ 15	2.0 ≤ LRC$\overline{\chi}$ / NCLR$\overline{\chi}$ ≤ 15 (LR side)
2.0 ≤HRC$\overline{\chi}$ / NC$\overline{\chi}$ ≤ 15	2.0 ≤ HRC$\overline{\chi}$ / NCHR$\overline{\chi}$ ≤ 15 (HR side)
2.0 ≤ (LRC and HRC) $\overline{\chi}$ / NC$\overline{\chi}$ ≤ 15	

- Calculate the appropriate LRC$\overline{\chi}$/NC$\overline{\chi}$ or HRC$\overline{\chi}$/NC$\overline{\chi}$ ratios for each of the probe sets. If the ratios are ≥2.0 and ≤ 15, proceed to the next step. If any of the ratios are <2.0 or >15, the assay is invalid for that specific probe and must be repeated. Repeat all patient specimens within the run.

  Note: Acceptable ranges for the Negative Calibrator and Positive Calibrators have been established only for a DML instrument.

CutOff Calculation

Once an assay has been validated according to the criteria stated above, the Cutoff Values for determining positive specimens are as follows:

- Combined-Probe Cocktail Method: $\frac{\text{(LRC replicates+ HRC replicates)}}{\text{\# of replicates}}$
- Dual Probe Method: Low-Risk HPV Probe Cutoff = LRC$\bar{X}$
  High-Risk HPV Probe Cutoff = HRC$\bar{X}$

Example CutOff Calculations

For:		Low Risk or High-Risk HPV Probe Dual Probe	Low-Risk HPV Probe CPC Method	High-Risk HPV Probe CPC Method	High-Risk HPV Probe CPC Method
	NC RLU Values	LRC or HRC RLU Values	LRC RLU Values	HRC RLU Values	LRC and HRC RLU Values
	97	312	330	235*	330
	101	335	305	295	305
	91	307	385	279	385
					295
					235*
					279
Mean RLU Value	96	318	340	287*	318.8*
%CV	4.9	4.7	12.0	3.9*	13.0
LRC$\bar{X}$/NC$\bar{X}$	N/A	3.31	3.54	3.00	3.32

* Mean %CV of all 6 replicates was 16.8. Replicate with a value of 235 was deleted as an outlier. % CV of replicates remaining was 13.0 with a mean of 318.8. The initial %CV of HRC was 11.5.

The mean RLU value for the Positive Calibrator determines the assay cutoff value. Therefore, the Positive Cutoff value is (LRC$\bar{X}$) = 318.

All specimen RLU values should be converted into a ratio to the appropriate Cutoff Value. For example, all assays tested with Low-Risk HPV Probe should be expressed as Specimen RLU/Low-Risk Cutoff Value. The same can be done with specimens tested with High-Risk HPV Probe or the CPC Probe.

Notes: RLU/CO values and Positive/Negative results for all specimens are reported in the DML instrument *Data Analysis Report*.

For the Rapid Capture System instrument application, the RCS HPV software protocol has been programmed to apply a Calibration Adjustment Factor (CAF) of 0.8 to the mean of 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 instrument application. 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 appropriated Cutoff (CO) Value. For example, all assays should be expressed as Specimen RLU/CO Value.

Quality Control

Quality control samples are supplied with the *digene* HC2 HPV DNA Test. Consult the appropriate user manual for instructions on how to input the Lot Numbers and Expiration Dates of the quality controls. These quality controls must be included in each test run, and the RLU/CO of each quality control must fall within the following acceptable ranges for the run to be considered valid. If the quality controls do not fall within these ranges, the assay is invalid and must be repeated. Accordingly, no patient results should be reported for any invalid run.

Quality Control	HPV Type	Expected Result (RLU/Cutoff Value) Low-Risk HPV Probe			
		MINIMUM	MAXIMUM	AVERAGE	%CV
QC1-LR	LOW-RISK (HPV 6)	2	8	5.0	25
QC2-HR	HIGH-RISK (HPV 16)	0.001	0.999	0.5	25

Quality Control	HPV Type	Expected Result (RLU/Cutoff Value) High-Risk HPV Probe			
		MINIMUM	MAXIMUM	AVERAGE	%CV
QC1-LR	LOW-RISK (HPV 6)	0.001	0.999	0.5	25
QC2-HR	HIGH-RISK (HPV 16)	2	8	5.0	25

Quality Control	HPV Type	Expected Result (RLU/Cutoff Value) CPC HPV Probe			
		MINIMUM	MAXIMUM	AVERAGE	%CV
QC1-LR	LOW-RISK (HPV 6)	2	8	5.0	25
QC2-HR	HIGH-RISK (HPV 16)	2	8	5.0	25

- The Quality Control materials provided in the kit are cloned HPV DNA targets and are not derived from wild-type HPV. This is the same type of material used for the calibrators supplied with the *digene* HC2 HPV DNA Test.
- This quality control material will not act as an appropriate control for the processing of PreservCyt Solution or SurePath Preservative Fluid.
- The Quality Controls provided with this test kit must be used for internal quality control. Additional quality controls may be tested according to guidelines or requirements of local, and/or country regulations or accrediting organizations.

Interpretation of Specimen Results

Note: The *digene* HC2 HPV DNA Test cutoff of 1pg/ml is equivalent to 100,000 HPV copies/ml or 5,000 HPV copies per assay.

- STM Specimens with RLU/Cutoff Value ratios ≥ 1.0 with Low-Risk HPV Probe only are considered "Positive" for 1 or more of HPV types 6, 11, 42, 43 or 44.
- STM Specimens with RLU/Cutoff Value ratios ≥ 1.0 with High-Risk HPV Probe only are considered "Positive" for 1 or more of HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.
- When testing PreservCyt specimens, if the RLU/CO ratio of a specimen is ≥1.0 and <2.5, QIAGEN recommends that the specimen 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.
- If the RLU/Cutoff ratio of a specimen is close to but less than 1.0 and high-risk HPV infection is suspected, consider alternate testing methods and/or a repeat specimen.
- STM Specimens with RLU/Cutoff Value ratios ≥ 1.0 for both Low-Risk HPV Probe and High-Risk HPV Probe are considered "Positive" for 1 or more HPV types from each group of probes.
- STM Specimens with RLU/Cutoff Value ratios ≥ 1.0 with the Combined-Probe Cocktail are considered positive for 1 or more of HPV types 6, 11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 56, 58, 59, and 68.
- Specimens with RLU/Cutoff Value ratios <1.0 for Combined-Probe Cocktail or both Low-Risk HPV Probe and High-Risk HPV Probe are considered "Negative" or "No HPV DNA detected" for the 18 HPV types tested. HPV DNA sequences are either absent or the HPV DNA levels are below the detection limit of the assay.

Performance Characteristics

Data Supporting the Low-Risk and High-Risk HPV Indication

Clinical 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 the U.S. 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 *digene* HC2 HPV DNA Test were obtained from women attending several Kaiser clinic facilities. The initial Pap smears were evaluated according to the Bethesda Classification. For cervical cancer screening equivalent terminology in the European Community refer, to the European Guidelines for Quality Assurance in Cervical Cancer Screening[40]. Women (15 years or older) with Pap smear results of ASC-US (atypical cells of undetermined significance) 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.

HPV DNA testing was performed on the initial specimen and only High-Risk HPV Probe was used. HPV DNA testing was performed with a prototype of the *digene* HC2 HPV DNA Test that contained probes to 11 of the 13 HPV types included in the High-Risk HPV Probe, 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.

HPV 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 performance characteristics of the *digene* HC2 HPV DNA Test for STM and PreservCyt media, assay performance is presented for only PreservCyt Solution.

Table 3 shows that among those presenting with an ASC-US referral Pap smear, the negative predictive value of the *digene* HC2 HPV DNA Test for having HSIL or greater disease at colposcopy is 99%.

Table 3. Comparison of *digene* HC2 HPV DNA Test versus Consensus Histology. ASC-US Referral Pap Population. Kaiser Study, PreservCyt Solution Specimens

		HSIL or greater at the time of colposcopy		
		+	-	Total
High-Risk HPV	+	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 4 shows theoretical positive and negative predictive values based on various prevalences for an initial ASC-US being found to be HSIL or higher based on High-Risk HPV Probe results.

Table 4. Theoretical Positive and Negative Predictive Value. High-Risk HPV Probe. ASC-US Pap Smear Results

Theoretical Prevalence for HSIL	Initial ASC-US Pap Smear Result	
	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 5 illustrates the variation between the various age groups contained in this study:

Table 5. Kaiser Study Data. *digene* HC2 High-Risk HPV DNA Test Performance versus Consensus Histology Results (HSIL). 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)

Clinical Sensitivity and Specificity for the Determination of the Risk of High-Grade Disease in Women with LSIL or HSIL Pap Smears

A multi-center clinical study using the *digene* HC2 HPV DNA Test was conducted using specimens collected from several large, high cervical disease and HPV prevalence hospital and medical center colposcopy clinics (3 sites) in the western and southern US. HPV testing was performed at 3 investigational sites not affiliated with the colposcopy clinics from which the specimens were collected. The population for this clinical study was comprised of women diagnosed as either LSIL or HSIL based on a recent Pap smear and referred for follow-up colposcopy. Of 702 patients enrolled, 327 had Pap smear results greater than ASC-US and had adequate information available; 96 of these had a final disease status of HSIL or greater. Exfoliated cervical cell specimens were obtained with either the *digene* HC2 DNA Collection

Device and placed into STM or with a broom device and rinsed in PreservCyt Solution. Specimens were collected at the time of colposcopy. Specimens were tested with the *digene* HC2 HPV DNA Test and the results compared to the disease status determined for each patient. Disease status was based on the results of histologic evaluation, however, when histology was negative or in the absence of a histology result, disease status was determined by cytology at the time of colposcopic examination (see *Table 6*). The *digene* HC2 HPV DNA Test was performed at 3 large metropolitan medical centers not affiliated with the sites collecting the specimens upon colposcopy. Cytology was performed at a reference pathology laboratory and the histology was performed at the institutions performing the colposcopy. Test results were compared to disease status to assess the test's sensitivity, specificity, and negative and positive predictive values for detecting high-grade cervical neoplasia. Due to the similarities between the performance characteristics of the *digene* HC2 HPV DNA Test for STM and PreservCyt media, assay performance is presented for only PreservCyt.

No difference was observed in High-Risk HPV Probe results from STM specimens and PreservCyt Solution specimens. The following table shows the results of the High-Risk HPV Probe in this population:

Table 6. Patient Disease Status Algorithm

Cytology Result	Histology Result	Disease Status
NEG	NEG or Not Done*	NEG
LSIL	NEG	LSIL
HSIL	NEG	HSIL
Cancer	NEG	HSIL+
NEG	LSIL	LSIL
LSIL	Not Done*	LSIL
LSIL	LSIL	LSIL
HSIL	LSIL	LSIL
Cancer	LSIL	LSIL
NEG	HSIL	HSIL
LSIL	HSIL	HSIL
HSIL	HSIL	HSIL
HSIL	Not Done*	HSIL
Cancer	HSIL	HSIL
NEG	Cancer	HSIL+
LSIL	Cancer	HSIL+
HSIL	Cancer	HSIL+

* Biopsy and/or Endocervical Curettage (ECC) not done because no abnormalities were observed upon colposcopy or histology result was not available.

Tables 7 and 8 represent the performance of the *digene* HC2 HPV DNA Test determined using 327 PreservCyt specimens, 96 of which were collected from women diagnosed with high-grade cervical disease. The comparisons were done using all study patients with abnormal referral Pap smear results. Comparisons are shown for PreservCyt specimens tested with High-Risk HPV Probe.

Table 7. Results of the High-Risk HPV Probe

	Final Disease Status						
Referral Pap Smear Result	HSIL	LSIL	Negative	Referral Pap Smear Result	HSIL	LSIL	Total
High Risk HPV Results	POS	NEG	POS	NEG	POS	NEG	
LSIL	44	4	78	33	28	37	224
HSIL	45	3	29	14	5	7	103
Total	89	7	107	47	33	44	327
	96		154		77		

Table 8 shows that the *digene* HC2 HPV DNA Test using the High-Risk HPV Probe demonstrated approximately 93% overall sensitivity for identifying women with high-grade neoplasia in a population referred for colposcopy on the basis of a Pap smear diagnosis of LSIL, HSIL or equivalent. The test also demonstrated a negative predictive value of nearly 93% in this population.

Table 8. Performance Characteristics. *digene* HC2 High-Risk HPV DNA Test Among Patients Having a Referral Pap Smear of LSIL or Higher and a Final Disease Status of HSIL

	Referral Pap LSIL or HSIL → HSIL Disease		
	+	-	Total
High-Risk HPV Probe Result			
+	89	140	229
-	7	91	98
Total	96	231	327

Sensitivity [TP/(TP+FN)] = 92.7% (89/96)
95% CI = 85.6 to 97.0
Specificity [TN/(TN+FP)] = 39.4% (91/231)
95% CI = 33.1 to 46.0
Disease Prevalence for Referral LSIL to final HSIL = 21.4%
Disease Prevalence for Referral HSIL to final HSIL = 46.6%
Overall Positive Predictive Value = 38.9% (89/229)
Overall Negative Predictive Value = 92.8% (91/98)

While the specificity of the *digene* HC2 HPV DNA Test appeared to be somewhat low, a strict correlation between absence of neoplasia and a negative HPV result is not expected. HPV DNA can be present in women who have not progressed to higher grade disease. In fact, when HPV Polymerase Chain Reaction (PCR) testing (a research use only assay) was performed on specimens with positive HPV results and whose corresponding disease status was less than low-grade neoplasia, nearly 75% were positive.

Table 9 indicates theoretical High-Risk HPV Probe positive and negative predictive values for an initial LSIL or HSIL being found on colposcopy to be HSIL or more severe disease.

Table 9. Theoretical Positive and Negative Predictive Value. High-Risk HPV Probe. Initial LSIL or HSIL Pap Smear Results

Theoretical Prevalence for HSIL	Initial LSIL or HSIL Pap Smear Result	
	Assay Positive	Assay Negative
	Predictive Value	Predictive Value
5	7.4	99.0
10	14.5	97.9
15	21.2	96.8
20	27.6	95.5
25	33.7	94.1
30	39.6	92.6
35	45.1	90.9
40	50.4	89.0
45	55.5	86.8
50	60.4	84.3

Data Supporting the High-Risk HPV Primary Screening Indication

Clinical Performance When Screening Patients with Normal Pap Smear Results as an Aid in the Assessment of Risk for Patient Management

The results of eight independent clinical studies conducted by prominent medical, academic, and government institutions at centers in the United States and abroad are described below. The studies utilized the established Pap methods in use in the countries in which the study was conducted. In all but two cases, the Bethesda Grading System was utilized to interpret the Pap results. In addition, high-grade cervical disease was diagnosed through the use of colposcopy-directed biopsy for each study. These studies assessed the clinical usefulness of the *digene* HC2 High-Risk HPV DNA Test in comparison to the Pap smear for older women (generally over 30–35 years old). All but one study also performed prospective HPV testing using the *digene* HC2 High-Risk HPV DNA Test.

The studies were cross-sectional general population screening studies utilizing the *digene* HC2 High-Risk HPV DNA Test, unless otherwise noted below. As indicated, 2 of the 8 screening studies were conducted in the United States; 2 in Europe, 2 in Latin America, 1 in Africa, and 1 in Asia.

The performance of the *digene* HC2 High-Risk HPV DNA Test observed from six cross-sectional studies are summarized, in Tables 10 and 11, for women aged 30 years and over and diagnosed with histologically-confirmed high-grade cervical neoplasia (defined as CIN3 or more severe).

Table 10. Performance Estimates of *digene* HC2 High-Risk HPV DNA Test Sensitivity and Specificity

Population	*n*		*Sensitivity(%)*			*Specificity(%)*		
			PAP Alone	*HPV Alone*	*HPV + PAP*	*PAP Alone*	*HPV Alone*	*HPV + PAP*
Western Europe 1	7592		51.6 (14/27)	96.3 (26/27)	100 (27/27)	98.5 (7453/7565)	96.2 (275/7565)	95.1 (7193/7565)
		95% CI	32.0–71.3	81.0-99.9	87.2–100	98.2–98.8	95.7-96.6	94.6–95.6
Latin America 1	6115		58.4 (45/77)	94.8 (73/77)	97.4 (75/77)	98.7 (5962/6038)	93.9 (5669/6038)	93.4 (5637/6038)
		95% CI	46.68–69.6	87.2–98.6	90.9–99.7	98.4–99.0	93.3–94.5	92.7–94.0
Latin America 2†	6176		77.9 (53/68)	89.7 (61/68)	94.1 (64/68)	94.1 (5745/6108)	94.0 (5742/6108)	89.9 (5490/6108)
		95% CI	66.2–87.1	79.9–95.8	85.6–98.4	93.4–94.6	93.4–94.6	89.1–90.6
Africa	2925		84.1 (90/107)	89.7 (96/107)	92.5 (99/107)	86.4 (2436/2818)	80.0 (2253/2818)	76.4 (2152/2818)
		95% CI	75.8–90.5	82.4–94.8	85.8–96.7	85.1–87.7	78.4–81.4	74.8–77.9
Asia	1936		97.6 (41/42)	100 (42/42)	100 (42/42)	76.3 (1445/1894)	83.0 (1572/1894)	68.0 (1287/1894)
		95% CI	87.4–99.9	91.6–100.0	91.6–100.0	74.3–78.2	81.2–85.0	65.8–70.1
U.S. 1	1040		50.0 (1/2)	100 (2/2)	100 (2/2)	97.6 (1013/1038)	96.2 (999/1038)	95.5 (991/1038)
		95% CI	1.26–98.7	15.8–100.0	15.8–100.0	96.5–98.4	94.9–97.3	94.0–96.7

† HC2 data where available, HCS data used otherwise; data combined.

Table 11. Performance Estimates of *digene* HC2 High-Risk HPV DNA Test Positive and Negative Predictive Value

Population	*n*		*Prevalence(%)*		*Positive Predictive Value (%)*			*Negative Predictive Value (%)*	
			CIN 3	*PAP Alone*	*HPV Alone*	*HPV + PAP*	*PAP Alone*	*HPV Alone*	*HPV + PAP*
Western Europe 1	7592		0.36 (27/7592)	11.1 (14/126)	8.23 (26/316)	6.77 (27/399)	99.83 (7453/7466)	99.99 (7275/7276)	100.0 (7193/7193)
		95% CI	0.23–0.52	6.21–17.9	5.45–11.8	4.51–9.69	99.70–99.91	99.92–100.0	99.95–100.0
Latin America I	6115		1.26 (77/6115)	37.2 (45/121)	16.5 (73/442)	15.8 (75/476)	99.47 (5962/5994)	99.93 (5669/5673)	99.96 (5637/5639)
		95% CI	0.99–1.57	28.6–46.4	13.2–20.3	12.6–19.4	99.25–99.63	99.82–99.98	99.87–100.0
Latin America 2†	6176		1.10 (68/6176)	12.7 (53/416)	14.3 (61/427)	9.4 (64/682)	99.74 (5745/5760)	99.88 (5742/5749)	99.93 (5490/5494)
		95% CI	0.86–1.39	9.69–16.3	11.1–18.0	7.30–11.8	99.57–99.85	99.75–99.95	99.81–99.98
Africa	2925		3.66 (107/2925)	19.1 (90/472)	14.5 (96/661)	12.9 (99/765)	99.31 (2436/2453)	99.51 (2253/2264)	99.63 (2152/2160)
		95% CI	3.01–4.40	15.6–22.9	11.9–17.4	10.6–15.5	98.89–99.60	99.13–99.76	99.27–99.84
Asia	1936		2.17 (42/1936)	8.37 (41/490)	11.5 (42/364)	6.47 (42/649)	99.93 (1445/1446)	100.0 (1572/1572)	100.0 (1287/1287)
		95% CI	1.57–2.92	6.07–11.2	8.44–15.3	4.70–8.65	99.62–100.0	99.77–100.0	99.71–100.0
U.S. 1	1040		0.19 (2/1040)	3.85 (1/26)	4.88 (2/41)	4.08 (2/49)	99.90 (1013/1014)	100.0 (999/999)	100.0 (991/991)
		95% CI	0.02–0.69	0.10–19.6	0.60–16.5	0.50–14.0	99.45–100.0	99.63–100.0	99.63–100.0

† HC2 data where available, HCS data used otherwise; data combined

Across all studies, there is a uniform, and often very significant improvement in sensitivity of the *digene* HC2 High-Risk HPV DNA Test over Pap alone. As with sensitivity, the Negative Predictive Value (NPV) of HPV exceeds that of Pap alone in all cases, approaching 100%. This NPV demonstrates the high probability of the absence of high-grade cervical disease or cancer in cytologically normal women that are free of HPV infection.

Although the specificity of the *digene* HC2 High-Risk HPV DNA Test is lower than for Pap alone, likelihood ratio analysis has demonstrated that the decrease in specificity observed is not significant enough to affect the clinical utility of using the test to identify women who are at little or no risk of having or developing cervical disease. Nonetheless, it is important that the decision to refer a patient to colposcopy is based on all clinical and risk information and patient history available to the physician. Important variables include a history of HPV infection and/or abnormal Pap smears age at first intercourse, number of sexual partners, and concurrent sexually transmitted diseases .[27,28]

Although the prevalence of high-grade disease does not vary significantly amongst the studies from which performance was determined, the prevalence of HPV infection in a population may affect performance, and typically varies with the patient population. In addition, the prevalence of HPV infection has been shown to decrease dramatically with age.[28, 30-37, 41] Positive predictive values decrease when testing populations with low prevalence or individuals with little risk of infection.

Longitudinal analyses were performed using the results of two studies; one conducted in the United States by the National Cancer Institute (NCI) in Portland, Oregon and the other conducted in France at the Laboratoire Pol Bouin C.H.U. de Reims. These longitudinal analyses were undertaken to demonstrate that Pap negative/HPV negative patients are at a lower risk of having cervical disease compared to traditionally-defined low-risk women whose HPV status is not known and compared to Pap negative/HPV positive patients.

The results of these longitudinal analyses are presented in Tables 12 and 13 below.

Table 12. Summary of Results: NCI and France Studies Relative Risk of High-Grade Disease

Study Group	Age	Low Risk Classification	n	Cases of CIN 3+	Rate (per 100 Patient Years)	Relative Risk (95% CI)
NCI	30 and over	Pap Normal HPV Negative	12,054	28	0.043	0.897 (0.596, 1.348)
		Consecutive Normal Paps*	9,429	19	0.048	1.000
	All	Pap Normal, HPV Negative	17,594	48	0.056	0.678 (0.514, 0.894)
		Consecutive Normal Paps*	13,392	44	0.082	1.000
France	30 and over	Pap Normal, HPV Negative	1,690	3	0.084	0.849 (0.307, 2.35)
		Consecutive Normal Paps*	2,026	4	0.099	1.000
	All	Pap Normal, HPV Negative	2,180	3	0.066	0.491 (0.221, 1.09)
		Consecutive Normal Paps*	2,650	7	0.136	1.000

*Three normal annual Paps over approximately 2 years

Table 13. Summary of Results: NCI and France Studies Disease Rates Stratified by HPV Status at Baseline

Study Group	Age	Baseline Status	n	Cases of CIN 3+	Rate (per 100 Patient Years)	Relative Risk (95% CI)
NCI	30 and over	Pap Normal, HPV positive	1,078	24	0.451	10.50 (6.13, 18.0)
		Pap Normal, HPV negative	12,054	28	0.043	1.00
	All	Pap Normal, HPV positive	2,561	63	0.096	10.64 (7.33 – 15.5)
		Pap Normal, HPV negative	17,594	48	0.056	1.00
France	30 and over	Pap Normal, HPV positive	419	14	2.346	27.3 (8.41, 88.3)
		Pap Normal, HPV negative	1696	3	0.084	1.00
	All	Pap Normal, HPV positive	619	22	2.520	37.0 (11.8, 116)
		Pap Normal, HPV negative	2180	3	0.066	1.00

The clinical utility of the HPV test result is further demonstrated by the increased risk of cervical disease in HPV positive women as compared to HPV negative women.

Analytical Sensitivity

A non-clinical panel of cloned HPV plasmid DNA was tested to determine if each of the 18 HPV types are detectable by the *digene* HC2 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 pg/ml, 0.5 pg/ml and 0.2 pg/ml) of each of the 18 HPV DNA types (6, 11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 56, 58, 59 and 68) was run in triplicate with Low-Risk HPV Probe or High-Risk HPV Probe, as appropriate. The mean signal in RLU for each concentration of each HPV type was calculated and compared to the Positive Calibrator for the appropriate side of the assay.

The detectable limit of each HPV type in STM is shown in Table 14. 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 1.09 pg of HPV DNA target per 1 ml of STM specimen. The mean detectable limit of all 18 HPV DNA types was 1.09 pg/ml with a standard deviation of 0.05.

Table 14. Summary of *digene* HC2 HPV DNA Test Detectable Limits of Sensitivity for each HPV DNA Type in STM

HPV DNA Type	Detectable HPV DNA Concentration (pg/ml)	Standard Deviation	95% Confidence Range
6	1.33	0.03	1.22–1.46
11	1.13	0.05	1.00–1.29
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
42	1.20	0.05	1.02–1.44
43	0.85	0.03	0.86–1.07
44	1.17	0.04	1.02–1.36
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)	1.09	0.05	0.97–1.27

Combined-Probe Cocktail (CPC) Performance

The same nonclinical HPV plasmid DNA panel described above was tested to determine the analytical sensitivity of each of the 18 HPV types in the *digene* HC2 HPV DNA Test following the Combined-Probe Cocktail (CPC) protocol as described in this insert. The analytical sensitivity of CPC protocol varied from 0.58 pg/ml to 1.39 pg/ml and all HPV types were detectable at an estimated level of 0.95 pg/ml of HPV DNA target per 1 ml of specimen. The mean detectable limit for all 18 HPV types was 0.95 pg/ml with a standard deviation of 0.07. This sensitivity is equivalent to the analytical sensitivity found for the Dual Probe method of the *digene* HC2 HPV DNA Test.

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 their respective processing/denaturation procedures described in these instructions for use and tested with the *digene* HC2 HPV DNA Test using High-Risk HPV Probe. 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 *digene* HC2 HPV DNA Test.

SurePath Specimen Result Correlation with STM Specimens in a Clinical Population

A two-phase clinical evaluation was conducted using 6 collection centers and 3 testing sites within the United States. Patients attending an STD clinic, obstetrics/gynecological clinic, colposcopy clinic, hospital, or family planning center were eligible for enrollment according to predetermined inclusion and exclusion criteria. The feasibility phase, intended to determine an appropriate *digene* HC2 High-Risk HPV DNA assay cutoff for use with SurePath specimens, enrolled approximately 400 patients. The clinical validation phase, enrolling approximately 1500 patients to validate the chosen assay cutoff value, began after an interim analysis of the feasibility demonstrated that an assay cutoff value of 1.0 RLU/CO using SurePath specimens produced acceptable agreement with STM specimen results.

In both evaluation phases, paired SurePath and STM cervical specimens were obtained from each consenting female participant. The SurePath specimen was then sent to a cytology lab for slide preparation. After cytological preparation, the remaining SurePath Specimen and the corresponding STM specimen were tested with the *digene* HC2 High-Risk HPV DNA Test using an assay cutoff of 1.0 RLU/CO.

Table 15 provides the SurePath result correlation with the paired STM specimen observed in the final results eligible for data analysis obtained from the total enrolled population.

Table 15. SurePath Result Agreement with STM (all ages and cytological classification) (n=1490)

Positive Agreement % 95% CI (n/N)		Negative Agreement % 95% CI (n/N)	
All Positive	High Positive Subset (RLU/CO ≥ 2.5)	All Negative	Low Negative Subset RLU/CO (<0.80)
93.5 90.7, 95.6 (401/429)	96.4 94.1, 98.0 (378/392)	95.3 93.8, 96.5 (1011/1061)	96.0 94.6, 97.1 (1002/1044)

These results predict that the relative assay sensitivity and specificity using SurePath specimens will correlate highly with that obtained using the STM specimen type as evidenced by the lower limit of the 95% confidence interval for both positive and negative agreement.

Reproducibility

A multi-center reproducibility study was performed to determine the between days, between sites, and overall reproducibility of the *digene* HC2 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 *digene* HC2 HPV DNA Test kits on 3 different days with an identical reproducibility panel. The reproducibility panel included the following specimens: 12 denatured clinical STM specimen pools; 3 undenatured clinical PreservCyt Solution specimen pools; Negative Calibrator; and Positive Calibrators Low-Risk and High-Risk 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 using both the High-Risk HPV Probe and CPC methods. The results are shown in Table 16.

Table 16. Summary of Overall Statistics for Multi-center Reproducibility of the *digene* HC2 HPV DNA Test

Statistical Measure	High-Risk HPV Probe	Combined-Probe Cocktail (CPC)	Combined Results of High-Risk HPV Probe and CPC[a]
Proportion of expected positives with an observed positive result	100% (99.0-100.0)	99.8% (98.92-100.0)	99.9% (99.38-100.0)
Proportion of expected negatives with an observed negative result	99.0% (97.49-99.73)	98.9% (96.79-99.77)	99.0% (97.88-99.58)
Agreement	99.5% (98.70-99.86)	99.5% (98.70-99.86)	99.5% (99.0-99.78)
Kappa	0.990	0.989	0.990

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

This indicates that the reproducibility of the *digene* HC2 HPV DNA Test with clinical specimens collected in STM is very good.

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 *digene* HC2 HPV DNA Test. All microorganisms were assayed at concentrations of $1x10^5$ and $1x10^7$ organisms per ml. Purified DNAs of viruses and plasmids were assayed at a concentration of 4 ng/ml.

Below is a list of the bacteria tested. All bacteria tested negative in the *digene* HC2 HPV DNA Test.

Acinetobacter anitratus	*Mycoplasma hyorhinis*
Acinetobacter lwoffi (ATCC 17908)	*Neisseria gonorrhoeae* (ATCC 19424)
Bacteroides fragilis (ATCC 25285)	*Neisseria lactamica* (NRL 2118)
Bacteroides melaninogenicus	*Neisseria meningitidis* (ATCC 13077)
Candida albicans (ATCC 14053 or 10231)	*Neisseria sicca* (ATCC 29256)
Chlamydia trachomatis	*Peptostreptococcus anaerobius*
Enterobacter cloacae	*Proteus vulgaris* (ATCC 21117, 8427, 33420)
Escherichia coli (HB101)*	*Serratia marcescens*
Escherichia coli	*Staphylococcus aureus* (Cowan strain)
Fusobacterium nucleatum	*Staphylococcus epidermidis*
Gardnerella vaginalis	*Streptococcus faecalis (ATCC 14508)*
Haemophilus ducreyi	*Streptococcus pyogenes (ATCC27762)*
Klebsiella pneumoniae	*Treponema pallidum*
Lactobacillus acidophilus	*Trichomonas vaginalis*
Mobiluncus curtisii	*Ureaplasma urealyticum*
Mobiluncus mulieris	*Mycoplasma hominis*

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

The only viruses or plasmids that showed cross-reactivity in the *digene* HC2 HPV DNA Test were HPV type 13 and plasmid pBR322. HPV 13 DNA reacted with Low-Risk HPV Probe only. HPV 13 is commonly detected in lip lesions of certain ethnic groups, but has not been detected in the anogenital tract.[29] Thus, the cross-reactivity observed between HPV 13 and *digene* HC2 HPV DNA Test Low-Risk HPV Probe would not be expected to cause a clinically confusing result for anogenital specimens. Cross-reactivity between pBR322 and *digene* HC2 HPV DNA Test Low-Risk and High-Risk HPV Probes 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 *digene* HC2 HPV DNA Test Low-Risk and High-Risk HPV Probes showed that no positive results were due to pBR322 when tested with a pBR322 probe. Thus, the likelihood of *digene* HC2 HPV DNA Test false-positive result due to homologous pBR322 sequences in clinical specimens appears to be low.

Cross-Hybridization

Each of the 18 HPV types was tested with both Low-Risk and High-Risk HPV Probes at concentrations of 4 ng/ml of HPV DNA. All of the HPV targets were expected to be positive with the appropriate probe group whereas none of the specimens were expected to be positive with the opposite probe group. This study demonstrated that there is a small amount of cross-hybridization between HPV types 6 and 42 (low-risk HPV types) and the high-risk probe group (High-Risk HPV Probe). Specimens with high levels (4 ng/ml or higher of HPV 6 or HPV 42 DNA may be positive for both probe groups. The clinical significance of this is that patients with 4 ng/ml or higher of HPV 6 or HPV 42 DNA may be referred to colposcopy.

In addition, High-Risk HPV Probe has 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[38]. Patients whose specimens contain high levels of these HPV DNA types may incorrectly be referred to colposcopy. It has also been reported in the literature that complex probes similar to that used in this test may cause false-positive results due to cross-hybridization with HPV types 11, 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 referred to colposcopy.

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 *digene* HC2 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 nonclinical 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 since 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 *digene* HC2 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 4 agents at any concentration. Furthermore, substances inherent in some clinical specimens do not inhibit the detection of the HPV DNA by the *digene* HC2 HPV DNA Test.

Reproducibility of *digene* hc2 HPV DNA Test with Clinical Specimens Collected in STM

The reproducibility of the *digene* HC2 HPV DNA Test with clinical specimens collected in STM was determined in a study using 20 clinical pools (10 positive and 10 negative) prepared by combining previously denatured and tested cervical brush specimens collected in STM. Specimens were tested in replicates of 4 on each of 5 days for a total of 20 replicates per specimen. Testing was performed using the Combined-Probe Cocktail method. Means, standard deviation and 95% confidence intervals about the mean (CIs) were calculated for each specimen within day and over 5 days and is shown in Table 17.

Table 17. 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 5 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 5 specimens with a mean RLU/CO within 20% above or below the assay cutoff (Nos. 6-10), 60 of 100 (60%) 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 using the *digene* HC2 HPV DNA Test.

Reproducibility of *digene* hc2 HPV DNA Test with Clinical Specimens Collected in PreservCyt Solution

The reproducibility of the *digene* HC2 HPV DNA Test with clinical specimens collected in PreservCyt Solution 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 4 on each of 5 days, for a total of 20 replicates per specimen. On each of the 5 days of the study, an 8 ml aliquot from each specimen was processed and tested according to the *digene* HC2 Sample Conversion Kit instructions for use using High-Risk HPV Probe only. Means, standard deviations, and 95% confidence intervals (CIs) were calculated for each specimen within each day and over all 5 days and replicates. The mean RLU/CO, confidence interval about the mean, and the percent of positive replicates is shown in Table 18 for each specimen, in descending order based on the mean RLU/CO.

Table 18. 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	100 (20/20)
5	18	1.36	1.23-1.48	90 (18/20)
6	15	1.32	1.16-1.49	95 (19/20)
7	23	1.17	1.06-1.27	85 (17/20)
8	16	1.14	1.07-1.20	75 (15/20)
9	20	1.10	0.96-1.21	75 (15/20)
10	19	1.06	0.95-1.17	85 (17/20)
11	22	1.05	0.99-1.10	45 (9/19)
12	11	1.04	0.96-1.11	70 (14/20)
13	14	0.94	0.86-1.01	65 (13/20)
14	24	0.77	0.73-0.81	25 (5/20)
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 6 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 7 specimens with a mean RLU/CO within 20% above or below the assay cutoff (Nos. 7-13), 88 of 139 (63.3%) of the replicates were positive and 51 of 139 (36.7%) were negative. For the 4 specimens within 10% above or below the cutoff (No. 10–13), 41 of 79 (51.9%) of the replicates were positive and 38 (48.1%) 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 using the *digene* HC2 HPV DNA Test.

Reproducibility of *digene* hc2 high-risk HPV DNA Test with Specimens Collected in Surepath Preservative Fluid

Reproducibility evaluations were conducted to characterize the ability of 3 different laboratories to obtain a similar diagnostic result on different days and with different runs from an identical set of specimens of known positive/negative HPV status when using an assay cutoff of 1.0 RLU/CO. The reproducibility specimen panel consisted of 5 HPV positive specimens, 2 specimens with HPV DNA concentrations near the assay cutoff and 5 negative HPV specimens.

Panel members were prepared by combining unique SurePath patient specimens with a known negative and positive HPV status to obtain the desired target RLU/CO values. Each panel member was tested in duplicate, two times each day over a period of five days at each of the three participating laboratories.

Table 19. Reproducibility Study SurePath Specimens Qualitative Results by Panel Member

Panel Member	Mean RLU/CO	Expected Result	HPV Positive n (%)	HPV Negative n (%)
1	0.20	negative	0 (0)	60 (100)
2	0.21	negative	0 (0)	60 (100)
3	0.22	negative	0 (0)	60 (100)
4	0.28	negative	2 (3.3)	58 (96.7)
5	0.36	negative	2 (3.3)	58 (96.7)
6	0.83	negative	13 (21.7)	47 (78.3)
7	1.17	positive	26 (43.3)	34 (56.7)
8	19.47	positive	60 (100)	0 (0)
9	25.65	positive	60 (100)	0 (0)
10	81.52	positive	60 (100)	0 (0)
11	154.18	positive	60 (100)	0 (0)
12	765.29	positive	60 (100)	0 (0)

SurePath Result in Reproducibility When Using the Rapid Capture System for Assay Processing

The reproducibility of SurePath specimen results when using the Rapid Capture System for assay processing were compared with the results obtained when using manual assay processing. Two comparative tests were performed on separate aliquots of the same processed specimen.

Table 20. Within-Specimen SurePath Result Agreement with RCS (RCS vs. Manual Assay)

Positive Agreement % 95% CI (n/N)		Negative Agreement % 95% CI (n/N)	
All Positive	High Positive Subset (RLU/CO ≥ 2.5)	All Negative	Low Negative Subset RLU/CO (<0.80)
99.0	100	97.7	98.7
417/421	375/375	1057/1079	1050/1064
97.6, 99.7	99.0, 100	96.9, 98.7	97.8, 99.28

Limitations of the Procedure

For In Vitro Diagnostic Use

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

- The *digene* HC2 HPV DNA Test for human papillomavirus types 6, 11,16, 18, 31, 33, 35, 39, 42, 43, 44 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.
- The *digene* HC2 HPV DNA Test distinguishes between 2 groups of HPV types: HPV 6/11/42/43/44 and 16/18/31/33/35/39/45/51/52/56/58/59/68. It will not distinguish among the viral types within these groups.
- The *digene* HC2 HPV DNA Test can only be used with cervical specimens collected using the *digene* HC2 DNA Collection Device or with biopsies collected in STM or cervical specimens collected using a broom-type collection device or brush/spatula combination and placed in PreservCyt Solution or cervical specimens collected in SurePath Preservative Fluid. Biopsy specimens may be assayed only if they are placed immediately in STM and stored at –20°C until assayed.
- The *digene* HC2 DNA Collection Device should not be used for collection of specimens from pregnant women.
- Infection with HPV is not a definitive indicator of the presence of high-grade cervical disease, nor does it imply in all cases that high-grade disease or cancer will develop.

- A small amount of cross-hybridization between HPV types 6, 11, 40, 42, 53, 54, 55, 56, MM4, MM7, MM8, and MM9, and the High-Risk HPV Probe exists. Patients having specimens containing high levels of these HPV types may incorrectly be referred to colposcopy[38].
- The *digene* HC2 HPV DNA Test is designed to detect low-risk and high-risk HPV types, including 39, 58, 59, and 68. Analytical studies conducted by QIAGEN, using cloned HPV plasmid DNA, demonstrate that this 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 *digene* HC2 HPV DNA Test. QIAGEN 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[36].), the performance characteristics of the *digene* HC2 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 both the *digene* HC2 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.

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Troubleshooting Guide

		Comments and suggestions
Incorrect or no color change observed during denaturation.		
a)	Denaturation Reagent not prepared properly	Make sure that the Denaturation Reagent contains the Indicator Dye and is a dark purple color.
b)	Denaturation Reagent not added	Make sure 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.
c)	Specimen contains blood or other materials that mask the color change.	The exact color change described is not expected with these types of specimens; *digene* HC2 HPV DNA Test results should not be adversely affected.
d)	Specimen pH may be unusually acidic.	If neither of the other causes applies, 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 since improper specimen pH will adversely affect the test results.
Quality Controls give incorrect results		
a)	Incorrect software protocol chosen for test (i.e., used CPC protocol for dual 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, with the correct protocol.
b)	Reverse placement of QC1-LR and QC2-HR	Retest specimens.
c)	Reverse placement of LRC and QC1-LR and/or HRC and QC1-HR	Retest Specimens.
Incorrect color change observed during hybridization.		
a)	Inadequate mixing of Probe Mix with denatured Calibrators, Controls, and/or specimens; or Probe Mix not added; or incorrect volume of Reagent added.	Shake hybridization microplate or microtube rack for an additional 2 minutes. If there are wells which still remain purple, add an additional 25 µl of the appropriate Probe Mix 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.
b)	Specimen contains blood or other materials that mask the color change.	The exact color change described is not expected with these types of specimens; *digene* HC2 HPV DNA Test results should not be adversely affected.

		Comments and suggestions
c)	Specimen had <1000 µl STM.	Check the volume of the original specimen. Volume should be 1350 µl ±20 µl (after removing 75 µl for Low and High-Risk HPV Probes). If volume is <1350 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.		
a)	No Probe added to Probe Diluent.	Prepare Probe Mixes as described in these instructions for use. Label tubes carefully.
b)	Probe contaminated with RNase during preparation	Use aerosol-barrier pipette tips when pipetting probe and wear gloves. Only use clean, new disposable reagent reservoirs.
c)	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.
d)	Inadequate mixing of diluted Probe and denatured specimen	After adding Probe Mix and specimen to each Hybridization Microplate well 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/microplate well.
e)	Incorrect time or temperature during hybridization step	Hybridize for 60 ±5 minutes at 65 ±2°C. Check temperature of Microplate Heater I or water bath. Make sure that the Microplate Heater I or water bath is set to heat specimens to correct temperature and is preheated for 60 minutes prior to use. Make sure that water level is adequate to heat specimens to correct temperature. Water baths should be calibrated periodically.
f)	Switched Probes/Probe Mixes/Hybridization Tubes	Prepare Probe Mixes carefully and label Probe Mix tubes accordingly. Be careful to add the correct Probe to the correct set of Hybridization Tubes. Label Probe Mix tubes, Hybridization Tubes and/or racks to minimize the potential for switches.
g)	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 30–45 minutes.
h)	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.
i)	Luminometer malfunction or incorrect programming	Refer to the appropriate User Manual for further instructions or call your local QIAGEN Representative.
Elevated RLU values in Calibrator, Quality Controls, and/or specimens (≥200 RLUs in many or all wells). Assay may fail validation criteria.		

		Comments and suggestions
a)	Denaturation Reagent not added; or, incorrect volume of reagent added; or, inadequate mixing of Denaturation Reagent with specimens or Calibrators.	Make sure 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.
b)	Light leak in the Luminometer. Door not sealed. Seal around door broken	Check background reading of the luminometer by reading an empty microplate. A reading of greater than 50 RLUs indicates that a light leak exists. Refer to appropriate User Manual for further instructions, or call your local QIAGEN Representative.
c)	Contamination of Detection Reagent 2 or Capture Microplate wells by Detection Reagent 1 or exogenous alkaline phosphatase	Refer to Contamination Check in this Troubleshooting section.
d)	Contaminated Wash Buffer	Refer to Contamination Check in this Troubleshooting section.
e)	Contaminated Automated Plate Washer	Refer to Contamination Check in this Troubleshooting section.
f)	Inadequate washing of Capture Microplate wells after Detection Reagent 1 incubation	Wash Microplate wells 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 *Automated Plate Washer User Manual* for instructions on testing for contamination or malfunctions.
g)	Detection Reagent 1 contamination of Microplate wells	Make sure that all work surfaces are clean and dry. Use care when using Detection Reagent 1. Avoid aerosols.
h)	Blotting hybridization solution on some area of Kimtowels Wipers or equivalent lint-free paper towels	Do not reblot on previously used Kimtowels Wipers or equivalent lint-free paper towels area.
i)	Used incorrect blotting towels	Use Kimtowels Wipers or equivalent lint-free paper towels for blotting.
Low PC/NC ratios or high number of low positive specimens with ratios <2.0 (>20%). Assay may fail validation criteria.		
a)	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. For PreservCyt Solution specimens, make sure that proper mixing and resuspension of the cell pellet is completed prior to denaturation incubation. Consult *digene* HC2 Sample Conversion Kit instructions for use for protocol details. A distinct color change from clear to dark purple should be seen. Incubate for 45 ± 5 minutes at 65 ± 2°C.

		Comments and suggestions
b)	Probe inadequately mixed or insufficient Probe added to assays	Prepare Probe Mixes as described. Mix thoroughly by vortexing to make sure that a visible vortex is produced. Probe Mixes must be added to tubes with a positive displacement pipettor or a multichannel pipettor to make sure of accurate delivery.
c)	Inadequate volume of diluted Probe added to each Hybridization Microtube	Make sure that the 8-channel pipettor is delivering accurately prior to adding Probe Mix to hybridization microplate or microtubes. Add 25 µl of Probe Mix to each microplate well or microtube containing denatured Calibrators, Quality Controls, and clinical specimens. Make sure that the 8-channel pipettor is delivering accurately prior to adding Probe Mix to Hybridization Microplate wells. Color change should be from dark purple to yellow upon addition and thorough mixing of Probe Mix. PreservCyt Solution specimens should turn pink instead of yellow.
d)	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.
e)	Insufficient capture	The capture step should be performed using the Rotary Shaker I set at 1100 ± 100 rpm. Validate shaker speed by calibration.
f)	Inadequate washing	Wash Microplate wells thoroughly with Wash Buffer 6 times, filling the wells to overflowing each time or using the Automated Plate Washer.
g)	Contaminated Wash Buffer	Refer to Contamination Check in this Troubleshooting section.
Series of positive specimens with RLU values approximately the same.		
a)	Contamination of Capture Microplate wells 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.
b)	Detection Reagent 2 contamination	Be careful not to contaminate the stock when pipetting Detection Reagent 2 into Capture Microplate wells. Avoid contamination of Detection Reagent 2 by aerosols from Detection Reagent 1 or from laboratory dust, etc.
c)	Automated Plate Washer malfunction	Refer to Contamination Check in this Troubleshooting section or see *Automated Plate Washer User Manual* for instructions on testing for contamination or malfunctions.
Wide %CVs between replicates.		
a)	Incorrect pipetting.	Check pipettor to make sure that reproducible volumes are being delivered. Calibrate pipettors routinely.
b)	Insufficient mixing	Mix thoroughly at all steps. Vortex prior to denaturation incubation and after adding Probe Mix. Make sure that a visible vortex is produced.

		Comments and suggestions
c)	Incomplete transfer of liquid from Hybridization Microtubes to Capture Microplate wells	Take care during transfer step from Hybridization Microplate wells or microtubes to Capture Microplate wells to make sure reproducible volumes are transferred.
d)	Improper washing conditions	Wash Microplate wells thoroughly with Wash Buffer 6 times, filling to overflowing each time or using Automated Plate Washer and proper Automated Plate Washer protocols.
e)	Detection Reagent 1 contamination of Microplate wells	Make sure that all work surfaces are clean and dry. Use care when using Detection Reagent 1. Avoid aerosols.
False-positive results obtained from known negative specimens.		
a)	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.
b)	Detection Reagent 1 contamination of Microplate wells	Wash Microplate wells 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 microplate wells after washing.
c)	Blotting on same area of Kimtowels Wipers or equivalent lint-free paper towels over several rows	Do not blot on area that has been previously used as contamination could occur.
d)	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 either the manual method or the MST Vortexer 2 method (for the manual vortexer method, invert tube one time). For PreservCyt Solution specimens, make sure that proper mixing and resuspension of the cell pellet is completed prior to denaturation incubation. Consult *digene* HC2 Sample Conversion Kit instructions for use 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. For SurePath specimens make sure that the specimens are incubated for 90±5 minutes at 65 ±2°C.
e)	Improper washing conditions	Wash Microplate wells 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.

		Comments and suggestions
f)	Contamination of pipette tip with un-denatured 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 these instructions for use. Improper specimen vortexing, tube inversion and agitation can result in incomplete denaturation of non-specific RNA:DNA hybrids endogenous to cervical specimens. When using PreservCyt Solution or SurePath Preservative Fluid 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-pipet 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.
Elevated Negative Calibrator RLU values (>200 RLUs). Remainder of assay performs as expected.		
a)	Detection Reagent 2 was incubated at a temperature greater than 20-25°C.	Rerun the test and make sure that capture and detection steps are incubated at 20-25°C.
b)	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.
c)	Detection Reagent 2 or Wash Buffer was contaminated with alkaline phosphatase or Detection Reagent 1	Refer to Contamination Check in this Troubleshooting section.
Assay fails validation criteria. Elevated PC/NC Ratio		
Reverse placement of the HRC and the QC2-HR and/or the LRC and the QC1-LR		Rerun the test and make sure that capture and detection steps are incubated at 20–25°C.

Contamination Check

Reagent Evaluated	Contamination Check Procedure	Interpretation of Results
Note: Take care when pipetting Detection Reagent 2 to avoid contamination. Wear gloves and avoid touching pipette tips on any work surfaces.		
Detection Reagent 2	1. Pipette 75 µl of the aliquoted, residual and or original vial of Detection Reagent 2 into a blank Capture Microplate well. 2. Incubate 20–25°C for 15 minutes. Avoid direct sunlight. 3. 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	1. Pipette 75 µl of Detection Reagent 2 into 4 separate Capture Microplate wells. 2. Label wells 1–4. 3. Well 1 serves as the Detection Reagent 2 control. 4. Pipette 10 µl of wash buffer from the wash bottle into well 2. 5. Allow wash buffer to flow through the washer tubing. 6. Pipette 10 µl of the wash buffer from the tubing into well 3. 7. Obtain an aliquot of the water used to prepare the wash buffer. Pipette 10 µl of the water into well 4. 8. 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 well 2, 3 and 4 to the Detection Reagent 2 control RLU value (well 1). The individual RLU values for wells 2, 3 & 4 should not exceed 50 RLUs of the Detection Reagent 2 control RLU value (well 1). Values exceeding 50 RLUs of the Detection Reagent 2 control indicate contamination. See Reagent Preparation and Storage for instructions on cleaning and maintenance of Wash Apparatus.

Reagent Evaluated	Contamination Check Procedure	Interpretation of Results
Automated Plate Washer	1. Pipette 75 µl of Detection Reagent 2 into 5 separate Capture Microplate wells. 2. Label wells 1–5. 3. Well 1 serves as the Detection Reagent 2 control. 4. Pipette 10 µl of wash buffer from the plate washer bottle labeled Wash into well 2. 5. Pipette 10 µl of the rinse liquid from the plate washer bottle labeled Rinse into well 3. 6. Depress the Prime key on the Plate Washer key pad, allowing wash buffer to flow through the lines. 7. Pipette 10 µl of the wash buffer from the trough into well 4. 8. Depress the Rinse key on the Plate Washer key pad, allowing the rinse liquid to flow through the lines. 9. Pipette 10 µl of the wash buffer from the trough into well 5. 10. 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 well 2, 3, 4 and 5 to the Detection Reagent 2 control RLU value (well 1). The individual RLU values for wells 2, 3, 4 & 5 should not exceed 50 RLUs of the Detection Reagent 2 control RLU value (well 1). • Values exceeding 50 RLUs of the DR2 control indicate contamination of the Plate Washer. See *Automated Plate Washer User Manual*, Decontamination Procedure.

Summary of *digene* HC2 HPV DNA Test

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

	Procedure	
	Manual Vortex Method	Multi-Specimen Tube (MST) Vortexer 2 Method
DENATURATION (For PreservCyt Solution specimens, see *digene* HC2 Sample Conversion Kit instructions for use)	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, calibrator and quality control individually for 5 seconds at high speed (see these instructions for use for details). Check that all tubes s how a purple color. ↓ Incubate at 65 ±2°C for 45 ±5 minutes. ↓ Prepare HPV Probe Mix. ↓	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. ↓ Incubate at 65 ±2°C for 45 ±5 minutes. ↓ Prepare HPV Probe Mix ↓
HYBRIDIZATION Combined-Probe Cocktail Method	Water Bath Method Mix denatured specimen well and pipette 75 µl into tubes. ↓ Incubate for 10 minutes at 20–25°C	Microplate Heater I Method Mix denatured specimen well and pipette 75 µl into microplate wells. ↓ Incubate for 10 minutes at 20–25°C
Dual Probe Method	↓	↓
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 these instructions for use for details). ↓	
HYBRID DETECTION	Pipette 75 µl Detection Reagent 1 into each well of capture microplate. Cover capture microplate with Plate Lid, 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 these instructions for use for details). ↓ Wash 6 times. ↓ Blot on lint-free paper towels ↓	Automated Plate Washer Method Place plate on washer and press “START/STOP” to begin. ↓ Go to next step. ↓ ↓ ↓ ↓
SIGNAL AMPLIFICATION	Pipette 75 µl Detection Reagent 2 into each well of capture microplate. Incubate at 20–25°C for 15–30 minutes. ↓	
READING	Read capture microplate on DML Instrument. ↓ Validate assay and interpret specimen results.	

Explanation of Symbols

	Use by
	Batch code
	Manufacturer
	European Authorized Representative
	Catalog number
	In vitro diagnostic medical device
	CE-IVD marked symbol
	Global Trade Item Number
	Contains sufficient for 96 tests
	Temperature limitation
	Consult instructions for use

Ordering Information

Product	Contents	Cat. no.
digene® HC2 HPV DNA Test	For 96 reactions	5196-1330
digene® HC2 DNA Collection Device	50 Cervical Samplers	See QIAGEN.com
digene® Sample Conversion Kit	Conversion components required for PreservCyt processing	See QIAGEN.com
digene® Specimen Transport Medium	1 x 30 ml	See QIAGEN.com
digene® Wash Buffer Concentrate	2 x 100 ml	See QIAGEN.com

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History	
Rev. 8 02/2019	New GHS information in Warnings and Precautions on pages 16-17. Can use Cervical Biopsies ups to 5 mm in cross section on page 24. Added new symbols on 108. Added ordering information on page 110. Revised Trademarks section to remove Patents on page 111.