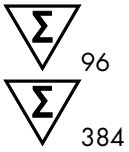
August 2015

digene® HC2 High-Risk HPV DNA Test Instructions For Use



IVD

An in vitro nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of 13 high-risk types of human papillomavirus (HPV) DNA in cervical and vaginal specimens

For use with:

- *digene* HC2 DNA Collection Device
- *digene* Specimen Transport Medium
- Hologic PreservCyt® Solution
- BD SurePath® Preservative Fluid

CE

REF

5197-1330 (1-plate kit)
618111 (4-plate kit)

QIAGEN
19300 Germantown Road
Germantown, MD 20874
USA

EC REP

QIAGEN GmbH
QIAGEN Strasse 1
40724 Hilden
GERMANY

1058538 Rev. 12

Key changes from previous instructions for use revision:

- Added the sample preparation procedure of SurePath post-gradient cell pellet samples using the QIAsymphony® DSP HPV Media Kit along with the associated performance data.
- Updated for compliance with the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

Contents

Intended Use

For in vitro diagnostic (IVD) use.

The *digene* HC2 High-Risk 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 13 high-risk types of HPV DNA in cervical and vaginal specimens.

Cervical and vaginal specimens that may be tested with the *digene* HC2 High-Risk HPV DNA Test include the following:

- Cervical specimens collected by a physician with the *digene* HC2 DNA Collection Device
- Self-collected vaginal specimens collected with the *digene* HC2 DNA Collection Device
- Biopsies collected in *digene* Specimen Transport Medium (STM)
- Specimens collected using a broom-type collection device or brush/spatula combination collection device, then placed in PreservCyt Solution or SurePath Preservative Fluid

The use of this 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 High-Risk 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.

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).

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 types 16 and 18 have been regarded as high-risk cancer-associated types (8-10). HPV types 31, 33, and 35 have been demonstrated to have an intermediate association with cancer (2, 11–14). This intermediate association is due to the fact that these types are more frequently detected in high-grade squamous intraepithelial lesions rather than in cancers. Therefore, induction of cancers due to the presence of these types is less likely than when high-risk HPV DNA types are present (15). These 5 HPV types together account for about 73% of HPV infections (16, 17). Additional HPV types, including 39, 45, 51, 52, 56, 58, 59 and 68, have been identified as the principal HPV types detectable in the remaining lesions (17–27). These HPV types can also be categorized into intermediate- and high-risk groups based on their relative distribution in various histopathological diagnosis categories (16, 17, 24–28).

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, 16, 29). Prospective studies have shown that 15–28% of women that tested positive for HPV DNA developed squamous intraepithelial lesions (SIL) within 2 years compared to only 1–3% of women who tested negative for HPV DNA (30, 31). In particular, the risk of progression for HPV types 16 and 18 was greater (approximately 40%) than for other HPV types (30).

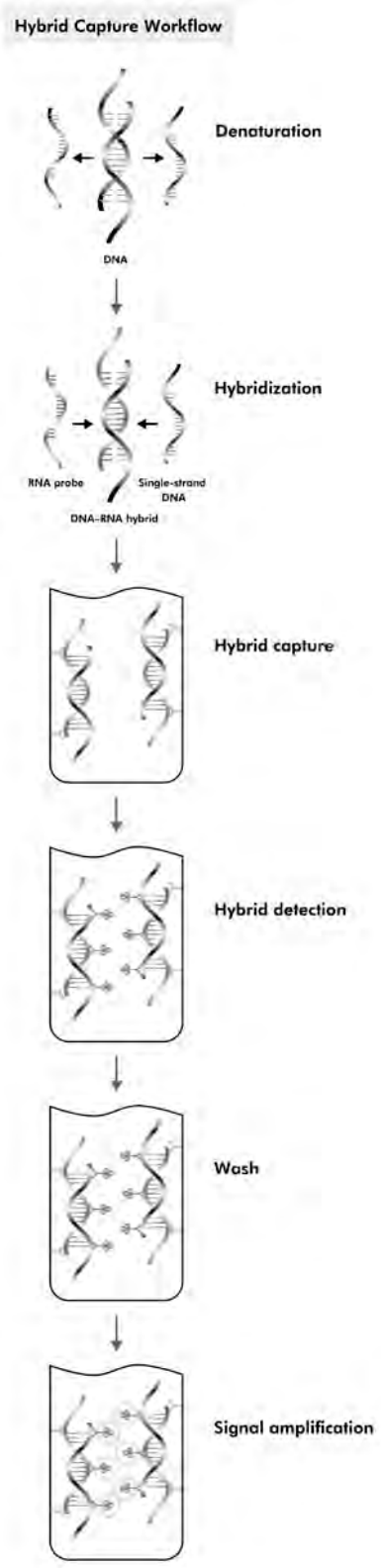
Pathogen information

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.

Principle of the Procedure

The *digene* HC2 High-Risk HPV DNA Test, using HC2 technology, is a nucleic acid hybridization assay with signal amplification that utilizes microplate chemiluminescent detection. Specimens containing the target DNA hybridize with a specific HPV RNA probe. 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 (RLU) by a *digene* Microplate Luminometer (DML) instrument. 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 assay cutoff (CO) indicates the presence of high-risk HPV DNA sequences in the specimen. An RLU measurement less than the assay CO indicates the absence of the specific high-risk HPV DNA sequences tested or HPV DNA levels below the detection limit of the test.


Hybrid Capture Workflow
Denaturation
DNA
Hybridization
RNA probe
Single-strand DNA
DNA–RNA hybrid
Hybrid capture
Hybrid detection
Wash
Signal amplification

Sample preparation using the QIAsymphony SP

Automated sample preparation of PreservCyt specimens can be performed using the QIAsymphony SP with the QIAsymphony DSP HPV Media Kit or the QIAsymphony DSP AXpH DNA Kit.

Sample preparation using the QIAsymphony DSP HPV Media Kit

The QIAsymphony DSP HPV Media Kit provides sample extracts on the hybridization microplate that are ready for automated testing using the Rapid Capture® System (RCS) with the *digene* HC2 High-Risk HPV DNA Test. The QIAsymphony SP performs all steps of the sample preparation procedure for up to 88 samples, in batches of up to 24, in a single run.

The QIAsymphony SP processes 88 PreservCyt samples in 2 hours and 15 minutes with no user intervention required once the instrument is loaded with samples.

The QIAsymphony SP processes 88 SurePath samples in 1 hour and 45 minutes with no user intervention required once the instrument is loaded with samples. The sample preparation using the QIAsymphony SP is immediately followed by a 90 minute incubation of the sample extracts in the hybridization microplate on a microplate heater. During the sample extract incubation, the calibrators and quality controls are denatured separately in a waterbath and are then manually pipetted into the first column of the hybridization microplate once the sample extract incubation is complete. Sample preparation of SurePath specimens with QIAsymphony SP and the QIAsymphony DSP HPV Media Kit can occur either before starting cytology processing or after cytology processing is complete.

Important: The sample extracts produced as a result of sample preparation of PreservCyt and SurePath specimens using the QIAsymphony DSP HPV Media Kit may only be tested using the RCS. Manual performance of the test with sample extracts is not validated.

When performing automated sample preparation using the QIAsymphony, refer to the applicable QIAsymphony user manuals and *QIAsymphony DSP HPV Media Kit Instructions for Use (Handbook)*, in addition to these instructions for use, for necessary procedural and descriptive information.

Sample preparation using the QIAsymphony DSP AXpH DNA Kit

The QIAsymphony DSP AXpH DNA Kit provides DNA eluates on the hybridization microplate that are ready for manual or RCS-automated testing with the *digene* HC2 High-Risk HPV DNA Test. The QIAsymphony SP performs all steps of the sample preparation procedure for up to 88 samples, in batches of up to 24, in a single run. The QIAsymphony SP processes 88 samples in 4 hours and 30 minutes with no user intervention required once the instrument is loaded with samples.

When performing automated sample preparation using the QIAsymphony, refer to the applicable QIAsymphony user manuals and *QIAsymphony DSP AXpH DNA Kit Handbook*, in addition to these instructions for use, for necessary procedural and descriptive information.

Testing using the Rapid Capture System

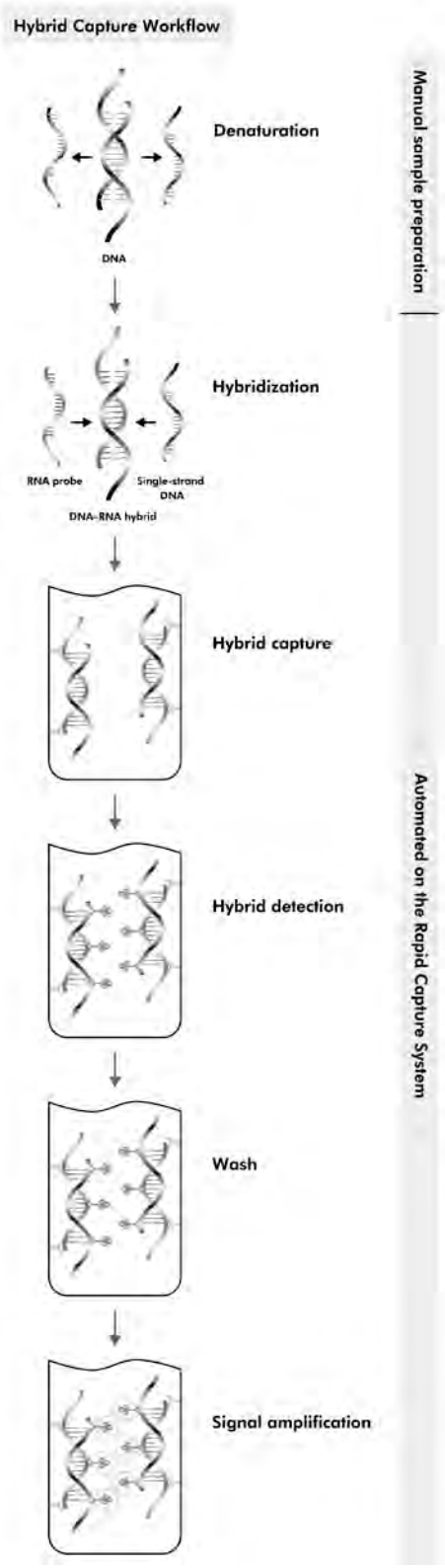
High-volume sample-throughput testing with the *digene* HC2 High-Risk HPV DNA Test can be performed using the RCS. The 4-plate kit (cat. no. 618111) can only be used with the RCS and cannot be used for manual testing.

The RCS is a general use automated pipetting and dilution system that can be used with the *digene* HC2 High-Risk HPV DNA Test for high-volume sample-throughput testing. This system processes up to 352 specimens in 8 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.

Sample preparation is performed independently of the RCS prior to placement on the RCS deck. In addition, chemiluminescent signal detection and results reporting are performed using an offline DML instrument common to both manual and RCS-automated testing.

Each of the *digene* HC2 High-Risk HPV DNA Test steps are performed in the exact sequence as manual testing. The RCS allows for the staggered processing of up to 4 microplates, with each microplate containing samples and the required test calibrators and quality controls.

When performing RCS-automated testing, refer to *Rapid Capture System User Manual* and *Rapid Capture System User Manual — Performing* digene *HC2 DNA Tests Using QIAsymphony SP Processed Samples*, in addition to these instructions for use, for necessary procedural and descriptive information.


Hybrid Capture Workflow
Denaturation
DNA
Hybridization
RNA probe
Single-strand DNA
DNA–RNA hybrid
Hybrid capture
Hybrid detection
Wash
Signal amplification
Manual sample preparation
Automated on the Rapid Capture System

Materials Provided

1-plate kit

There are 96 tests in the 1-plate *digene* HC2 High-Risk HPV DNA Test (cat. no. 5197-1330).

When performing manual testing using the 1-plate kit, the smallest number of tests recommended for each use is 24. If fewer than 24 tests per use are desired, the total number of tests per kit may be reduced due to limited reagent volumes. The number of patient results will vary, depending on the number of uses per kit, as specified below:

Number of uses	Number of patient results
1	88
2	80
3	72
4	64

When performing RCS-automated testing with the 1-plate kit, full-kit use requires testing a full microplate (88 samples) per RCS run. Testing of a partial microplate is acceptable; however, the entire kit is used due to the void volume required for operation of the instrument.

4-plate kit

There are 384 tests in the 4-plate *digene* HC2 High-Risk HPV DNA Test (cat. no. 618111).

The 4-plate kit can only be used for RCS-automated testing. To achieve 384 tests, the 4-plate kit must be used in 1 or 2 RCS runs. If more than 2 runs are desired, the total number of tests per kit may be reduced due to limited reagent volumes.

Kit contents

***digene* HC2 High-Risk HPV DNA Test**		
Catalog number	**5197-1330**	**618111**
Number of tests	**96**	**384**
Indicator Dye Contains 0.05% (w/v) sodium azide	0.35 ml	2.0 ml
Denaturation Reagent* Dilute sodium hydroxide (NaOH) solution	50 ml	2 x 100 ml
Probe Diluent* Buffered solution with 0.05% (w/v) sodium azide	5 ml	20 ml
High-Risk HPV Probe HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 RNA probe in buffered solution (red cap)	200 µl	3 x 200 µl
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 ml	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 ml	1 ml
Negative Calibrator Carrier DNA in STM with 0.05% (w/v) sodium azide	2 ml	2 ml
High-Risk HPV Calibrator 1 pg/ml cloned HPV 16 DNA and carrier DNA in STM with 0.05% (w/v) sodium azide	1 ml	2 ml
Capture Microplate Coated with goat polyclonal anti-RNA–DNA hybrid antibodies	1	4
Detection Reagent 1 Alkaline phosphatase–conjugated antibodies to RNA-DNA hybrids in buffered solution with 0.05% (w/v) sodium azide	12 ml	40 ml
Detection Reagent 2 CDP-Star® with Emerald II (chemiluminescent substrate)	12 ml	40 ml
Wash Buffer Concentrate* Contains 1.5% (w/v) sodium azide	100 ml	2 x 100 ml

* See "Warnings and Precautions," page 20, for health and safety information.

Materials Required but Not Provided

Important: Make sure that the instruments used in this procedure have been checked and calibrated according to the manufacturer's recommendations.

In vitro diagnostic equipment and materials

Only equipment and materials validated with the *digene* HC2 High-Risk HPV DNA Test are available from QIAGEN.

- *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 User Manual*
- Hybrid Capture System Rotary Shaker I
- Hybrid Capture System Microplate Heater I
- Hybrid Capture System Automated Plate Washer
- Hybrid Capture System Multi-Specimen Tube (MST) Vortexer 2 (optional)*
- Conversion Rack and Lid (optional)*
- *digene* Specimen Rack and Lid (optional)*
- EXPAND-4 pipet and stand (optional)†
- Tube Sealer Dispenser and cutting device (optional, used with the MST Vortexer 2)
- Rapid Capture System (required for use with the 4-plate kit; optional for the 1-plate kit)
- Wash Apparatus
- Hybridization microplates
- Microplate lids
- RCS microplate well strips*
- RCS reagent troughs*
- RCS reagent trough lids*
- RCS disposable tips*
- RCS drop-on caps*

* Required for performing RCS-automated testing.

† Custom item used for transfer of STM samples to hybridization microplate. Other custom, expandable, multi-channel pipets can be used, provided tip spacing of 3.2 cm is achievable when expanded.

- Buffer N2*
- Buffer D2*
- Blue RCS Washer Boat†
- Extra-long pipet tips
- Specimen collection tubes
- Specimen collection tube rack
- Specimen collection tube screw caps
- Disposable reagent reservoirs
- DuraSeal™ tube sealer film
- Hybridization microtubes‡
- Microtube rack‡
- Plate sealers‡

General laboratory use equipment and materials

- 65 ± 2°C waterbath of sufficient size to hold a specimen rack [21 cm wide x 32 cm deep x 18 cm high (8.25 x 12.25 x 4.9 in.)]
- Microcentrifuge
- Vortexer with cup attachment
- Single-channel pipet; variable settings for 20–200 µl and 200–1000 µl volumes
- Repeating positive displacement pipet, such as Eppendorf® Repeater® pipet
- 8-channel pipet: variable settings for 25–200 µl volumes
- Timer
- Sodium hypochlorite solution, 0.5% v/v
- Parafilm® or equivalent
- Disposable aerosol-barrier pipet tips for single-channel pipet (20–200 µl and 200–1000 µl)
- Disposable tips for repeating positive displacement pipet (12.5, 5, 2.5 and 1.25 ml)
- Disposable tips for 8-channel pipet (25–200 µl)
- Kimtowels® wipers or equivalent low-lint paper towels
- Disposable bench cover
- Powder-free, disposable gloves
- 5 ml and/or 15 ml snap-cap, round-bottom, polypropylene tubes
- Tube rack to hold 10 ml or 15 ml tubes
- 50 ml polypropylene conical tubes

* Required for performing testing with samples prepared using the QIAsymphony DSP AXpH DNA Kit.
† Required for RCS-automated testing of samples processed using the QIAsymphony DSP HPV Media Kit.
‡ Required for performing hybridization using microtubes and waterbath.

Additional equipment and materials for PreservCyt sample preparation

Refer to *QIAsymphony DSP HPV Media Kit Instructions for Use (Handbook)* for automated sample preparation using the QIAsymphony DSP HPV Media Kit.

Refer to *QIAsymphony DSP AXpH DNA Kit Handbook* for automated sample preparation using the QIAsymphony DSP AXpH DNA Kit.

Refer to the digene *HC2 Sample Conversion Kit* instructions for use for manual sample preparation.

Additional equipment and materials for SurePath sample preparation

Refer to *QIAsymphony DSP HPV Media Kit Instructions for Use (Handbook)* for automated sample preparation using the QIAsymphony DSP HPV Media Kit.

Manual SurePath sample preparation requires the following additional equipment and materials:

- Swinging bucket centrifuge capable of reaching 800 ± 15 x *g* and holding 15 ml conical, polypropylene centrifuge tubes
- *digene* HC2 Sample Conversion Tubes or 15 ml VWR® or Corning® polypropylene tubes

  Important: The *digene* HC2 Sample Conversion Tubes available from QIAGEN must be used with the MST Vortexer 2 or the RCS.
- 7 ml standard-tipped transfer pipets or equivalent
- *digene* Specimen Transport Medium

Warnings and Precautions

For in vitro diagnostic use.

Read all instructions carefully before using the test.

Warnings

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

Specimens

CAUTION **Risk of infectious agents**

Specimens may contain infectious agents and should be handled accordingly. Consider all specimens 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 applicable national and 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 pipet 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 (32, 33).
- Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with national and local regulations.

Following denaturation and incubation, the specimens are no longer considered infectious (34); however, lab personnel should still adhere to national and local precautions.

Sodium azide

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 U.S. 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.
4. Flush well with water.

Buffer N2

CAUTION **Risk of highly reactive compounds**

Do not add bleach or acidic solutions directly to any solution or waste containing Buffer N2.

Buffer N2 contains guanidine hydrochloride which can form highly reactive compounds when combined with bleach.

If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water and then with 1% (v/v) sodium hypochlorite.

RCS-automated testing

Refer to *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 High-Risk HPV DNA Test kit:

Wash Buffer Concentrate

Contains: Sodium azide. Warning! Harmful if swallowed. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Dispose of contents/ container to an approved waste disposal plant.

Denaturation Reagent

Contains: Sodium hydroxide. Danger! Causes severe skin burns and eye damage. May be corrosive to metals. Dispose of contents/container to an approved waste disposal plant. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. Immediately call a POISON CENTER or doctor/physician. Store locked up. Wear protective gloves/protective clothing/ eye protection/face protection.

Probe Diluent

Contains: Acetic acid; Polyacrylic acid. Danger! Causes severe skin burns and eye damage. Dispose of contents/container to an approved waste disposal plant. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. Immediately call a POISON CENTER or doctor/physician. Store locked up. Wear protective gloves/protective clothing/eye protection/face protection.

High-Risk HPV Calibrator

Warning! Causes mild skin irritation. If skin irritation occurs: Get medical advice/attention.

High-Risk HPV Quality Control

Warning! Causes mild skin irritation. If skin irritation occurs: Get medical advice/attention.

Low-Risk HPV Quality Control

Warning! Causes mild skin irritation. If skin irritation occurs: Get medical advice/attention.

Negative Calibrator

Warning! Causes mild skin irritation. If skin irritation occurs: Get medical advice/attention.

Precautions

The user must always adhere to the following precautions when performing the *digene* HC2 High-Risk HPV DNA Test:

- Do not use the reagents beyond the expiration date indicated next to the ⌛ symbol on the outer box label or the expiration date of the prepared reagents.
- Performing the test outside the time and temperature ranges provided may produce invalid results. Tests not falling within the established time and temperature ranges are invalid and must be repeated.
- The *digene* HC2 High-Risk HPV DNA Test procedure, assay calibration, quality control and the interpretation of specimen results must be followed closely to obtain reliable test results.
- It is important to pipet 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.

- With the exception of the Wash Buffer Concentrate, the kit components have been tested as a unit. Do not interchange components from other sources or from different lots. It is, however, acceptable to combine components from kits of the same lot number to have the required reagent volumes to test multiple microplates in a single RCS run.
- Nucleic acids are very sensitive to environmental nuclease degradation. Nucleases are present on human skin and on surfaces or materials handled by humans. Clean and cover work surfaces with a disposable bench cover and wear powder-free gloves when performing all test steps.
- Make sure to prevent contamination of the capture microplate and the Detection Reagent 2 (DR2) with exogenous alkaline phosphatase during performance of the test. Substances that may contain alkaline phosphatase include Detection Reagent 1 (DR1), bacteria, saliva, hair and oils from the skin. Covering the capture microplate after the wash step and during the DR2 incubation is especially important because exogenous alkaline phosphatase may react with the DR2, producing false-positive results.
- Protect the DR2 from prolonged exposure to direct light. Use the DR2 immediately after aliquoting and avoid direct sunlight.
- Prime the repeating pipet in advance of reagent delivery and check for large air bubbles periodically. Excessive amounts of large air bubbles in the repeating pipet tip may cause inaccurate delivery and can be avoided by filling the pipet, dispensing all liquid and refilling. Refer to the pipet user manual for specific instructions for use.
- Perform multi channel pipetting using the reverse pipetting technique (see “Hybrid detection,” page 50) for dispensing DR1 and DR2. Check each pipet tip on the multi-channel pipet for proper fit and filling.
- Make sure that each capture microplate well is washed thoroughly (see “Washing,” page 51). Inadequate washing will result in increased background and may cause false-positive results. Residual Wash Buffer in the capture microplate wells may result in reduced signal or poor reproducibility.

Reagent Storage and Handling

Kit components

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. All reagents are provided ready-to-use, except the Denaturation Reagent (DNR), the Probe Mix and the Wash Buffer.

Prepared reagents

Once prepared, the DNR is stable for 3 months at 2–8°C.

Once prepared, the Wash Buffer is stable for 3 months at 2–30°C.

If testing PreservCyt samples processed using the QIAsymphony DSP HPV Media Kit or QIAsymphony DSP AXpH DNA Kit, the opened, undenatured calibrators and quality controls are stable for 3 months at 2–8°C.

If testing samples processed using the QIAsymphony DSP AXpH DNA Kit, the prepared Denaturation Reagent 2 (DNR2) is stable for 8 hours at 15–30°C.

Specimen Collection and Preparation

Collect and transport cervical and vaginal specimens for testing with the *digene* HC2 High-Risk HPV DNA Test using one of the following sampling devices:

- *digene* HC2 DNA Collection Device (consisting of a cervical brush and STM)
- Biopsies collected in *digene* STM
- A broom-type collection device or combination brush/spatula collection device placed in PreservCyt Solution or SurePath Preservative Fluid

Specimens collected with other sampling devices or transported in other transport media have not been qualified for use with this test. The performance characteristics of this test were established only with the collection kits indicated.

The *digene* HC2 DNA Collection Device must not be used for pregnant women. Cervical specimens must be collected prior to the application of acetic acid or iodine if colposcopy examination is performed. Refer to the *digene* HC2 DNA Collection Device instructions for use for additional specimen collection and handling procedures.

Cervical and vaginal specimens collected in STM do not require sample conversion prior to testing with the *digene* HC2 High-Risk HPV DNA Test. PreservCyt and SurePath specimens require sample conversion prior to testing with the *digene* HC2 High-Risk HPV DNA Test.

Cervical and vaginal specimens in STM

Important: Do not collect an STM cervical or vaginal specimen if high concentrations of anti-fungal cream, contraceptive jelly or douche are present.

STM specimens may be held for up to 2 weeks at room temperature and shipped without refrigeration to the testing laboratory. Ship specimens in an insulated container using either an overnight or 2-day delivery vendor.

At the testing laboratory, store specimens at 2–8°C if the test will be performed within 1 week. If the test will be performed later than 1 week, cover the specimen tube caps with Parafilm and store specimens at –20°C for up to 3 months. When removing specimens from the freezer for testing, replace caps immediately with specimen collection tube screw caps.

A preservative has been added to the STM to retard bacterial growth and to retain the integrity of the DNA. It is not intended to preserve viability of organisms or cells.

Cervical biopsies

Freshly collected cervical biopsies 2–5 mm in cross section may be tested with the *digene* HC2 High-Risk HPV DNA Test. Do not use biopsies less than 2 mm in diameter. Immediately place the biopsy specimen into 1.0 ml of STM, cover the specimen tube cap with Parafilm to prevent the cap from popping off and store frozen at –20°C. Ship biopsy specimens at 2–30°C for overnight delivery to the testing laboratory.

At the testing laboratory, store at –20°C until processed. When removing specimens from the freezer for testing, immediately replace the caps with specimen collection tube screw caps.

Cervical specimens in PreservCyt Solution

Important: Do not collect a PreservCyt cervical specimen for sample preparation with the QIAsymphony DSP HPV Media Kit if high concentrations of anti-fungal cream, vaginal lubricating jelly or blood are present.

Important: Do not collect a PreservCyt cervical specimen for sample preparation with the QIAsymphony DSP AXpH DNA Kit if contraceptive jelly is present.

Collect specimens in the routine manner, and prepare the ThinPrep® Pap Test slides according to the instructions for use provided by the manufacturer.

Following collection, store PreservCyt specimens for up to 3 months at 2–30°C prior to sample preparation for the *digene* HC2 High-Risk HPV DNA Test. PreservCyt specimens cannot be frozen.

The following methods are available for sample preparation:

- Automated sample preparation using the QIAsymphony SP and the QIAsymphony DSP HPV Media Kit

  The result is a sample extract (containing magnetic particles, STM and DNR) that is ready to proceed to the denaturation step of the test.
- Automated sample preparation using the QIAsymphony SP and the QIAsymphony DSP AXpH DNA Kit

  The result is a DNA eluate ready to proceed to the denaturation step of the test.
- Manual sample preparation using the *digene* HC2 Sample Conversion Kit

  The result of manual sample preparation is a denatured sample ready to proceed to the hybridization step of the test.

Specimen volume requirements are based on the sample preparation method as follows:

- Automated sample preparation using the QIAsymphony DSP HPV Media Kit requires 3 ml of specimen
- Automated sample preparation using the QIAsymphony DSP AXpH DNA Kit requires 4 ml of specimen
- Manual sample preparation using the *digene* HC2 Sample Conversion Kit requires at least 4 ml of specimen

Specimens with less than the required specimen volume after the Pap Test has been prepared contain insufficient material for testing and could cause a false-negative result in the *digene* HC2 High-Risk HPV DNA Test.

Cervical specimens in SurePath Preservative Fluid

Important: Do not collect a SurePath cervical specimen for sample preparation with the QIAsymphony DSP HPV Media Kit if contraceptive jelly, anti-fungal cream or anti-inflammatory cream are present.

Collect specimens in SurePath Preservative Fluid according to the applicable instructions for use.

Sample preparation of SurePath specimens can occur either before starting cytology processing or after completing cytology processing.

If before starting cytology processing, use a sample from the original SurePath specimen that has not been processed using any other diagnostic method, including the BD PrepMate® System and BD PrepStain® Slide Processor. In these instructions for use, these samples are referred to as "SurePath samples" to prevent confusion.

If after completing cytology processing, use a sample from the remaining post-gradient cell pellet after a SurePath specimen has been prepared according to the appropriate instructions for the BD PrepMate System and the BD PrepStain Slide Processor. In these instructions for use, these samples are referred to as "SurePath post-gradient cell pellet samples" to prevent confusion.

The following methods are available for sample preparation:

- Automated sample preparation of SurePath samples using the QIAsymphony SP and the QIAsymphony DSP HPV Media Kit.

  The result is a denatured sample extract (containing magnetic particles, STM and DNR) that is ready to proceed to the hybridization step of the test.
- Automated sample preparation of Sure Path post-gradient cell pellet samples using the QIAsymphony SP and the QIAsymphony DSP HPV Media Kit.

  The result is a denatured sample extract (containing magnetic particles, STM and DNR) that is ready to proceed to the hybridization step of the test.
- Manual sample preparation of SurePath post-gradient cell pellet samples.

  The result of manual sample preparation is a denatured sample ready to proceed to the hybridization step of the test.

Sample volume requirements are based on the sample preparation method as follows:

- Automated sample preparation using the QIAsymphony DSP HPV Media Kit requires 950 µl
- Manual sample preparation requires 2.8 ml of SurePath post-gradient cell pellet sample

Using less than the required volume could cause a false-negative result in the *digene* HC2 High-Risk HPV DNA Test.

Automated sample preparation of SurePath specimens

Following collection, store SurePath specimens for up to 4 weeks at 5–25°C prior to sample preparation using the QIAsymphony SP and the QIAsymphony DSP HPV Media Kit. The SurePath specimen used must not have been processed using any other diagnostic method, including the BD PrepMate and BD PrepStain Slide Processor. Automated sample preparation requires 950 µl of the SurePath specimen.

Automated sample preparation of SurePath post-gradient cell pellet samples

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

The post-gradient cell pellet with SurePath Preservative Fluid may be stored for up to 4 weeks at 5–25°C prior to sample preparation for the *digene* HC2 High-Risk HPV DNA Test. Automated sample preparation requires 950 µl of the SurePath post-gradient cell pellet.

Manual sample preparation of SurePath post-gradient cell pellet samples

Important: Immediately after SurePath Pap slide preparation, pipet 2.0 ml of SurePath Preservative Fluid 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 High-Risk 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 High-Risk 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.

Procedure

Things to do before starting

- For manual testing, allow at least 60 minutes for the Microplate Heater I to equilibrate to 65°C ± 2°C from a cold start. Not allowing time for this warm-up period could result in melting of the hybridization microplate. Refer to *Microplate Heater I User Manual* for additional instructions.
- If using a waterbath during the denaturation and hybridization steps, make sure that the waterbath is at 65°C and the water level is adequate to immerse the entire volume of specimen in the tube.

Reagent preparation

- Remove the specimens and all required reagents from the refrigerator prior to beginning the test. Allow them to reach 20–25°C for 15–30 minutes. Prepare PreservCyt and SurePath samples prior to equilibrating any previously denatured specimens and reagents to room temperature.
- If combining the ready-to-use reagents for a multiple-plate RCS run, mix individual bottles thoroughly and then combine the applicable volume of reagent into a clean, disposable, polypropylene conical tube.
- For manual testing, the Wash Buffer and Probe Mix reagents are prepared during particular steps of the testing. For RCS-automated testing, all reagents are prepared prior to starting the RCS run and placed on the RCS deck.
- Prepare the DNR and DNR2, as applicable, before preparing other reagents.
- Discard all prepared reagents (unless specified differently) and reagent aliquots at the end of the test.
- Use Tables 1–5, below, to determine the volume required for each reagent based on the number of tests/microplates and testing method. The volumes for RCS-automated testing include the reagent void volume required by the instrument.

Table 1. Required volumes of prepared and ready-to-use reagents for manual testing of STM specimens and manually prepared PreservCyt and SurePath post-gradient cell pellet samples

Number of tests/strips	Probe mix	Wash Buffer	DR1	DR2
24/3	1.04 ml	>1 liter	3 ml	3 ml
48/6	2.08 ml	>1 liter	5 ml	5 ml
72/9	3.12 ml	>1 liter	7 ml	7 ml
96/12	4.16 ml	>1 liter	12 ml	12 ml

Table 2. Required volumes of prepared and ready-to-use reagents for RCS-automated testing of STM specimens, manually prepared PreservCyt and SurePath post-gradient cell pellet samples, and SurePath and SurePath post-gradient cell pellet samples prepared using the QIAsymphony DSP HPV Media Kit

Number of microplates	Probe Mix	Wash Buffer	DR1	DR2
≤1	5.20 ml	3 liters	10 ml	10 ml
≤1.5	6.24 ml	3 liters	14 ml	14 ml
≤2	8.32 ml	3 liters	18 ml	18 ml
≤2.5	9.36 ml	6 liters	22 ml	22 ml
≤3	10.40 ml	6 liters	26 ml	26 ml
≤3.5	12.48 ml	6 liters	30 ml	30 ml
≤4	13.52 ml	6 liters	34 ml	34 ml

Table 3. Required volumes of prepared and ready-to-use reagents for RCS-automated testing of PreservCyt samples prepared using the QIAsymphony DSP HPV Media Kit

Number of microplates	DNR	Probe Mix	Wash Buffer	DR1	DR2
≤1	2.2 ml	5.20 ml	3 liters	10 ml	10 ml
≤1.5	2.2 ml	6.24 ml	3 liters	14 ml	14 ml
≤2	2.4 ml	8.32 ml	3 liters	18 ml	18 ml
≤2.5	2.4 ml	9.36 ml	6 liters	22 ml	22 ml
≤3	2.6 ml	10.40 ml	6 liters	26 ml	26 ml
≤3.5	2.6 ml	12.48 ml	6 liters	30 ml	30 ml
≤4	2.8 ml	13.52 ml	6 liters	34 ml	34 ml

Table 4. Required volumes of prepared and ready-to-use reagents for manual testing of PreservCyt samples prepared using the QIAsymphony DSP AXpH DNA Kit

Number of tests/strips	DNR	DNR2	Probe Mix	Wash Buffer	DR1	DR2
24/3	0.6 ml	1.0 ml	1.04 ml	>1 liter	3 ml	3 ml
48/6	0.6 ml	2.0 ml	2.08 ml	>1 liter	5 ml	5 ml
72/9	0.6 ml	2.5 ml	3.12 ml	>1 liter	7 ml	7 ml
96/12	0.6 ml	5.0 ml	4.16 ml	>1 liter	12 ml	12 ml

Table 5. Required volumes of prepared and ready-to-use reagents for RCS-automated testing of PreservCyt samples prepared using the QIAsymphony DSP AXpH DNA Kit

Number of microplates	DNR	DNR2	Probe Mix	Wash Buffer	DR1	DR2
≤1	2.2 ml	5.0 ml	5.20 ml	3 liters	10 ml	10 ml
≤1.5	2.2 ml	5.5 ml	6.24 ml	3 liters	14 ml	14 ml
≤2	2.4 ml	6.5 ml	8.32 ml	3 liters	18 ml	18 ml
≤2.5	2.4 ml	7.7 ml	9.36 ml	6 liters	22 ml	22 ml
≤3	2.6 ml	8.8 ml	10.40 ml	6 liters	26 ml	26 ml
≤3.5	2.6 ml	10.0 ml	12.48 ml	6 liters	30 ml	30 ml
≤4	2.8 ml	11.0 ml	13.52 ml	6 liters	34 ml	34 ml

Denaturation Reagent

The 1-plate kit comes with 50 ml of Denaturation Reagent and the 4-plate kit comes with 2 x 100 ml of Denaturation Reagent. Make sure to prepare the DNR according to the volume provided with the applicable kit.

Notes:

- Once prepared, the DNR is stable for 3 months at 2–8°C.
- If color fades, add 3 additional drops of Indicator Dye and mix thoroughly before using.

50 ml bottle

1. Add 5 drops of Indicator Dye to the 50 ml bottle of Denaturation Reagent.
2. Mix thoroughly.

   The DNR should be a uniform, dark purple color.
3. Label the DNR with the new expiration date.

100 ml bottle

1. Add 10 drops of Indicator Dye to the 100 ml bottle of Denaturation Reagent.
2. Mix thoroughly.

   The DNR should be a uniform, dark purple color.
3. Label the DNR with the new expiration date.

Denaturation Reagent 2

Note: DNR2 is only required for testing PreservCyt samples prepared using the QIAsymphony DSP AXpH DNA Kit.

1. Label a clean disposable polypropylene conical tube as "DNR2".
2. Add the required volume of Buffer N2 (see Table 6, below) to the labeled container.

Table 6. Preparation of DNR2

Volume of DNR2 required	Volume of Buffer N2	Volume of Buffer D2	Indicator Dye
1.0 ml	0.4 ml	0.6 ml	1–2 drops
2.0 ml	0.8 ml	1.2 ml	1–2 drops
2.5 ml	1.0 ml	1.5 ml	1–2 drops
5.0 ml	2.0 ml	3.0 ml	1–2 drops
5.5 ml	2.2 ml	3.3 ml	1–2 drops
6.5 ml	2.6 ml	3.9 ml	1–2 drops
7.7 ml	3.1 ml	4.6 ml	1–2 drops
8.8 ml	3.5 ml	5.3 ml	1–2 drops
10.0 ml	4.0 ml	6.0 ml	1–2 drops
11.0 ml	4.4 ml	6.6 ml	1–2 drops

3. Add the required volume of Buffer D2 (see Table 6, above) to the labeled container.
4. Add the required amount of Indicator Dye (see Table 6, above) to the labeled container.

   Note: Use the Indicator Dye supplied with the *digene* HC2 High-Risk HPV DNA Test kit.
5. Vortex for no less than 10 seconds.

   Note: Once prepared, the DNR2 is stable for 8 hours at 15–30°C.

Probe Mix

- For manual testing, prepare the Probe Mix during specimen denaturation incubation (as applicable, see "Denaturation of calibrators, quality controls and STM specimens," page 43, or "Denaturation of calibrators, quality controls and DNA eluates for manual testing," page 41).
- Take extreme care to prevent RNase contamination. Use aerosol-barrier pipet tips when pipetting probe.
- Probe Diluent is viscous. Make sure a visible vortex is achieved when preparing Probe Mix; incomplete mixing may result in reduced signal.
- If combining multiple vials of the probe for RCS-automated testing, pool the probe into one vial and mix by pipetting.

1. To avoid trapping the probe in the vial lid, centrifuge each vial of probe briefly to bring liquid to the bottom of the vial.
2. Tap the vial gently to mix.
3. Determine the amount of Probe Mix required.

   Recommendation: Make extra Probe Mix to account for the volume that may be lost in the pipet tips or on the side of the vial. The volumes specified in Tables 1–5, above, include the recommended extra volume.

   Manual testing: Determine the volumes required for a 1:25 dilution of probe in Probe Diluent to prepare the Probe Mix (25 µl/test). Volumes are provided in Table 1, page 30, and Table 4, page 31, as applicable.

   RCS-automated testing: Use the volumes specified in Table 2, page 31, Table 3, page 31, or Table 5, page 32, as applicable.
4. Label a new, disposable container as "High-Risk HPV Probe Mix".

   Depending on the number of tests, either a 5 ml or 15 ml snap-cap, round-bottom, polypropylene tube is recommended.
5. Add the required amount of Probe Diluent (see Table 7, below) to the labeled tube.
6. Pipet the required amount of the High-Risk HPV Probe into the Probe Diluent (see Table 7, below) by placing the pipet tip against the inner wall of the tube just above the meniscus and expelling the contents.

   Important: Do not immerse the tip into the Probe Diluent.

Table 7. Preparation of Probe Mix

Volume of Probe Mix required	Volume of Probe Diluent	Volume of High-Risk HPV Probe
1.04 ml	1.0 ml	40 µl
2.08 ml	2.0 ml	80 µl
3.12 ml	3.0 ml	120 µl
4.16 ml	4.0 ml	160 µl
5.20 ml	5.0 ml	200 µl
6.24 ml	6.0 ml	240 µl
8.32 ml	8.0 ml	320 µl
9.36 ml	9.0 ml	360 µl
10.40 ml	10.0 ml	400 µl
12.48 ml	12.0 ml	480 µl
13.52 ml	13.0 ml	520 µl

7. Vortex for at least 5 seconds at maximum speed to mix thoroughly.

   A visible vortex must be produced.

Wash Buffer

- For manual testing, prepare the Wash Buffer during the hybrid capture step (see "Hybrid capture," page 49).
- To minimize exposure, add water to the Wash Buffer Concentrate when preparing.
- For the manual microplate washing method, prepare 3 liters of the Wash Buffer in the Wash Apparatus.

  Recommendation: Every 3 months, clean the Wash Apparatus and tubing with 0.5% sodium hypochlorite solution and rinse thoroughly with distilled or deionized water to prevent possible contamination from alkaline phosphatase present in bacteria and molds.
- For the Automated Plate Washer, prepare the Wash Buffer and store in a covered container, or prepare 1 liter and place in the Automated Plate Washer wash reservoir.
- For RCS-automated testing, prepare the specified amount (as applicable, see Table 2, page 31, Table 3, page 31, or Table 5, page 32) in the RCS Wash Bottle.

1. Mix the Wash Buffer Concentrate well and add the required volume of Wash Buffer Concentrate (see Table 8, below) to the specified container.
2. Add the required volume of distilled or deionized water (see Table 8, below) to the specified container.

Table 8. Preparation of Wash Buffer

Volume of Wash Buffer required	Volume of Wash Buffer concentrate	Volume of distilled or deionized water
1 liter	33.3 ml	966.7 ml
2 liters	66.6 ml	1933.4 ml
3 liters	100.0 ml	2900.0 ml
6 liters	200.0 ml	5800.0 ml

3. Place a clean, low-lint paper towel over any openings of the container and mix well.
4. Seal the container to prevent contamination or evaporation, or place on the respective instrument, as applicable.
5. Label the Wash Buffer with the new expiration date.

   Note: Once prepared, the Wash Buffer is stable for 3 months at 2–30°C.

Create the plate layout

1. Create a plate layout using the *digene* assay analysis software with *digene* assay protocols for HPV.

   Refer to the applicable software user manual for instructions on creating a plate layout with the proper positions for the calibrators, quality controls and specimens.

   Notes:

   - The calibrators, quality controls and specimens are run in an 8-well column configuration.
   - Test the calibrators and quality controls in the following positions on the microplate (see Figure 1, below):
     - Negative Calibrator (NC) replicates in microplate wells A1, B1, C1
     - High-Risk HPV Calibrator (HRC) replicates in microplate wells D1, E1, F1
     - Low-Risk HPV Quality Control (QC1-LR) in microplate well G1
     - High-Risk HPV Quality Control (QC2-HR) in microplate well H1

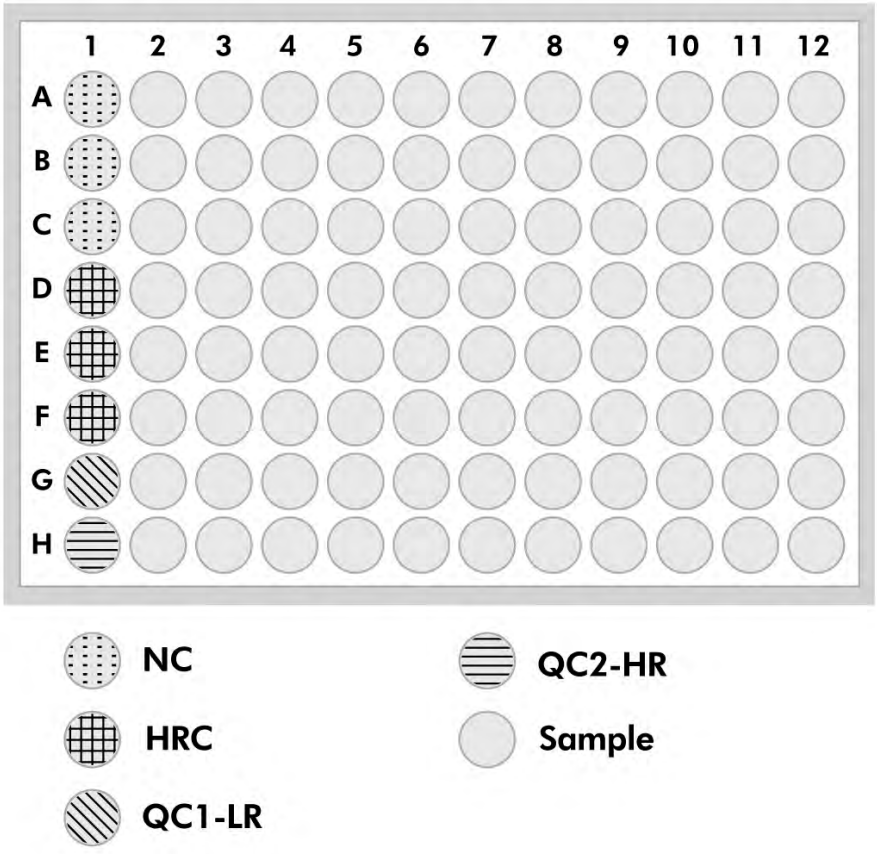


Figure 1. Position of calibrators, quality controls and samples on the microplate.

Important: When performing RCS-automated testing, use RCS-specific assay protocols to create the plate layout and generate results. The defined parameters of the RCS-specific assay protocols are different from those for the manual testing assay protocols (see "Cutoff calculation," page 56).

2. Place calibrators, quality controls and specimens to be tested in a specimen collection tube rack or specimen rack in the order in which they will be tested.

   Important: When performing RCS-automated testing, it is critical that the plate layout corresponds with the correct specimens tested to prevent reporting inaccurate specimen results. For each specimen rack and lid used, confirm that the serial numbers match and, as applicable, label each specimen rack and lid according to the order to be tested on the RCS. Use a marker and label that will not wash off in the 65°C waterbath.

Sample preparation

PreservCyt and SurePath specimens require sample preparation prior to testing with the *digene* HC2 High-Risk HPV DNA Test. Depending on the type of sample preparation performed, the prepared samples are ready for different steps of the *digene* HC2 High-Risk HPV DNA Test.

The available methods of sample preparation are as follows:

- Automated sample preparation of PreservCyt specimens using the QIAsymphony DSP HPV Media Kit
- Automated sample preparation of SurePath specimens and post-gradient cell pellets using the QIAsymphony DSP HPV Media Kit
- Automated sample preparation of PreservCyt specimens using the QIAsymphony DSP AXpH DNA Kit
- Manual sample preparation of PreservCyt specimens
- Manual sample preparation of SurePath post-gradient cell pellets

Sample preparation of PreservCyt specimens using the QIAsymphony DSP HPV Media Kit

Refer to *QIAsymphony DSP HPV Media Kit Instructions for Use (Handbook)* for instructions to prepare PreservCyt samples using the QIAsymphony DSP HPV Media Kit.

Important: The sample extracts produced as a result of sample preparation of PreservCyt specimens using the QIAsymphony DSP HPV Media Kit may only be tested using the RCS. Manual performance of the test with sample extracts is not validated.

The result of sample preparation of PreservCyt specimens using the QIAsymphony DSP HPV Media Kit is sample extracts in a hybridization microplate with the first column empty. The sample extracts contain magnetic particles, STM and DNR and are ready for RCS-automated testing at the denaturation step. The calibrators, quality controls and sample extracts are denatured at the same time in the hybridization microplate during RCS-automated testing (see "Denaturation and hybridization of samples prepared using the QIAsymphony SP," page 41).

When performing RCS-automated testing of samples prepared using the QIAsymphony SP, refer to *Rapid Capture System User Manual — Performing* digene *HC2 DNA Tests Using QIAsymphony SP Processed Samples* for instructions to complete testing.

Sample preparation of SurePath specimens and SurePath post-gradient cell pellets using the QIAsymphony DSP HPV Media Kit

Refer to *QIAsymphony DSP HPV Media Kit Instructions for Use (Handbook)* for instructions to prepare SurePath samples and SurePath post-gradient cell pellet samples using the QIAsymphony DSP HPV Media Kit.

Important: The sample extracts produced as a result of sample preparation of SurePath specimens using the QIAsymphony DSP HPV Media Kit may only be tested using the RCS. Manual performance of the test with sample extracts is not validated.

The result of sample preparation of SurePath specimens and SurePath post-gradient cell pellet samples using the QIAsymphony DSP HPV Media Kit is calibrators, quality controls and sample extracts in a hybridization microplate ready for RCS-automated testing at the hybridization step of the test.

When performing RCS-automated testing of samples prepared using the QIAsymphony SP, refer to *Rapid Capture System User Manual — Performing* digene *HC2 DNA Tests Using QIAsymphony SP Processed Samples* for instructions to complete testing.

Sample preparation of PreservCyt specimens using the QIAsymphony DSP AXpH DNA Kit

Refer to *QIAsymphony DSP AXpH DNA Kit Handbook* for instructions on sample preparation of PreservCyt specimens.

The result of sample preparation of PreservCyt specimens using the QIAsymphony DSP AXpH DNA Kit is DNA eluates in a hybridization microplate with the first column empty. The DNA

eluates are ready for the denaturation step of the test. The calibrators, quality controls and DNA eluates are denatured at the same time on the hybridization microplate (see "Denaturation and hybridization of samples prepared using the QIAsymphony SP," page 41).

Manual sample preparation of PreservCyt specimens

Refer to the *digene* HC2 Sample Conversion Kit instructions for use for manual sample preparation of PreservCyt specimens.

Manual sample preparation of PreservCyt specimens using the *digene* HC2 Sample Conversion Kit results in samples ready for the hybridization step of the test. Prepare the calibrators and quality controls separately (see "Denaturation of calibrators, quality controls and STM specimens," page 43).

Manual sample preparation of SurePath post-gradient cell pellets

Manual sample preparation of SurePath post-gradient cell pellets results in samples ready for the hybridization step of the test. Prepare the calibrators and quality controls separately (see "Denaturation of calibrators, quality controls and STM specimens," page 43).

Important: If the post-gradient cell pellet of the SurePath specimen appears to contain less than 1 ml, the post-gradient cell pellet is not suitable for testing with the *digene* HC2 High-Risk HPV DNA Test as the SurePath Preservative Fluid was not added post-cytology.

1. Equilibrate the SurePath post-gradient cell pellets to room temperature and confirm the observed liquid volume equals approximately 2.8 ml.
2. Centrifuge the SurePath post-gradient cell pellets in a swinging bucket rotor at 800 ± 15 x *g* for 10 ± 1 minutes.
3. Remove the tubes from the centrifuge.
4. Immediately following centrifugation, carefully decant the supernatant and gently blot each tube approximately 3 times on Kimtowels wipers or equivalent low-lint paper towels to remove excess liquid. Observe the pellet in each tube.

   Important: Do not let the cell pellets slide down the tube during blotting.
5. Place the tubes into the rack.
6. Add 200 µl of STM to each pellet using a repeating or single-channel pipet.

7. Resuspend each pellet by vortexing each tube individually for 15 seconds at high speed.

   If the pellet is difficult to resuspend, vortex for an additional 5–30 seconds or until the pellet floats loose from the bottom of the tube and appears to dissolve.

   Note: Tubes may be mixed without capping.

8. Pipet 100 µl of DNR into each SurePath specimen using a repeating or single-channel pipet.

   Important: Make sure not to touch the sides of the tube or cross-contamination of specimens could occur.

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

   Note: Tubes can be mixed without capping.

10. Label *digene* HC2 Sample Conversion Tubes or 15 ml conical tubes with applicable sample identification and type (example "SP" for a SurePath specimen) and place tubes in a tube rack.

    Important: For RCS-automated testing, *digene* HC2 Sample Conversion Tubes must be used.

11. Transfer the entire volume to the applicable 15 ml conical tube using a disposable, 7 ml standard-tipped transfer pipet or equivalent.

12. Cap the conical tubes and place in a tube rack.

13. Incubate the tubes in a 65 ± 2°C waterbath for 90 ± 5 minutes.

    Note: This incubation time is longer than required for other approved specimen types.

    If testing will be completed on the same day, denature the calibrators and quality controls (see "Denaturation of calibrators, quality controls and STM specimens," page 43).

14. Remove the tube rack from the waterbath after the incubation.

    If using a specimen rack, do not allow to cool before removing the rack lid. Immediately continue with testing or remove the rack lid and the DuraSeal tube sealer film.

    Note: If the specimen rack cools, the tubes may stick to the rack lid and subsequently spill.

    The prepared SurePath samples may be:

    - Tested immediately (proceed to "Hybridization of prepared STM samples and manually prepared PreservCyt and SurePath post-gradient cell pellet samples," page 46)
    - Stored (see "Optional stop point of prepared STM samples and manually prepared PreservCyt and SurePath post-gradient cell pellet samples," page 45)

Denaturation and hybridization of samples prepared using the QIAsymphony SP

The result of preparing samples on the QIAsymphony SP is a hybridization microplate containing, at a minimum, the prepared samples.

If PreservCyt samples were prepared using the QIAsymphony SP, the first column of the hybridization microplate is empty. The contents of the microplate are ready for the denaturation step of the test. The calibrators and quality controls are added to the hybridization microplate either manually or during RCS-automated testing, and then the denaturation step is performed.

If SurePath samples or SurePath post-gradient cell pellet samples were prepared using the QIAsymphony SP, the plate contains the prepared samples with the denatured calibrators and quality controls pipetted into the first column of the hybridization microplate. The contents of the microplate are ready for RCS-automated testing at the hybridization step of the test.

Important: The sample extracts produced as a result of sample preparation using the QIAsymphony DSP HPV Media Kit can only be tested using the RCS. Manual performance of the test with sample extracts is not validated.

When performing RCS-automated testing of samples prepared using the QIAsymphony SP, refer to *Rapid Capture System User Manual — Performing* digene *HC2 DNA Tests Using QIAsymphony SP Processed Samples* for instructions to complete testing.

Denaturation of calibrators, quality controls and DNA eluates for manual testing

- This procedure is for manual testing of PreservCyt specimen samples prepared using the QIAsymphony DSP AXpH DNA Kit. If performing RCS-automated testing, refer to *Rapid Capture System User Manual — Performing* digene *HC2 DNA Tests Using QIAsymphony SP Processed Samples* for instructions to complete testing.
- Denaturation of calibrators and quality controls is performed using DNR, while the denaturation of the DNA eluates is performed using DNR2.

1. Vortex each calibrator and quality control for 10 seconds at the maximum setting.
2. Invert each tube to retrieve material from the cap of the tube.

3. Remove the caps from the calibrator and quality control tubes and discard.
4. Using a single-channel pipet, add 50 µl of the applicable calibrator or quality control to the bottom of the empty hybridization microplate well according to the created plate layout.

   If the calibrator and quality controls will be used for additional testing, cap the tubes with new specimen collection tube screw caps, label with a new expiration date and store at 2–8°C.

   Note: The opened, undenatured calibrators and quality controls are stable for 3 months at 2–8°C.
5. Thoroughly vortex the prepared DNR and DNR2, and aliquot each into an appropriately labeled disposable reagent reservoir.

   Important: Make sure to add the correct reagent to the correct column of the eluate microplate.
6. Using an 8-channel pipet, add 25 µl of DNR to the first column of the hybridization microplate containing the calibrators and quality controls.
7. Using an 8-channel pipet, add 25 µl of DNR2 to each hybridization microplate well containing a DNA eluate.
8. Cover the hybridization microplate with a microplate lid and shake for 30 seconds on the Rotary Shaker I set at 1100 ± 100 rpm.
9. Place the microplate in the Microplate Heater I equilibrated to 65 ± 2°C, making sure not to cause splashing. Incubate the hybridization microplate for 45 ± 5 minutes.

   Prepare the Probe Mix during this incubation (see "Probe Mix," page 33).
10. Remove the hybridization microplate from the Microplate Heater I.

    The denatured calibrators, quality controls and DNA eluates may be:

    - Stored (see "Optional stop point of DNA eluates," page 42)
    - Tested immediately (proceed to "Hybridization of DNA eluates," page 43)

Optional stop point of DNA eluates

Denatured DNA eluates, including calibrators and quality controls, covered with a microplate lid may be stored at 2–8°C for 2 weeks.

Hybridization of DNA eluates

1. If the hybridization microplate containing the denatured calibrators, quality controls and DNA eluates has been stored, remove the microplate lid and allow the hybridization microplate to equilibrate to 20–25°C.
2. Thoroughly vortex the Probe Mix and aliquot into a disposable reagent reservoir.
3. Carefully pipet 25 µl of the Probe Mix into each hybridization microplate well using an 8-channel pipet and new tips for each Probe Mix addition.

   Avoid back-splashing and touching the sides of the hybridization microplate wells.
4. Cover the hybridization microplate with a microplate lid and shake for 3 ± 2 minutes on the Rotary Shaker I set at 1100 ± 100 rpm.

   After shaking, the calibrators, quality controls and DNA eluates should turn yellow.

   Samples that remain purple may not have received the proper amount of Probe Mix. Add an additional 25 µl of Probe Mix to the samples that remain purple and shake again. If a sample remains purple after following this procedure, retest the specimen.
5. Place the microplate in the Microplate Heater I equilibrated to 65 ± 2°C, making sure not to cause splashing. Incubate the hybridization microplate for 60 ± 5 minutes.
6. Proceed to "Hybrid capture," page 49, to continue testing.

Denaturation and hybridization of STM specimens and manually prepared PreservCyt and SurePath post-gradient cell pellet samples

- When testing manually prepared PreservCyt and SurePath post-gradient cell pellet samples, the denaturation step is not required for the samples. However, the calibrators and quality controls required with the test are denatured according to the instructions below.
- Some STM specimens may contain blood or other biological material that may mask the color changes upon addition of DNR. Specimens that exhibit a dark color prior to the addition of DNR 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 test. Proper mixing can be verified by observing the color change of the calibrators and quality controls.

Denaturation of calibrators, quality controls and STM specimens

- Do not remove the specimen collection device from the specimen tube at any time.
- To avoid false-positive results, it is critical that all specimen material come into contact with the DNR. Mixing after the DNR addition is a critical step.
- STM specimens denatured using the MST Vortexer 2 method must use the "Hybridization using a microplate and the Microplate Heater I" method on page 46. The "Hybridization

using microtubes and waterbath" method (page 48) has not been validated with STM specimens denatured using the MST Vortexer 2.

1. Remove and discard the caps from the tubes.

   Important: Consider caps removed from STM specimen tubes as potentially infectious (see "Warnings and Precautions," page 20, for additional information).

2. Pipet the specified volume (see Table 9, below) of DNR into the tubes using a repeating or adjustable pipet.

   Make sure not to touch the sides of the tubes or cross-contamination of specimens could occur.

   Important: The 1-plate and 4-plate kits have different volumes for the High-Risk HPV Calibrator. Make sure to add the correct volume of DNR.

   Note: The volume of DNR added is equivalent to half the liquid volume in the tube.

Table 9. Addition of DNR

Calibrator, quality control or STM specimen	Volume of DNR required
Negative Calibrator, 2 ml	1000 µl
High-Risk HPV Calibrator, 1 ml	500 µl
High-Risk HPV Calibrator, 2 ml	1000 µl
Low-Risk HPV or High-Risk HPV Quality Control, 1 ml	500 µl
STM specimen, 1 ml	500 µl

3. Mix the tubes using either the MST Vortexer 2 method or the manual, individual tube vortexing method.

   MST Vortexer 2 method

   a. Cover the tubes with DuraSeal tube sealer film by pulling the film over the tubes in the specimen rack.

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

   c. Move the red-handled lever to the UP position so that it is horizontal.

   d. Place the specimen rack securely within the guides on the MST Vortexer 2 and with the largest notched corner of the rack located in the right-front corner. Secure the specimen rack by moving the red-handled lever to the "down" position so that it is vertical.

   e. Make sure that the speed setting is at 100 (maximum speed), and power ON the MST Vortexer 2.

   f. Vortex the tubes for 10 seconds.

   g. Power OFF the MST Vortexer 2.

   h. Remove the specimen rack from the MST Vortexer 2 by moving the red-handled lever to the "up" position.

Manual, individual tube vortexing method

a. Recap the tubes with new specimen collection tube screw caps.

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

   Important: During mixing, a visible vortex of liquid must be observed that washes the entire inner surface of the tube.

c. Invert each tube one time to wash the inside of the tube, cap and rim.

d. Return the tube to the rack.

The liquid in the tube should turn purple.

4. Incubate the tubes in a rack in a 65 ± 2°C waterbath for 45 ± 5 minutes.

   For manual testing, prepare the Probe Mix during this incubation (see "Probe Mix," page 33).

5. Remove the tubes from the waterbath after the incubation.

   If using a specimen rack, do not allow to cool before removing the rack lid. Immediately continue with testing or remove the rack lid and the DuraSeal tube sealer film.

   Note: If the specimen rack cools, tubes may stick to the rack lid and subsequently spill.

   The denatured calibrators, quality controls, and STM specimens may be:

   - Stored (see "Optional stop point of prepared STM samples and manually prepared PreservCyt and SurePath post-gradient cell pellet samples," page 45)
   - Tested immediately (proceed to "Hybridization of prepared STM samples and manually prepared PreservCyt and SurePath post-gradient cell pellet samples," page 46)

Optional stop point of prepared STM samples and manually prepared PreservCyt and SurePath post-gradient cell pellet samples

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

All prepared samples, including calibrators and quality controls, may be 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.

For overnight storage at 2–8°C in the specimen rack, cover the samples with DuraSeal tube sealer film and replace the rack lid.

For storage at –20°C in the specimen rack, remove the rack lid and the DuraSeal tube sealer film and place an applicable cap on the tubes.

Hybridization of prepared STM samples and manually prepared PreservCyt and SurePath post-gradient cell pellet samples

When performing RCS-automated testing of STM samples or manually prepared PreservCyt and SurePath post-gradient cell pellet samples, refer to *Rapid Capture System User Manual* for instructions to complete testing.

If the denatured calibrators, quality controls or specimens have been stored, allow them to equilibrate to 20–25°C and, if stored in a specimen rack, remove and discard the caps from the tubes.

- Two hybridization methods are available for STM samples and manually prepared PreservCyt and SurePath post-gradient cell pellet samples: "Hybridization using microplate and Microplate Heater I" and "Hybridization using microtubes and waterbath."
- STM specimens denatured using the MST Vortexer 2 method must use "Hybridization using a microplate and the Microplate Heater I." on page 46. "Hybridization using microtubes and waterbath" (page 48) has not been validated with STM specimens denatured using the MST Vortexer 2.
- Probe Mix is viscous. Make sure that the Probe Mix is thoroughly mixed and that the required amount is completely dispensed into each hybridization microplate well or hybridization microtube.
- When transferring the sample to the hybridization microplate or hybridization microtube, avoid touching the sides of the hybridization microplate wells or hybridization microtubes as false-positive results can occur if samples are not carefully transferred. Limit the formation of air bubbles. Use a clean, extra long pipet tip for each transfer to avoid cross-contamination.

Hybridization using a microplate and the Microplate Heater I

1. Obtain and label a hybridization microplate.
2. Vortex using one of the following methods:

   Calibrators, quality controls or STM samples with the MST Vortexer 2

   a. As applicable, cover the tubes with DuraSeal tube sealer film and secure the rack lid on the specimen rack.

   b. Vortex the specimen rack for a minimum of 5 seconds on the maximum speed setting.

   c. Immediately place the specimen rack on the bench top and release the latches. Lift the rack lid approximately 1 cm, and move it gently left and right to release any tubes that may have adhered to the DuraSeal tube sealer film. Remove the rack lid by lifting it straight up until it clears the specimen rack.

   d. Carefully peel the DuraSeal tube sealer film from the rack lid and discard.

PreservCyt or SurePath post-gradient cell pellet samples with MST Vortexer 2

a. As applicable, cover the tubes with DuraSeal tube sealer film and secure the rack lid on the specimen rack.

b. Vortex the Conversion Rack for a minimum of 10 seconds on the maximum speed setting.

c. Immediately place the specimen rack on the bench top and release the latches. Lift the rack lid approximately 1 cm and move gently left and right to release any tubes that may have adhered to the DuraSeal tube sealer film. Remove the rack lid by lifting it straight up until it clears the specimen rack.

d. Carefully peel the DuraSeal tube sealer film from the rack lid and discard.

Any sample type with vortexer

a. Vortex each tube individually for at least 5 seconds.

3. Using the EXPAND-4 pipet or a single-channel pipet with an extra-long pipet tip, transfer 75 µl of each calibrator, quality control or sample to the bottom of an empty hybridization microplate well according to the created plate layout.

   If the samples will be stored, cap the denatured calibrators, quality controls and STM samples with new specimen collection tube screw caps, and place the original cap for each sample on the PreservCyt and SurePath post-gradient cell pellet samples.

   Note: Store the samples according to the limits detailed in "Optional stop point of prepared STM samples and manually prepared PreservCyt and SurePath post-gradient cell pellet samples", page 45.

4. After transferring the last sample, cover the hybridization microplate with a microplate lid and incubate for 10 minutes at 20–25°C.

5. Thoroughly vortex the Probe Mix and aliquot into a disposable reagent reservoir.

6. Carefully pipet 25 µl of the Probe Mix into each hybridization microplate well using an 8-channel pipet and new tips for each Probe Mix addition.

   Avoid back-splashing and touching the sides of the hybridization microplate wells.

7. Cover the hybridization microplate with a microplate lid and shake for 3 ± 2 minutes on the Rotary Shaker I set at 1100 ± 100 rpm.

   After shaking, the calibrators, quality controls, STM samples and SurePath post-gradient cell pellet samples should turn yellow and the PreservCyt samples should turn pink.

   Samples that remain purple may not have received the proper amount of Probe Mix. Add an additional 25 µl of Probe Mix to the samples that remain purple and shake again. If a sample remains purple after following this procedure, retest the specimen.

8. Place the microplate in the Microplate Heater I equilibrated to 65 ± 2°C, making sure not to cause splashing. Incubate the hybridization microplate for 60 ± 5 minutes.
9. Proceed to "Hybrid capture," page 49, to continue testing.

Hybridization using microtubes and waterbath

1. Label and place the required number of clean hybridization microtubes into the microtube rack.
2. Vortex each calibrator, quality control and sample tube individually for at least 5 seconds prior to removing sample.
3. Using a single-channel pipet with an extra-long pipet tip, transfer 75 µl of each calibrator, quality control or sample to the bottom of the applicable hybridization microtube according to the created plate layout.

   If the samples will be stored, cap the denatured calibrators, quality controls and STM samples with new specimen collection tube screw caps, and place the original cap for each sample on the PreservCyt and SurePath post-gradient cell pellet samples.

   Note: Store the samples according to the limits detailed in "Optional stop point of prepared STM samples and manually prepared PreservCyt and SurePath post-gradient cell pellet samples," page 45.
4. After transferring the last sample, incubate the hybridization microtubes for 10 minutes at 20–25°C.
5. Thoroughly vortex the Probe Mix and aliquot into a disposable reagent reservoir.
6. Carefully pipet 25 µl of the Probe Mix into each hybridization microtube using an 8-channel pipet and new tips for each row.

   Avoid back-splashing and touching the sides of the hybridization microtubes.

   Inspect the rack from underneath to make sure that all hybridization microtubes have received the correct amount of Probe Mix.
7. Cover the hybridization microtubes with a plate sealer. Place the rack cover on top of the rack. Shake the microtube rack for 3 ± 2 minutes on the Rotary Shaker I set at 1100 ± 100 rpm.

   After shaking, the calibrators, quality controls, STM samples and SurePath post-gradient cell pellet samples should turn yellow and the PreservCyt samples should turn pink.

   Samples that remain purple may not have received the proper amount of Probe Mix. Add an additional 25 µl of Probe Mix to the samples that remain purple and shake again. If a sample remains purple after following this procedure, retest the specimen.

8. Incubate the microtube rack for 60 ± 5 minutes in a 65 ± 2°C waterbath.

   Make sure that the water level in the waterbath is sufficient to cover the entire hybridization microtube volume.

   Note: The microtube rack will float in the waterbath.

9. Proceed to "Hybrid capture" to continue testing.

Hybrid capture

1. Remove all but the required number of capture microplate wells from the plate frame.
2. Return the unused capture microplate wells to the original bag and reseal.
3. With a marker, sequentially number each column and label the capture microplate with an applicable identifier.

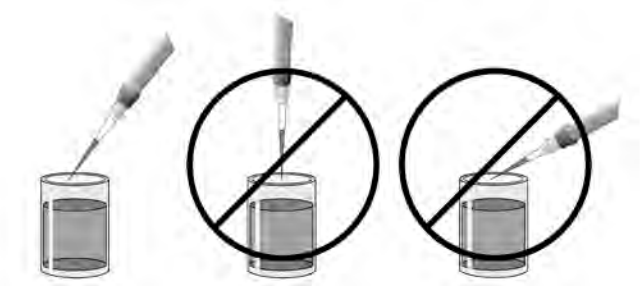
   The samples will be added to the capture microplate wells according to the created plate layout.

4. As applicable, carefully remove the hybridization microplate from the Microplate Heater I or the microtube rack from the waterbath.

   Immediately remove the microplate lid and place it on a clean surface or remove the rack lid and slowly pull the plate sealer up and across the microtube rack.

5. Using an 8-channel pipet, transfer the entire contents (approximately 100 µl) of the hybridization microplate wells or the hybridization microtubes to the bottom of the corresponding capture microplate wells.

   Use new pipet tips for each transfer and allow each pipet tip to drain to make sure there is complete sample transfer. If desired, the pipet may be steadied by resting the middle of the pipet tips on the top edge of the capture microplate wells (see Figure 2, below).



Correct | Do not pipet vertically to avoid back-splashing. | Do not allow tip to touch side of well.

Figure 2. Correct pipetting.

6. Cover the capture microplate with the microplate lid or a new plate sealer and shake for 60 ± 5 minutes on the Rotary Shaker I at 1100 ± 100 rpm at 20–25°C.

   Prepare the Wash Buffer during this incubation (see "Wash Buffer," page 35).

7. When the incubation is complete, remove the capture microplate from the Rotary Shaker I and carefully remove the microplate lid or plate sealer.
8. Remove the liquid from the capture microplate wells by discarding into a sink; fully invert the capture microplate over the sink and shake hard with a downward motion.

   Important: Do not reinvert the microplate.

   Make sure not to cause a back-splash by decanting too closely to the bottom of the sink.

9. Blot by tapping firmly 2–3 times on clean Kimtowels wipers or equivalent low-lint paper towels.

   Make sure that all liquid is removed from the capture microplate wells and the top of the capture microplate is dry.

10. Proceed to "Hybrid detection" to continue testing.

Hybrid detection

- Make reagent additions across the capture microplate in a left-to-right direction using an 8-channel pipet. Wipe tips on disposable reagent reservoir to remove excess reagent before delivery to the microplate.
- If an 8-channel pipet is not used, a repeating pipet may be substituted. Aliquot the DR1 into a polypropylene tube of sufficient size to hold the required volume.
- It is recommended that the reverse pipetting technique be utilized to improve consistency of reagent delivery. The procedure is described below.
- If desired, the pipet may be steadied by resting the middle of the pipet tips on the top edge of the capture microplate wells. Make sure not to touch the sides of the capture microplate wells as cross contamination of samples could occur (see Figure 2, page 49).

1. Mix the DR1 thoroughly, and carefully transfer the applicable volume (as applicable, see Table 1, page 30, or Table 4, page 31) into a clean, disposable reagent reservoir.
2. Carefully pipet 75 µl of DR1 into each capture microplate well using the reverse pipetting technique, as follows:
   a. Attach tips onto an 8-channel pipet; make sure all tips are firmly seated.
   b. Push the plunger of the pipet past the first stop to the second stop.
   c. Immerse tips into the reagent.
   d. Release plunger slowly and allow the reagent to fill the tips.

e. Dispense the reagent into the microplate wells by pressing the plunger to the first stop. Do not release the plunger until the pipet tips have been immersed into the reagent.

f. Refill tips and repeat until all microplate wells are filled.

Make sure that all capture microplate wells have been filled by observing the intensity of the pink color. All capture microplate wells should have a similar pink intensity.

3. Cover the capture microplate with a microplate lid, clean Parafilm or equivalent and incubate for 30–45 minutes at 20–25°C.
4. Proceed to "Washing" to continue testing.

Washing

Wash the capture microplate using one of the methods below.

Automated Plate Washer method

Always keep the Automated Plate Washer powered 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. Refer to *Automated Plate Washer User Manual* for additional instructions.

- Make sure that the wash reservoir is filled at least to the 1 liter mark with Wash Buffer. If not, prepare the Wash Buffer (see "Wash Buffer," page 35).
- Make sure that 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 before each wash and rinse after each wash.
- If only a partial strip of capture microplate wells is being used, place the empty microplate wells in the capture microplate to complete the column prior to washing.

1. Remove the microplate lid and place the capture microplate on the Automated Plate Washer platform.
2. Make sure that the Automated Plate Washer is powered ON and that the display reads **Digene Wash Ready** or **P1**.
3. Select the number of strips to be washed by pressing the **Rows** button and then **+** or **–** to adjust.
4. Press the **Rows** button to return to **Digene Wash Ready** or **P1**.

5. Press the **Start/Stop** button to begin.

   The Automated Plate Washer will perform 6 fill-and-aspirate cycles taking approximately 10 minutes. There will be a brief pause during the program; do not remove the microplate prematurely.

   When the Automated Plate Washer is finished washing, it will read **Digene Wash Ready** or **P1**.

6. Remove the capture microplate from the Automated Plate Washer platform when the program is finished.

   The capture microplate should appear white, and no residual pink liquid should remain in the capture microplate wells.

7. Proceed to "Signal amplification," page 53, to continue testing.

Manual washing method

1. Remove the DR1 from the capture microplate wells by placing clean Kimtowels wipers or equivalent low-lint paper towels on top of the capture microplate.
2. Make sure that the paper towels are in contact with the entire surface area of the capture microplate and carefully invert.
3. Allow the capture microplate to drain for 1–2 minutes.
4. Blot well on clean Kimtowels wipers or equivalent low-lint paper towels.

   Carefully discard the used paper towels to avoid alkaline phosphatase contamination.

5. Using the Wash Apparatus, manually wash the capture microplate 6 times.

   To wash properly, overflow each capture microplate well with Wash Buffer. This will remove the DR1 from the tops of the capture microplate wells. Washing begins at capture microplate well A1 and continues in a serpentine fashion to the right and downward. After all capture microplate wells have been filled, decant the liquid into the sink with a strong downward motion. The second wash is started at capture microplate 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 capture microplate well.

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

   The capture microplate should appear white, and no pink residual liquid should remain in the capture microplate wells.

8. Proceed to "Signal amplification," page 53, to continue testing.

Signal amplification

- Use a new pair of gloves for handling the DR2.
- Make reagent additions across the capture microplate in a left-to-right direction using an 8-channel pipet.
- If an 8-channel pipet is not used, a repeating pipet may be substituted. Aliquot the DR2 into a polypropylene tube of sufficient size to hold the required volume.
- Add the DR2 without interruption. The incubation time of all capture microplate wells must be as close as possible.
- Make sure to not touch the sides of the capture microplate wells or splash reagent onto the tips because cross-contamination of specimens could occur (see Figure 2, page 49).

1. Mix the DR2 thoroughly, and transfer the applicable volume (as applicable see Table 1, page 30, or Table 4, page 31) into a clean, disposable reagent reservoir.
2. Carefully pipet 75 µl of DR2 into each capture microplate well using the reverse pipetting technique previously described (see "Hybrid detection," page 50).

   Make sure that all capture microplate wells have been filled accurately by observing the intensity of the yellow color; all capture microplate wells should have a similar yellow intensity.
3. Cover the capture microplate with a microplate lid and incubate at 20–25°C for 15 minutes (and no later than 30 minutes of incubation).

   Important: Avoid direct sunlight.
4. Proceed to "Measuring the capture microplate and generating results" to continue testing.

Measuring the capture microplate and generating results

1. Measure the capture microplate using a DML instrument.

   Refer to the respective software user manual for details about measuring a capture microplate and generating test result reports. The *digene* assay analysis software will allow the entry of pertinent test information.
2. If a full capture microplate was not used, remove used capture microplate wells from the microplate frame, rinse the microplate frame thoroughly with distilled or deionized water, dry and reserve for the next test.
3. Discard all reagent aliquots and prepared reagents, unless otherwise specified.

   Dilute the remaining DNR in the bottle prior to disposal according to national and local laboratory procedures.

Interpretation of Results

The *digene* HC2 High-Risk HPV DNA Test assay CO of 1pg/ml is equivalent to 100,000 HPV copies/ml or 5,000 HPV copies per assay.

Results of STM specimen testing

STM specimens with a RLU/CO value ≥1.0 are considered "positive" for 1 or more of HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68.

STM specimens with a RLU/CO value <1.0 are considered "negative" or "no HPV DNA detected" for the 13 HPV types tested. High-risk HPV DNA sequences are either absent or the HPV DNA levels are below the detection limit of the test.

Results of SurePath specimen testing

SurePath specimens with a RLU/CO value ≥1.0 are considered "positive" for 1 or more of HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68.

SurePath specimens with a RLU/CO value <1.0 are considered "negative" or "no HPV DNA detected" for the 13 HPV types tested. HPV DNA sequences are either absent or the HPV DNA levels are below the detection limit of the test.

Results of PreservCyt specimen testing

PreservCyt specimens with a RLU/CO value ≥1.0 are considered "positive" for 1 or more of HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68.

PreservCyt specimens with a RLU/CO value <1.0 are considered "negative" or "no HPV DNA detected" for the 13 HPV types tested. HPV DNA sequences are either absent or the HPV DNA levels are below the detection limit of the test.

For PreservCyt specimens with a RLU/CO value ≥1.0 and <2.5, QIAGEN recommends retesting the specimen, as follows:

- If the first retest RLU/CO is ≥1.0, report the specimen as "positive." No further testing is required.
- If the first retest RLU/CO is <1.0, a second retest (third result) is required. The second result is the final result (<1.0 is negative, ≥1.0 is positive) and is reported.

RLU/CO value close to 1.0

If the RLU/CO 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.

Other HPV types

Because this assay only detects high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68, be aware that other low-risk HPV types may be present in the specimen. If testing specifically for the presence of sexually transmitted low-risk HPV, use the *digene* HC2 HPV DNA Test, which detects low-risk and high-risk HPV DNA types.

Assay Calibration Verification

Assay calibration verification is performed to make sure that the reagents, calibrators and quality controls are functioning properly, permitting accurate determination of the assay CO. The *digene* HC2 High-Risk HPV DNA Test requires assay calibration with each test; therefore, it is necessary to verify each assay. This verification procedure is not intended as a substitute for internal quality control testing. Acceptable ranges for assay calibration and quality controls have been established only for DML instruments approved by QIAGEN.

Assay calibration is automatically performed by the *digene* assay analysis software and printed on the data analysis report. However, users with *digene* Qualitative Software version 1.03 or earlier must manually perform assay calibration verification before patient results may be reported. Contact QIAGEN Technical Services for more information.

The test must meet the specified assay calibration criteria. If any of the following criteria are invalid, the software will not interpret the specimen results.

Negative calibrator

The NC must be tested in triplicate with each test. The NC mean must be ≥10 and ≤250 RLU and the coefficient of variation (CV) must be ≤25%. If the CV is >25%, the software removes the RLU value furthest from the mean as an outlier and recalculates the mean and the CV using the remaining values.

If the CV remains >25%, the assay calibration is invalid and the test must be repeated for all patient specimens. Accordingly, do not report patient specimen results.

Positive calibrator

The HRC must be tested in triplicate with each test. The CV of the HRC must be ≤15%. If the CV is >15%, the software removes the RLU value furthest from the mean as an outlier and recalculates the mean and the CV using the remaining values.

If the CV remains >15%, the assay calibration is invalid and the test must be repeated for all patient specimens. Accordingly, do not report patient specimen results.

Positive calibrator mean/negative calibrator mean

The software uses the HRC$\overline{X}$ and the NC$\overline{X}$ to calculate the HRC$\overline{X}$/NC$\overline{X}$. A valid HRC$\overline{X}$/NC$\overline{X}$ is defined as 2.0 ≤ HRC$\overline{X}$/NC$\overline{X}$ ≤ 15. If the HRC$\overline{X}$/NC$\overline{X}$ is <2.0 or >15, the assay calibration is invalid and the test must be repeated for all patient specimens. Accordingly, do not report patient specimen results.

Cutoff calculation

The *digene* assay analysis software calculates and reports the RLU/CO and positive/negative results for all specimens. The CO for determining positive specimens is the HRC$\overline{X}$. The *digene* assay analysis software uses the specimen RLU values to express results as specimen RLU/CO.

For RCS-automated testing, the RCS HPV assay protocol applies a calibration adjustment factor (CAF) of 0.8 to the valid HRC$\overline{X}$. This CAF is necessary so that the performance characteristics of RCS-automated testing remain equivalent to the manual testing. The CAF is only applied to RCS-automated test results; therefore, it is critical to select the correct assay protocol in order to generate accurate test results.

Quality controls

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

Consult the applicable *digene* assay analysis software user manual for instructions on entering the lot numbers and expiration dates of the quality controls. For an assay to be valid, the RLU/CO of each quality control must fall within the defined criteria as specified in Table 10, below. If the quality controls are not within these ranges, the assay is invalid and the test must be repeated. Accordingly, do not report patient results.

Table 10. Quality control assay validity criteria

Quality control	Minimum RLU/CO	Maximum RLU/CO	CV (%)
QC1-LR	0.001	0.999	≤25
QC2-HR	2	8	≤25

Limitations

- The *digene* HC2 High-Risk HPV DNA Test for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 is not recommended for evaluation of suspected sexual abuse.
- Prevalence of HPV infection in a population may affect performance. Positive predictive values decrease when testing populations with low prevalence or individuals with no risk of infection.
- A negative test result does not exclude the possibility of HPV infection because very low levels of infection or specimen collection error may cause a false-negative test result. Also, this test does not detect DNA of low-risk HPV types (6, 11, 42, 43 and 44).
- 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 cervical disease or cancer will develop.
- A small amount of cross-hybridization exists between the High-Risk HPV Probe and HPV types 6, 11, 40, 42, 53, 54, 55, 66, MM4, MM7, MM8 and MM9. Patients having specimens containing high levels of these HPV types may incorrectly be referred to colposcopy (15, 35).
- The *digene* HC2 High-Risk HPV DNA Test is designed to detect high-risk HPV types, including 39, 58, 59 and 68. Analytical studies conducted by QIAGEN, using cloned HPV plasmid DNA, demonstrate that the test detects these types at concentrations 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 High-Risk 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 (28), the performance characteristics of the *digene* HC2 High-Risk HPV DNA Test for the detection of HPV types 39, 58, 59 and 68 have not been statistically confirmed.
- If high concentrations of anti-fungal cream, contraceptive jelly or douche are present at the time an STM specimen is collected for 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 CO.
- If high concentrations of anti-fungal cream, vaginal lubricating jelly or blood are present at the time a PreservCyt cervical specimen is collected for sample preparation with the QIAsymphony DSP HPV Media Kit, 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 CO.
- If contraceptive jelly is present at the time a PreservCyt cervical specimen is collected for sample preparation with the QIAsymphony DSP AXpH DNA Kit, a false-negative test result may occur.
- If contraceptive jelly, anti-fungal cream or anti-inflammatory cream are present at the time a SurePath cervical specimen is collected for sample preparation with the QIAsymphony DSP HPV Media Kit, a false-negative test result may occur.

- Cross-reactivity between the High-Risk HPV 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.
- When performing RCS-automated testing, failure to visually observe the hybridization plate to make sure of proper specimen transfer and failure to correct for any inadequate specimen transfer may result in false-negative results.

Performance Characteristics

Clinical performance when screening patients with normal Pap smear results as an aid in the assessment of risk for patient management

The results of 8 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 2 cases, the Bethesda Grading System was utilized to interpret the Pap results. For cervical cancer screening equivalent terminology in the European Community, refer to the European Guidelines for Quality Assurance in Cervical Cancer Screening (36). 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 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. Two of the studies were conducted in the United States, 2 in Europe, 2 in Latin America, one in Africa and one in Asia.

The performance of the *digene* HC2 High-Risk HPV DNA Test observed from 6 cross-sectional studies is summarized (see Tables 11 and 12, below) for women aged 30 years and over and diagnosed with histologically confirmed high-grade cervical neoplasia, which is defined as cervical intraepithelial neoplasia (CIN) 3 or more severe.

Table 11. Performance estimates — sensitivity and specificity

		Sensitivity (%) (n/N) 95% Confidence Interval(CI)			Specificty (%) (n/N) 95% CI		
Population	**n**	**Pap alone**	**HPV alone**	**HPV + Pap**	**Pap alone**	**HPV alone**	**HPV + Pap**
Western Europe 1	7592	51.6 (14/27) 32.0–71.3	96.3 (26/27) 81.0–99.9	100.0 (27/27) 87.2–100.0	98.5 (7453/7565) 98.2– 98.8	96.2 (7275/7565) 95.7–96.6	95.1 (7193/7565) 94.6–95.6
Latin America 1	6115	58.4 (45/77) 46.68–69.6	94.8 (73/77) 87.2–98.6	97.4 (75/77) 90.9–99.7	98.7 (5962/6038) 98.4–99.0	93.9 (5669/6038) 93.3–94.5	93.4 (5637/6038) 92.7–94.0
Latin America 2*	6176	77.9 (53/68) 66.2–87.1	89.7 (61/68) 79.9–95.8	94.1 (64/68) 85.6–98.4	94.1 (5745/6108) 93.4–94.6	94.0 (5742/6108) 93.4–94.6	89.9 (5490/6108) 89.1–90.6
Africa	2925	84.1 (90/107) 75.8–90.5	89.7 (96/107) 82.4–94.8	92.5 (99/107) 85.8–96.7	86.4 (2436/2818) 85.1–87.7	80.0 (2253/2818) 78.4–81.4	76.4 (2152/2818) 74.8–77.9
Asia	1936	97.6 (41/42) 87.4–99.9	100.0 (42/42) 91.6–100.0	100.0 (42/42) 91.6–100.0	76.3 (1445/1894) 74.3–78.2	83.0 (1572/1894) 81.2–85.0	68.0 (1287/1894) 65.8–70.1
USA 1	1040	50.0 (1/2) 1.26–98.7	100.0 (2/2) 15.8–100.0	100.0 (2/2) 15.8–100.0	97.6 (1013/1038) 96.5–98.4	96.2 (999/1038) 94.9–97.3	95.5 (991/1038) 94.0–96.7

* *digene* HC2 High-Risk HPV DNA Test data where available, HCS data used otherwise; data combined.

Table 12. Performance estimates — positive and negative predictive value

Population	n	Prevalence CIN 3 (%) (n/N) 95% CI	Positive predictive value (%) (n/N) 95% CI			Negative predictive value (%) (n/N) 95% CI		
			Pap alone	HPV alone	HPV + Pap	Pap alone	HPV alone	HPV + Pap
Western Europe 1	7592	0.36 (27/7592) 0.23–0.52	11.1 (14/126) 6.2–17.9	8.23 (26/316) 5.5–11.8	6.77 (27/399) 4.5–9.7	99.83 (7453/7466) 99.7–99.9	99.99 (7275/7276) 99.9–100.0	100.0 (7193/7193) 99.9–100.0
Latin America 1	6115	1.26 (77/6115) 0.99–1.57	37.2 (45/121) 28.6–46.4	16.5 (73/442) 13.2–20.3	15.8 (75/476) 12.6–19.4	99.47 (5962/5994) 99.3–99.6	99.93 (5669/5673) 99.8–100.0	99.96 (5637/5639) 99.9–100.0
Latin America 2*	6176	1.10 (68/6176) 0.86–1.39	12.7 (53/416) 9.7–16.3	14.3 (61/427) 11.1–18.0	9.4 (64/682) 7.3–11.8	99.74 (5745/5760) 99.6–99.9	99.88 (5742/5749) 99.8–100.0	99.93 (5490/5494) 99.8–100.0
Africa	2925	3.66 (107/2925) 3.01–4.40	19.1 (90/472) 15.6–22.9	14.5 (96/661) 11.9–17.4	12.9 (99/765) 10.6–15.5	99.31 (2436/2453) 98.9–99.6	99.51 (2253/2264) 99.1–99.8	99.63 (2152/2160) 99.3–99.8
Asia	1936	2.17 (42/1936) 1.57–2.92	8.37 (41/490) 6.1–11.2	11.5 (42/364) 8.4–15.3	6.47 (42/649) 4.7–8.7	99.93 (1445/1446) 99.6–100.0	100.0 (1572/1572) 99.8–100.0	100.0 (1287/1287) 99.7–100.0
USA 1	1040	0.19 (2/1040) 0.02–0.69	3.85 (1/26) 0.1–19.6	4.88 (2/41) 0.6–16.5	4.08 (2/49) 0.5–14.0	99.90 (1013/1014) 99.5–100.0	100.0 (999/999) 99.6–100.0	100.0 (991/991) 99.6–100.0

* *digene* HC2 High-Risk HPV DNA Test 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 of HPV exceeds that of Pap alone in all cases, approaching 100%. This negative predictive value demonstrates the high probability of the absence of high-grade cervical disease or cancer in women with a normal cytology 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 history of HPV infection and/or abnormal Pap smear, age at first intercourse, number of sexual partners and concurrent sexually transmitted diseases (37, 38).

Although the prevalence of high-grade disease does not vary significantly among 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 (17, 24–29, 38–40). Positive predictive values decrease when testing populations with low prevalence or individuals with little risk of infection.

Longitudinal analysis was performed using the results of 2 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 (see Tables 13 and 14, below).

Table 13. Longitudinal analysis — 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*	9429	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	1690	3	0.084	0.849 (0.307–2.35)
		Consecutive normal Paps†	2026	4	0.099	1.000
	All	Pap normal, HPV negative	2180	3	0.066	0.491 (0.221–1.09)
		Consecutive normal Paps†	2650	7	0.136	1.000

* Three normal Paps over approximately 2 years.

† Two normal Paps over approximately 2 years.

Table 14. Longitudinal analysis — 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	1078	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	2561	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.

Clinical performance when 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 USA in 1996 under the direction of the Kaiser Foundation Research Institute and the Kaiser Permanente Medical Group. Cervical specimens for routine Pap smear and for the *digene* HC2 High-Risk HPV DNA Test were obtained from women attending several Kaiser clinic facilities. Initial Pap smears were evaluated according to the Bethesda Classification. For cervical cancer screening equivalent terminology in the European Community, refer to the European Guidelines for Quality Assurance in Cervical Cancer Screening (36). Women (15 years or older) with Pap smear results of atypical cells of undetermined significance (ASC-US) returned for colposcopy and biopsy. Colposcopy-directed histological specimens were examined by pathologists, and an initial diagnosis was made. Each histology specimen was also reviewed by an independent pathologist, and discrepancies between the initial review and the independent review were adjudicated by a third pathologist.

The initial specimen was tested with a prototype of the *digene* HC2 High-Risk HPV DNA Test that contained probes to 11 of the 13 HPV types (excluding HPV types 59 and 68). This difference would not be expected to result in a significantly different performance profile for the test.

High-risk HPV DNA test results and histological diagnoses were available from 885 women with ASC-US Pap smears. Testing on the majority of patients was performed with specimens collected in both STM and PreservCyt Solution. Due to the similarities between the performance characteristics of the *digene* HC2 High-Risk HPV DNA Test for STM and PreservCyt Solution, assay performance is only presented for PreservCyt Solution.

Among those presenting with an ASC-US referral Pap smear, the negative predictive value of the *digene* HC2 High-Risk HPV DNA Test for having HSIL or greater disease at colposcopy is 99% (see Table 15, below).

Table 15. Comparison of the *digene* HC2 High-Risk HPV DNA Test versus consensus histology; ASC-US referral Pap population; Kaiser study, PreservCyt specimens

		HSIL or greater at the time of colposcopy		
		+	–	Total
***digene* HC2 High-Risk HPV DNA Test result**	+	66	317	**383**
	–	5	497	**502**
	Total	71	814	**885**

Sensitivity [TP/(TP+FN)] = 93.0% (66/71)
95% CI = 84.3–97.7
Specificity [TN/(TN+FP)] = 61.1% (497/814)
95% CI = 57.7–64.4
Disease prevalence = 8.0% (71/885)
Assay positive predictive value = 17.2% (66/383)
Assay negative predictive value = 99.0% (497/502)

The 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 test results is determined (see Table 16, below).

Table 16. Theoretical positive and negative predictive value of high-risk HPV testing of 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

The variation between the various age groups contained in this study is determined (see Table 17, below).

Table 17. 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 (%) **(n/N)**	100 (35/35)	88.46 (23/26)	80.0 (8/10)
95% CI	90.0–100.0	69.9–97.6	44.4–97.5
Specificity (%) **(n/N)**	31.4 (79/252)	66.2 (132/207)	79.15 (281/355)
95 % CI	25.7–37.5	59.3–72.6	74.6–83.3
Negative predictive value (%) **(n/N)**	100.0 (79/79)	97.86 (137/140)	99.29 (281/283)
Positive predictive value (%) **(n/N)**	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 multicenter clinical study using the *digene* HC2 High-Risk HPV DNA Test was conducted using specimens collected from several large, high cervical disease and HPV prevalent hospital and medical center colposcopy clinics (3 sites) in the western and southern United States. 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 then placed into STM, or with a broom device that was then rinsed in PreservCyt Solution. Specimens were collected at the time of colposcopy. Specimens were tested with the *digene* HC2 High-Risk HPV DNA Test, and the results were compared to the disease status determined for each patient. Disease status was based on the results of histological evaluation. However, when histology was negative or in the absence of a histology result, disease status was determined by cytology at the time of colposcopy examination (see Table 18, below).

The *digene* HC2 High-Risk 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 *digene* HC2 High-Risk HPV DNA Test's performance characteristics for STM and PreservCyt Solution, assay performance is presented for only PreservCyt Solution. No difference was observed in high-risk HPV testing results from STM specimens and PreservCyt specimens.

Table 18. Patient disease status algorithm

Cytology result	Histology result	Disease status
Negative	Negative or not done*	Negative
LSIL	Negative	LSIL
HSIL	Negative	HSIL
Cancer	Negative	HSIL+
Negative	LSIL	LSIL
LSIL	Not done*	LSIL
LSIL	LSIL	LSIL
HSIL	LSIL	LSIL
Cancer	LSIL	LSIL
Negative	HSIL	HSIL
LSIL	HSIL	HSIL
HSIL	HSIL	HSIL
HSIL	Not done*	HSIL
Cancer	HSIL	HSIL
Negative	Cancer	HSIL+
LSIL	Cancer	HSIL+
HSIL	Cancer	HSIL+
Cancer	Not done*	HSIL+
Cancer	Cancer	HSIL+

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

The performance of the *digene* HC2 High-Risk HPV DNA Test was determined using 327 PreservCyt specimens, 96 of which were collected from women diagnosed with high-grade cervical disease (see Tables 19 and 20, below). The comparisons were done using all study patients with abnormal referral Pap smear results.

Table 19. Results of high-risk HPV testing

			Final disease staus HSIL		Final disease status LSIL		Final disease status negative		
		High-risk HPV result	+	–	+	–	+	–	Total
Referral Pap smear result	LSIL		44	4	78	33	28	37	**224**
	HSIL		45	3	29	14	5	7	**103**
	Total		89	7	107	47	33	44	**327**
	Total		96		154		77		**327**

The *digene* HC2 High-Risk HPV DNA Test 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 (see Table 20, below). The test also demonstrated a negative predictive value of nearly 95% in this population.

Table 20. Performance characteristics of high-risk HPV DNA testing among patients having a referral Pap smear of LSIL or higher and a final disease status of HSIL

		Final disease status		
		HSIL	LSIL or negative	Total
digene HC2 High-Risk HPV DNA Test result	+	89	140	**229**
	–	7	91	**98**
	Total	96	231	**327**

Sensitivity [TP/(TP+FN)] = 92.7% (89/96)
95% CI = 85.6–97.0
Specificity [TN/(TN+FP)] = 39.4% (91/231)
95% CI = 33.1–46.0
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 High-Risk 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 *digene* HC2 High-Risk HPV DNA Test results and whose corresponding disease status was less than low-grade neoplasia, nearly 75% were positive.

The theoretical positive and negative predictive values of the *digene* HC2 High-Risk HPV DNA Test for initial LSIL or HSIL Pap smear results found to be HSIL or more severe disease on colposcopy were determined (see Table 21, below).

Table 21. Theoretical positive and negative predictive value of the *digene* HC2 High-Risk HPV DNA Test of initial LSIL or HSIL Pap smear results

	Initial LSIL or HSIL Pap smear result	
Theoretical prevalence for HSIL	Assay positive predictive value	Assay negative 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

Vaginal or self-collection performance

In the literature cited for *digene* HC2 High-Risk HPV DNA Test performance from self-collected vaginal specimens, over 141,000 women were enrolled between the ages of 16–54. The study cohorts included women from China (41, 42), Mexico (43, 44) and the United Kingdom (45). Study designs varied slightly, but in general women with a positive test result were offered further examination by colposcopy, and results were reported in terms of sensitivity and specificity versus the comparative method.

In two studies where data were available to compare self-collected versus physician-collected specimens, the results indicate high sensitivity for CIN2+ for both methods (42, 45), 81–85% for self-collected specimens vs. 96–100% for physician-collected specimens. Specificity results were similar for CIN2+ for both methods (42, 45), 81–82% for self-collected vs. 83–85% for physician-collected specimens. In other studies with only self-collected performance data available, performance of the *digene* HC2 High-Risk HPV DNA Test sensitivity for CIN2+ was 3.4 times greater than cytology (43) and 98% sensitivity before verification bias adjustment (44).

Analytical sensitivity

A non-clinical panel of cloned HPV plasmid DNA was tested to determine if each of the 13 HPV types are detectable by the *digene* HC2 High-Risk HPV DNA Test and to determine the analytical sensitivity of the assay for each of the HPV types. Each HPV target concentration (100 pg/ml, 10 pg/ml, 2.5 pg/ml, 1.0 pg/ml, 0.5 pg/ml and 0.2 pg/ml) of each of the 13 HPV DNA types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) was run in triplicate. The mean RLU for each concentration of each HPV type was calculated and compared to the Positive Calibrator.

The detectable limit of each HPV type in STM was determined (see Table 22, below). The detectable limits varied from 0.62 pg/ml to 1.39 pg/ml, depending on the HPV type tested. The mean detectable limit of all 13 HPV DNA types was 1.08 pg/ml with a standard deviation of 0.05 pg/ml.

Table 22. Summary of the detectable limits of sensitivity for each HPV DNA type in STM

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

Equivalence between specimen types

Equivalence between STM and PreservCyt specimens

Equivalence between STM and PreservCyt specimens was examined for equal recovery of HPV 18 DNA. Approximately 106 positive HeLa cells containing integrated HPV 18 genomes were spiked into STM and into a PreservCyt negative cell pool. Each specimen type was processed according to its respective sample preparation and denaturation procedures as described in the applicable instructions for use and tested with the *digene* HC2 High-Risk HPV DNA Test. The results demonstrated that recovery of HPV 18 DNA from human carcinoma cells is equivalent for the two media and that PreservCyt Solution sample preparation does not affect the analytical sensitivity of the *digene* HC2 High-Risk HPV DNA Test.

Equivalence between manual sample preparation of PreservCyt specimens and sample preparation of PreservCyt specimens using the QIAsymphony DSP HPV Media Kit

Studies were performed using PreservCyt specimens collected from a subpopulation of women with normal cytology (n=1276) and a subpopulation of women with a cytology of ASC-US or greater than ASC-US (n=402). Manual sample preparation and sample preparation using the QIAsymphony DSP HPV Media Kit were performed for each specimen followed by RCS-automated testing with the *digene* HC2 High-Risk HPV DNA Test (see Table 23, below).

Table 23. PreservCyt specimen result agreement between manual sample preparation and sample preparation using the QIAsymphony DSP HPV Media Kit (n=1678)

Positive agreement (%) (n/N) 95% CI		Negative agreement (%) (n/N) 95% CI	
All positives	Strong positive region RLU/CO ≥ 2.5	All negatives	Strong negative region RLU/CO < 0.8
96.0	97.6	96.2	99.1
(409/426)	(372/381)	(1204/1252)	(1173/1184)
93.7–97.5	95.6–98.8	95.0–97.1	98.3–99.5

The relative assay sensitivity and specificity of PreservCyt specimens prepared using the QIAsymphony DSP HPV Media Kit correlates highly with the results obtained using the manual sample preparation method as evidenced by the lower limit of the 95% CI for both positive and negative agreement.

Equivalence between manual sample preparation of PreservCyt specimens and sample preparation of PreservCyt specimens using the QIAsymphony DSP AXpH DNA Kit

Studies were performed using PreservCyt specimens collected from a subpopulation of women aged 30 years and older with normal cytology (n=1901) and a subpopulation of women with ASC-US cytology (n=398). Manual sample preparation and sample preparation using the QIAsymphony DSP AXpH DNA Kit were performed for each specimen followed by testing with the *digene* HC2 High-Risk HPV DNA Test (see Table 24, below).

Table 24. PreservCyt specimen result agreement between manual sample preparation and sample preparation using the QIAsymphony DSP AXpH DNA Kit (n=2299)

Positive agreement (%) (n/N) 95% CI		Negative agreement (%) (n/N) 95% CI	
All positives	Strong positive region RLU/CO ≥ 2.5	All negatives	Strong negative region RLU/CO < 0.8
92.7	96.5	99.1	99.9
(281/303)	(245/254)	(1978/1996)	(1967/1969)
89.3–95.2	93.4–98.1	98.6–99.4	99.6–100.0

The relative assay sensitivity and specificity of PreservCyt specimens prepared using the QIAsymphony DSP AXpH DNA Kit correlates highly with the results obtained using the manual sample preparation method as evidenced by the lower limit of the 95% CI for both positive and negative agreement.

Equivalence between STM and manual sample preparation of SurePath post-gradient cell pellet samples

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 applicable *digene* HC2 High-Risk HPV DNA Test CO value for use with SurePath post-gradient cell pellet samples, enrolled approximately 400 patients. The clinical validation phase, enrolling approximately 1500 patients to validate the chosen CO value, began after an interim analysis of the feasibility phase demonstrated that a CO of 1.0 RLU/CO using SurePath post-gradient cell pellet samples produced acceptable agreement with STM specimen results.

In both evaluation phases, paired SurePath and STM cervical specimens were collected from each consenting female participant. The SurePath specimen was then sent to a cytology lab for slide preparation. After cytological preparation, the remaining SurePath post-gradient cell pellet samples and the corresponding STM specimen were tested with the *digene* HC2 High-Risk HPV DNA Test using a CO of 1.0 RLU/CO (see Table 25, below).

Table 25. SurePath post-gradient cell pellet sample results agreement with STM specimen results (all ages and cytological classification) (n=1490)

Positive agreement (%) (n/N) 95% CI		Negative agreement (%) (n/N) 95% CI	
All positives	Strong positive region RLU/CO ≥ 2.5	All negatives	Strong negative region RLU/CO < 0.8
93.5	96.4	95.3	96.0
(401/429)	(378/392)	(1011/1061)	(1002/1044)
90.7–95.6	94.1–98.0	93.8–96.5	94.6–97.1

The relative assay sensitivity and specificity of testing SurePath post-gradient cell pellet samples correlates highly with the results obtained testing STM specimens as evidenced by the lower limit of the 95% CI for both positive and negative agreement.

Equivalence between manual sample preparation of SurePath post-gradient cell pellet samples and sample preparation of SurePath specimens using the QIAsymphony DSP HPV Media Kit

Studies were performed using SurePath specimens collected from the following subpopulations:

- Women with normal cytology (n=1189)
- Women with a cytology of ASC-US or greater than ASC-US (n=199)

For each SurePath specimen, sample preparation of the SurePath specimen using the QIAsymphony DSP HPV Media Kit and manual sample preparation of the post-gradient cell pellet sample were performed. RCS-automated testing with the *digene* HC2 High-Risk HPV DNA Test (see Table 26, below) was performed for each of the prepared samples.

Table 26. Result agreement between manual sample preparation of SurePath samples and sample preparation of SurePath specimens using the QIAsymphony DSP HPV Media Kit (n=1388)

Positive agreement (%) (n/N) 95% CI		Negative agreement (%) (n/N) 95% CI	
All positives	Strong positive region RLU/CO ≥ 2.5	All negatives	Strong negative region RLU/CO < 0.8
91.7	97.5	99.0	99.7
(222/242)	(192/197)	(1134/1146)	(1124/1127)
87.6–94.6	94.2–98.9	98.2–99.4	99.2–99.9

The relative assay sensitivity and specificity of SurePath specimens prepared using the QIAsymphony DSP HPV Media Kit correlates highly with the results obtained using the manual sample preparation method as evidenced by the lower limit of the 95% CI for both positive and negative agreement.

Equivalence between manual sample preparation of SurePath post-gradient cell pellet samples and sample preparation of SurePath post-gradient cell pellet samples using the QIAsymphony DSP HPV Media Kit

Studies were performed using SurePath specimens collected from the following subpopulations:

- Women with normal cytology (n=1200)
- Women with a cytology of ASC-US or greater than ASC-US (n=183)

Manual sample preparation and sample preparation using the QIAsymphony DSP HPV Media Kit were performed for each SurePath post-gradient cell pellet sample followed by RCS-automated testing with the *digene* HC2 High-Risk HPV DNA Test (see Table 27, below).

Table 27. SurePath post-gradient cell pellet sample result agreement between manual sample preparation and sample preparation using the QIAsymphony DSP HPV Media Kit (n=1383)

Positive agreement (%) (n/N) 95% CI		Negative agreement (%) (n/N) 95% CI	
All positives	Strong positive region RLU/CO ≥ 2.5	All negatives	Strong negative region RLU/CO < 0.8
92.6	97.4	94.4	99.3
(188/203)	(147/151)	(1114/1180)	(1078/1086)
88.2–95.5	93.4–99.0	92.9–95.6	98.6–99.6

The relative assay sensitivity and specificity of SurePath post-gradient cell pellet samples prepared using the QIAsymphony DSP HPV Media Kit correlates highly with the results obtained using the manual sample preparation method as evidenced by the lower limit of the 95% CI for both positive and negative agreement.

Agreement between test methods

A multicenter study (n=2270) was conducted to evaluate the clinical test results with the RCS compared with the test results using the manual method. Testing was performed at 3 sites, external to QIAGEN, with patient specimens collected from 5 collection sites. The data set consisted of 1269 cervical specimens collected in PreservCyt Solution and 1001 specimens collected in STM.

Statistical agreements, between matched specimens tested with the RCS and with the manual test, were calculated for this patient population (see Tables 28 and 29, below).

Table 28. Summary of agreement between RCS-automated and manual testing — STM specimens (n=1001)

Cytological classification	HPV prevalence (%)	Positive agreement (%) (n/N) 95% CI All positive	Positive agreement (%) (n/N) 95% CI Strong positive region (RLU/CO ≥ 2.5)	Negative agreement (%) (n/N) 95% CI All negative	Negative agreement (%) (n/N) 95% CI Strong negative region (RLU/CO < 0.8)
WNL* <30 years	21	99.3 (139/140) 96.1–100.0	99.1 (112/113) 95.2–100.0	99.3 (538/542) 98.1–99.8	100.0 (531/531) 99.3–100.0
WNL ≥30 years	15	92.0 (23/25) 74.0–99.0	93.8 (15/16) 69.8–99.8	100.0 (143/143) 97.5–100.0	100.0 (142/142) 97.4–100.0
ASC-US	65	98.1 (51/52) 89.7–100.0	100.0 (47/47) 92.4–100.0	96.4 (27/28) 81.7–99.9	100.0 (26/26) 86.8–100.0
LSIL+	96	100.0 (65/65) 94.5–100.0	100.0 (62/62) 94.2–100.0	66.7 (2/3) 9.4–99.2	66.7 (2/3) 9.4–99.2
Other	33	100.0 (1/1) 2.5–100.0	100.0 (1/1) 2.5–100.0	100.0 (2/2) 15.8–100.0	100.0 (2/2) 15.8–100.0
All STM specimens	28	98.6 (279/283) 96.4–99.6	99.2 (237/239) 97.0–99.9	99.2 (712/718) 98.2–99.7	99.9 (703/704) 99.2–100.0

* WNL = within normal limits.

Table 29. Summary of agreement between RCS-automated and manual testing — PreservCyt specimens (n=1269)

Cytological classification	HPV prevalence (%)	Positive agreement (%) (n/N) 95% CI: All positive	Positive agreement (%) (n/N) 95% CI: Strong positive region (RLU/CO ≥ 2.5)	Negative agreement (%) (n/N) 95% CI: All negative	Negative agreement (%) (n/N) 95% CI: Strong negative region (RLU/CO < 0.8)
WNL* <30 years	20	96.2 (75/78) 89.2–99.2	100.0 (64/64) 94.4–100.0	98.4 (301/306) 96.2–99.5	99.0 (293/296) 97.1–99.8
WNL ≥30 years	8	88.7 (47/53) 77.0–95.7	92.1 (35/38) 78.6–98.3	99.1 (578/583) 98.0–99.7	99.5 (571/574) 98.5–99.9
ASC-US	36	100.0 (48/48) 92.6–100.0	100.0 (46/46) 92.3–100.0	96.6 (84/87) 90.3–99.3	96.5 (83/86) 90.1–99.3
LSIL+	77	100.0 (64/64) 94.4–100.0	100.0 (62/62) 94.2–100.0	89.5 (17/19) 66.9–98.7	88.9 (16/18) 65.3–98.6
Other	11	100.0 (3/3) 29.2–100.0	100.0 (3/3) 29.2–100.0	100.0 (24/24) 85.6–100.0	100.0 (24/24) 85.8–100.0
All PreservCyt specimens†	20	96.4 (238/247) 93.2–98.3	98.6 (211/214) 96.0–99.7	98.5 (1007/1022) 97.6–99.2	98.9 (990/1001) 98.0–99.4

* WNL = within normal limits.

† Cytology data unavailable from 4 patients.

A supplemental clinical study was performed using archived residual PreservCyt specimens collected from a subpopulation of women aged 30 years and older with normal cytology (see Table 30, below) with an HPV prevalence of 4.8%.

Table 30. Summary of agreement between RCS-automated and manual testing — WNL women aged 30 years and older (n=2077)

Positive agreement (%) (n/N) 95% CI		Negative agreement (% (n/N) 95% CI	
All positives	Strong positive region (RLU/CO ≥ 2.5)	All negatives	Strong negative region (RLU/CO < 0.8)
92.0	91.8	99.3	99.7
(92/100)	(78/85)	(1964/1977)	(1944/1949)
84.84–96.48	83.77–96.62	98.88–99.65	99.40–99.92

There were 7 discordant results between the manual and RCS-automated testing results in the strong-positive region. The initial manual testing results for these 7 specimens were outside the recommended PreservCyt specimen retest algorithm; however, because the study design required testing all specimens in triplicate, repeat results were available for discrepant resolution.

The repeat testing data for each of the 7 discordant specimens suggests that all of the discordant specimens are negative for HPV DNA (see Table 31, below). Based on the repeat negative results obtained for both replicates, each of the initially positive manual test results was likely false-positive.

Table 31. Discordant PreservCyt specimens for WNL women aged 30 years and older (n=7)

		Manual testing (RLU/CO)			RCS automated testing (RLU/CO)		
Sample	Site	Initial	Repeat 1	Repeat 2	Initial	Repeat 1	Repeat 2
1	A	2.51	0.08	0.08	0.12	0.17	0.14
2	A	20.18	0.08	0.09	0.19	0.24	0.20
3	A	3.88	0.12	0.11	0.17	0.22	0.22
4	A	9.37	0.09	0.09	0.15	0.21	0.20
5	A	6.01	0.17	0.13	0.25	0.30	0.30
6	B	2.97	0.71	0.99	1.59	0.89	0.90
7	C	11.01	0.16	0.14	0.19	0.15	0.21

Results from this clinical study indicate an overall agreement between RCS-automated and manual testing using either STM or PreservCyt specimens.

Reproducibility

Overall reproducibility of manual testing

A multicenter reproducibility study was performed to determine the between days, between sites and overall reproducibility of the *digene* HC2 High-Risk HPV DNA Test using a panel of HPV DNA targets and HPV-positive and HPV-negative clinical STM specimens.

Three external laboratories performed the testing with the same lot of *digene* HC2 High-Risk 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 specimen pools
- Negative Calibrator
- Positive High-Risk HPV Calibrator at concentrations of 0.5, 1, 2.5, 5 and 10 pg/ml.

All panel members were tested each day in triplicate using the *digene* HC2 High-Risk HPV DNA Test. The results indicate that the reproducibility of the *digene* HC2 High-Risk HPV DNA Test with clinical specimens is very good (see Table 32, below).

Table 32. Overall reproducibility — multicenter reproducibility (all runs at all sites)

Statistical measure	Result
Expected positives with an observed positive result (95% CI)	100.0% (99.0–100.0)
Expected positives with an observed negative result (95% CI)	99.0% (97.49–99.73)
Agreement (95% CI)	99.5% (98.70–99.86)
Kappa	0.990

Reproducibility with clinical STM specimens

Manual testing

A study was performed to access the reproducibility of manual testing of clinical STM specimens with the *digene* HC2 High-Risk HPV DNA Test. A 20-member panel consisting of clinical pools (10 positive and 10 negative) were prepared by combining previously tested STM specimens. Specimens were tested in replicates of 4 on each of 5 days for a total of 20 replicates per specimen. Testing was performed using a combined Probe Mix consisting of the High-Risk HPV

Probe and a low-risk HPV probe. The reproducibility of the test would not be expected to differ when using only the Probe Mix in the *digene* HC2 High-Risk HPV DNA Test. The mean RLU/CO and the 95% CI about the mean were calculated (see Table 33, below).

Table 33. Reproducibility of STM specimens — manual testing (descending order by mean RLU/CO)

Specimen ID	Mean RLU/CO	95% CI	Positive test result (%) (n/N)
10	3.18	3.02–3.35	100 (20/20)
20	1.43	1.36–1.50	100 (20/20)
11	1.25	1.20–1.28	100 (20/20)
12	1.21	1.15–1.27	100 (20/20)
15	1.20	1.14–1.25	100 (20/20)
13	1.07	1.01–1.11	80 (16/20)
16	1.06	1.01–1.09	75 (15/20)
17	1.04	1.00–1.06	80 (16/20)
14	0.98	0.92–1.02	45 (9/20)
18	0.92	0.87–0.96	20 (4/20)
19	0.72	0.68–0.75	0 (0/20)
7	0.40	0.33–0.46	0 (0/20)
4	0.38	0.35–0.39	0 (0/20)
9	0.37	0.32–0.41	0 (0/20)
1	0.35	0.32–0.36	0 (0/20)
2	0.35	0.31–0.37	0 (0/20)
8	0.32	0.29–0.34	0 (0/20)
3	0.30	0.27–0.31	0 (0/20)
6	0.27	0.24–0.30	0 (0/20)
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 CO, 100 of 100 replicates (100.0%) were positive. For the 5 specimens with a mean RLU/CO within 20% above or below the CO, 60 of 100 (60%; 95% CI = 49.7–69.6) of the replicates were positive and 40 of 100 (40%) were negative. For the 10 specimens with the mean RLU/CO at 20% or more below the CO, 200 of 200 replicates (100%) were negative.

The results indicate that specimens at 20% or more away from the CO can be expected to yield consistent results. Specimens close to the CO yielded approximately equal numbers of positive and negative results. These data demonstrate that manual testing of STM specimens with the *digene* HC2 High-Risk HPV DNA Test yield reproducible results.

RCS-automated testing

A study was performed to assess the within-run, day-to-day and inter-laboratory reproducibility of RCS-automated testing of STM specimens with the *digene* HC2 High-Risk HPV DNA Test. A 16-member panel of pooled clinical specimens (see Table 34, below) was tested using a single lot of reagents, twice daily on 3 different days. Each panel member was tested in quadruplicate.

Table 34. Reproducibility of STM specimens — RCS-automated testing panel composition

Panel member	Approximate RLU/CO	Expected test result
1N	<0.4	Negative
2N	0.4–0.8	Negative
3P	0.8–1.2	High-negative/low-positive
4P	0.8–1.2	High-negative/low-positive
5P	0.8–1.2	High-negative/low-positive
6P	1.2–2.0	Low-positive
7P	1.2–2.0	Low-positive
8P	1.2–2.0	Low-positive
9P	2.0–5.0	Low-positive
10P	5.0–10.0	Mid-positive
11N	<0.4	Negative
12N	<0.4	Negative
13N	<0.4	Negative
14XR	Low-risk HPV DNA positive clinical material in STM clinical negative pool	High-negative/low-positive
15XR	Low-risk HPV DNA plasmid in STM clinical negative pool	High-negative/low-positive
16XR	Plasmid vector DNA control in STM clinical negative pool	High-negative/low-positive

Two panel members (14XR and 15XR) were included to evaluate the potential for cross-hybridization of the *digene* HC2 High-Risk HPV DNA Test's Probe Mix with specimens containing only low-risk HPV DNA types 6, 11, 42, 43 and 44. Panel member 16XR was composed of pGEM® DNA at a concentration of 1.49 ng/ml and served as a vector control for panel member 15XR. The results of this testing indicated no false-positive test results due to the presence of low-risk HPV DNA types in clinical specimens. These results are consistent with manual testing.

The reproducibility was calculated according to the method described by NCCLS E5-A (46) (see Table 35, below). This method requires the computation of variance components for each of

the sources of variability: laboratory, day, run, and error (defined as inter-assay and between-assay variation).

Table 35. Reproducibility of STM specimens — RCS-automated testing; quantitative reproducibility

			Standard deviation					
Panel member	n	Mean RLU/CO	Within run	Between run	Between day	Between lab	Total	Total CV (%)
1N	72	0.13	0.02	0.01	0.01	0.01	0.02	15.10
2N	72	0.36	0.03	0.01	0.03	0*	0.04	11.69
3P	72	0.96	0.06	0.06	0.04	0*	0.09	9.55
4P	72	1.03	0.06	0.18	0.06	0*	0.19	18.81
5P	72	1.41	0.11	0.14	0.15	0.06	0.24	17.00
6P	72	1.73	0.10	0.27	0*	0.11	0.31	18.10
7P	72	1.74	0.12	0.21	0*	0*	0.24	13.78
8P†	70	1.95	N/A‡	N/A‡	N/A‡	N/A‡	0.47	23.80
9P	72	5.21	0.34	0.44	0.21	0*	0.59	11.36
10P	72	7.67	0.46	0.63	0.71	0*	1.05	13.70
11N	72	0.13	0.01	0.01	0.01	0*	0.02	16.89
12N	72	0.17	0.03	0.06	0.03	0*	0.07	39.14
13N	72	0.15	0.02	0.02	0*	0.01	0.03	17.01

* Negative variance components are set equal to zero.

† Two invalid replicates for panel member 8P precluded variance component analysis due to unequal size groups under comparison.

‡ N/A: variance analysis not possible due to fewer replicates than other panel members.

Reproducibility of clinical PreservCyt specimens

Manual testing

The reproducibility of manual testing of PreservCyt specimens with the *digene* HC2 High-Risk HPV DNA Test was determined in a study using 24 mock specimens at various 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 sample from each specimen was prepared according to the *digene* HC2 Sample Conversion Kit instructions for use and tested. The mean and the 95% CI were calculated (see Table 36, below).

Table 36. Reproducibility of PreservCyt specimens — manual testing with manual sample preparation; qualitative reproducibility (descending order by mean RLU/CO)

Specimen ID	Mean RLU/CO	95% CI	Positive test result (%) (n/N)
21	3.51	3.19–3.83	100 (20/20)
12	1.58	1.48–1.69	100 (20/20)
13	1.42	1.32–1.52	100 (20/20)
17	1.38	1.23–1.53	90 (18/20)
18	1.36	1.23–1.48	95 (19/20)
15	1.32	1.16–1.49	85 (17/20)
23	1.17	1.06–1.27	75 (15/20)
16	1.14	1.07–1.20	75 (15/20)
20	1.10	0.96–1.21	85 (17/20)
19	1.06	0.95–1.17	45 (9/19)
22	1.05	0.99–1.10	70 (14/20)
11	1.04	0.96–1.11	65 (13/20)
14	0.94	0.86–1.01	25 (5/20)
24	0.77	0.73–0.81	0 (0/20)
3	0.28	0.25–0.30	0 (0/20)
1	0.27	0.24–0.30	0 (0/20)
7	0.27	0.25–0.30	0 (0/20)
2	0.27	0.25–0.28	0 (0/20)
5	0.26	0.24–0.28	0 (0/20)
4	0.24	0.22–0.25	0 (0/20)
9	0.23	0.21–0.25	0 (0/20)
8	0.22	0.18–0.27	0 (0/20)
10	0.22	0.20–0.25	0 (0/20)
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 CO, 114 of 120 replicates (95.0%) were positive. For the 7 specimens with a mean RLU/CO within 20% above or below the CO, 88 of 139 (63.3%; 95% CI = 54.3–70.9) of the replicates were positive and 51 of 139 (36.7%) were negative. For the 4 specimens within 10% above or below the CO 41 of 79 (51.9%) of the replicates were positive and 38 of 79 (48.1%) were negative. For the 11 specimens with the mean RLU/CO at 20% or more below the CO, 220 of 220 replicates (100%) were negative.

The results indicate that specimens at 20% or more away from the CO can be expected to yield consistent results. Specimens close to the CO yielded approximately equal numbers of positive

and negative results. These data demonstrate that manual testing of PreservCyt specimens with the *digene* HC2 High-Risk HPV DNA Test yield reproducible results.

RCS-automated testing with manual sample preparation

An internal study of RCS-automated testing was performed using clinical PreservCyt specimens obtained predominately from women with a cytology result of ASC-US or greater than ASC-US (HPV prevalence 57%). Specimens were divided into 2 aliquots; each aliquot was then individually processed using the *digene* HC2 Sample Conversion Kit and tested in duplicate with the *digene* HC2 High-Risk HPV DNA Test.

As with other qualitative IVD tests, variability in the *digene* HC2 High-Risk HPV DNA Test results obtained from clinical specimens is associated primarily with one or a combination of the following: specimen collection, sample preparation and the testing procedure. Because the test results compared were obtained from the same clinical specimen, the experimental design controlled for variability due to specimen collection. The repeatability of results obtained from 2 individually prepared sample aliquots from the same clinical specimen (referred to below as "between prepared aliquots") reflects variation due to the combination of sample preparation and the testing procedure. The repeatability of results obtained from the same sample aliquot (referred to below as "within prepared aliquot") reflects variation from the testing procedure only (see Table 37, below).

Table 37. Reproducibility of PreservCyt specimens — RCS-automated testing with manual sample preparation; qualitative reproducibility

	Analysis	Positive agreement (%) (n/N) 95% CI	Negative agreement (%) (n/N) 95% CI	Overall agreement (%) (n/N) 95% CI
Within prepared aliquot	All data	99.62 (261/262) 97.9–100.0	94.7 (160/169) 90.1–97.5	97.7 (421/431) 95.8–98.9
	Strong-positive and strong-negative regions	100.0 (249/249) 98.5–100.0	98.2 (160/163) 94.7–99.6	99.3 (409/412) 97.9–99.9
Between prepared aliquot	All data	99.6 (264/265) 97.9–100.0	98.2 (163/166) 94.8–99.6	99.1 (427/431) 97.6–99.8
	Strong-positive and strong-negative regions	100.0 (249/249) 98.5–100.0	99.4 (161/162) 96.6–100.0	99.8 (410/411) 98.7–100.0

An additional study was performed to evaluate the quantitative reproducibility of results obtained with RCS-automated testing of simulated PreservCyt specimens. Three testing sites, including QIAGEN, participated in the study.

Each testing laboratory performed both RCS-automated and manual testing of the *digene* HC2 High-Risk HPV DNA Test twice per day on 5 different days with a provided 6-member reproducibility panel. Each panel member was composed of cultured cells spiked into PreservCyt Solution intended to yield an approximate RLU/CO value (see Table 38, below).

The HPV DNA-positive panel members were prepared by adding varying amounts of HPV DNA-positive SiHa cells (from a laboratory cell line). The negative panel member was composed of HPV-negative Jurkat cells (from a different laboratory cell line). The final cellular concentration of all 6 panel members was approximately 5 x 10^4 cells/ml.

Table 38. Reproducibility of PreservCyt specimens — RCS-automated testing with manual sample preparation; quantitative reproducibility panel members

Panel member	Cell type	Approximate RLU/CO	Expected test result
1N	Jurkat	<1.0	Negative
2N	Jurkat	<1.0	Negative
3P	SiHa and Jurkat	5.0–8.0	Low-positive
4P	SiHa and Jurkat	5.0–8.0	Low-positive
5P	SiHa	30.0–50.0	Mid-positive
6P	SiHa	200.0	High-positive

The reproducibility was calculated according to the method described by NCCLS E5-A (46) (see Table 39, below). This method requires the computation of variance components for each of the sources of variability: laboratory, day, run and error (defined as inter-assay and between-assay variation). Each of the 6 panel members was tested in quadruplicate in each of the 10 runs (2 runs per day over 5 days of testing) at each of the 3 testing laboratories.

Table 39. Reproducibility of PreservCyt specimens — RCS-automated testing with manual sample preparation; quantitative reproducibility

			Standard deviation					
Panel member	n	Mean RLU/CO	Within run	Between run	Between day	Between lab	Total	Total CV (%)
1N	120	0.20	0.04	0.01	0.01	0.08	0.089	44.4
2N	120	0.20	0.06	0.01	0*	0.08	0.10	52.2
3P	120	4.05	0.76	1.17	0*	0.26	1.42	35.1
4P	120	4.23	0.74	0.86	0*	0.31	1.18	27.8
5P	120	28.6	5.00	5.61	4.41	0*	8.71	30.5
6P	120	214.6	33.95	27.25	18.09	25.53	53.61	25.0

* Negative variance components are set equal to zero.

To supplement this initial reproducibility study with data from specimens very close to the assay cutoff, an additional precision study was conducted at a site external to QIAGEN using the RCS.

The panel consisted of 1 negative, 2 negative or low-positive and 2 low-positive members. Each panel member was prepared by spiking cultured Jurkat and SiHa cells into PreservCyt Solution to yield the target RLU/CO values (see Table 40, below).

This external site completed RCS-automated testing using a single lot of the *digene* HC2 High-Risk HPV DNA Test reagents for each test run, performing the test 2 times per day on 3 different days with a provided 5-member panel of simulated PreservCyt specimens. Each panel member was divided into 4 samples and all 4 samples were tested on the same microplate (see Table 41, below).

Table 40. Reproducibility of PreservCyt specimens — RCS-automated testing with manual sample preparation; quantitative reproducibility near the assay CO panel members

Panel member	Approximate RLU/CO	Expected test result
1N	0.2	Negative
2N	0.8–1.2	High-negative/low-positive
3P	0.8–1.2	High-negative/low-positive
4P	1.2–2.0	Low-positive
5P	1.2–2.0	Low-positive

Table 41. Reproducibility of PreservCyt specimens — RCS-automated testing with manual sample preparation; quantitative reproducibility near assay CO

			Standard deviation				
Panel member	n	Mean RLU/CO	Within run	Between run	Between day	Total	Total CV (%)
1N	24	0.14	0.01	0*	0.02	0.02	15.12
2N	24	1.39	0.14	0.15	0*	0.21	14.84
3P	24	1.31	0.16	0*	0.11	0.19	14.70
4P	24	1.74	0.13	0.21	0.18	0.31	17.73
5P	24	1.63	0.24	0.20	0.26	0.40	24.63

* Negative variance components are set equal to zero.

Sample preparation using the QIAsymphony DSP HPV Media Kit

An internal study of sample preparation using the QIAsymphony DSP HPV Media Kit was performed using clinical PreservCyt specimens obtained from women with one of the two following cytology results:

- ASC-US or greater than ASC-US
- Negative for intraepithelial lesion or malignancy (NILM)

Two samples were removed from each specimen. Each sample was individually prepared using the QIAsymphony DSP HPV Media Kit and the results determined by RCS-automated testing with the *digene* HC2 High-Risk HPV DNA Test.

As with other qualitative IVD tests, variability in the *digene* HC2 High-Risk HPV DNA Test results obtained from clinical specimens is associated primarily with one or a combination of the following: specimen collection, sample preparation and the testing procedure. Because the test results compared were obtained from the same clinical specimen (referred to as "between samples"), the experimental design controlled for variability due to specimen collection. The reproducibility of results (see Table 42, below) obtained from 2 individually prepared samples from the same clinical specimen reflects variation due to sample preparation and the testing procedure.

Table 42. Reproducibility of PreservCyt specimens — sample preparation using the QIAsymphony DSP HPV Media Kit; qualitative reproducibility between samples

Positive agreement (%) (n/N) 95% CI	Negative agreement (%) (n/N) 95% CI	Overall agreement (%) (n/N) 95% CI
99.0 (95/96) 94.3–99.8	96.4 (161/167) 92.4–98.3	97.3 (256/263) 94.6–98.7

An additional study was performed to evaluate the reproducibility of results using simulated PreservCyt specimens. Sample preparation using the QIAsymphony DSP HPV Media Kit was followed by RCS-automated testing with the *digene* HC2 High-Risk HPV DNA Test. The 8 positive panel members were prepared by adding either HPV DNA positive SiHa or HeLa cells to HPV DNA negative C-33 A cells in PreservCyt Solution, while the 2 HPV DNA negative panel members contained only HPV DNA negative C-33 A cells.

Three different operators performed the testing on a single day using 3 different QIAsymphony SP instruments and 3 different QIAsymphony DSP HPV Media Kit lots with panel members 2N, 3E, 5P, 7P and 9P. Panel members 2N, 3E, 5P and 7P were tested with 18 replicates over 3 different runs, yielding 54 data points for each panel member. Panel member 9P was tested with 16 replicates over 3 different runs, yielding 48 data points.

One operator performed the *digene* HC2 High-Risk HPV DNA Test on 3 different days using 3 different QIAsymphony SP instruments and one QIAsymphony DSP HPV Media Kit lot with panel members 1N, 4E, 6P, 8P and 10P. Panel members 1N, 4E, 6P and 8P were tested with 18 replicates over 8 different runs, yielding 144 data points for each panel member. Panel member 10P was tested with 16 replicates over 8 different runs, yielding 128 data points.

For panel members with a mean RLU/CO at 20% or more above the CO, 572 of 572 (100.0%) were positive. For panel members with a mean RLU/CO within 20% above or below the CO, 98 of 198 (49.5%) were positive and 100 of 198 (50.5%) were negative. For panel members with a mean RLU/CO at 20% or more below the CO, 198 of 198 (100.0%) were negative (see Table 43, below).

Table 43. Reproducibility of PreservCyt specimens — sample preparation using the QIAsymphony DSP HPV Media Kit; qualitative reproducibility

Panel member	Cell type	Mean RLU/CO	Standard deviation	Positive test result (%) (n/N)
1N	C-33 A	0.37	0.05	0 (0/144)
2N	C-33 A	0.41	0.06	0 (0/54)
3E	HeLa and C-33 A	0.81	0.11	6 (3/54)
4E	SiHa and C-33 A	1.09	0.18	66 (95/144)
5P	HeLa and C-33 A	3.17	0.46	100 (54/54)
6P	SiHa and C-33 A	4.81	0.74	100 (144/144)
7P	HeLa and C-33 A	6.77	0.97	100 (54/54)
8P	SiHa and C-33 A	9.41	1.39	100 (144/144)
9P	HeLa and C-33 A	13.72	2.81	100 (48/48)
10P	SiHa and C-33 A	28.13	5.08	100 (128/128)

The results indicate that specimens at 20% or more away from the CO can be expected to yield consistent results. Specimens near the CO yielded approximately equal numbers of positive and negative results. These data demonstrate that sample preparation of PreservCyt specimens using the QIAsymphony DSP HPV Media Kit followed by testing with the *digene* HC2 High-Risk HPV DNA Test yields reproducible results.

The results of the internal study were also used to evaluate the quantitative reproducibility of results obtained with sample preparation of PreservCyt specimens using the QIAsymphony DSP HPV Media Kit (see Table 44 and Table 45, below).

Table 44. Reproducibility of PreservCyt specimens — sample preparation using the QIAsymphony DSP HPV Media Kit; quantitative reproducibility with same operator

			Standard deviation				
Panel member	n	Mean RLU/CO	Within runs	Between runs	Between combinations*	Estimated total standard deviation	Estimated total CV (%)
1N	144	0.37	0.04	0.03	0.03	0.06	14.92
4E	144	1.09	0.12	0.11	0.09	0.19	17.24
6P	144	4.81	0.49	0.40	0.42	0.77	15.92
8P	144	9.41	0.96	0.97	0.46	1.44	15.32
10P	128	28.13	4.00	2.04	2.54	5.16	18.35

* Between combinations of QIAsymphony SP instruments and different days.

Table 45. Reproducibility of PreservCyt specimens — sample preparation using the QIAsymphony DSP HPV Media Kit; quantitative reproducibility on same day

Panel member	n	Mean RLU/CO	Standard deviation			Estimated total CV (%)
			Within runs	Between runs[†]	Estimated total standard deviation	
2N	54	0.41	0.04	0.05	0.06	15.86
3E	54	0.81	0.08	0.08	0.12	14.48
5P	54	3.17	0.38	0.33	0.50	15.72
7P	54	6.77	0.92	0.38	1.00	14.73
9P	48	13.72	2.64	1.15	2.88	21.01

[†] A run consists of a combination of a QIAsymphony DSP HPV Media Kit, a QIAsymphony SP instrument and an operator.

The quantitative reproducibility is very high as indicated by all CV values remaining below 25%. Standard deviations between runs are comparable to the corresponding value within runs, which indicates consistent results regardless of the instrument or kit lot used.

Sample preparation using the QIAsymphony DSP AXpH DNA Kit

An internal study of sample preparation using the QIAsymphony DSP AXpH DNA Kit was performed using clinical PreservCyt specimens obtained either from women with cytology of ASC-US or NILM. Two samples were removed from each specimen. Each sample was individually prepared using the QIAsymphony DSP AXpH DNA Kit and the results determined by RCS-automated testing with the *digene* HC2 High-Risk HPV DNA Test.

As with other qualitative IVD tests, variability in the *digene* HC2 High-Risk HPV DNA Test results obtained from clinical specimens is associated primarily with one or a combination of the following: specimen collection, sample preparation and the testing procedure. Because the test results compared were obtained from the same clinical specimen (referred to as "between samples"), the experimental design controlled for variability due to specimen collection. The reproducibility of results (see Table 46, below) obtained from 2 individually prepared samples from the same clinical specimen reflects variation due to sample preparation and the testing procedure.

Table 46. Reproducibility of PreservCyt specimens — sample preparation using the QIAsymphony DSP AXpH DNA Kit; qualitative reproducibility between samples

Positive agreement (%) (n/N) 95% CI	Negative agreement (%) (n/N) 95% CI	Overall agreement (%) (n/N) 95% CI
95.3 (101/106) 89.4–98.0	96.7 (176/182) 92.3–98.5	96.2 (277/288) 93.3–97.9

An additional study was performed to evaluate the reproducibility of results using simulated PreservCyt specimens. Sample preparation using the QIAsymphony DSP AXpH DNA Kit was followed by RCS-automated testing with the *digene* HC2 High-Risk HPV DNA Test.

Three different operators performed the *digene* HC2 High-Risk HPV DNA Test on different days using different instruments and different reagent lots with a 9-member panel. Each panel member was tested in duplicate over 24 different runs, yielding 48 data points for each panel member. The 8 positive panel members were prepared by adding either HPV DNA-positive SiHa or HeLa cells to HPV DNA-negative H9 cells in PreservCyt Solution, while the HPV DNA-negative panel member contained only HPV DNA-negative H9 cells.

For panel members with a mean RLU/CO at 20% or more above the CO, 237 of 240 (98.8%) were positive. For panel members with a mean RLU/CO within 20% above or below the CO, 95 of 144 (66.0%) were positive and 49 of 144 (34.0%) were negative. For panel members with a mean RLU/CO at 20% or more below the CO, 48 of 48 (100.0%) were negative (see Table 47, below).

Table 47. Reproducibility of PreservCyt specimens — sample preparation using the QIAsymphony DSP AXpH DNA Kit; qualitative reproducibility

Panel member	Cell type	Mean RLU/CO	Standard deviation	Positive test result (%) (n/N)
1N	H9	0.17	0.03	0 (0/48)
2E	H9 and HeLa	1.00	0.16	56 (27/48)
3E	H9 and HeLa	1.16	0.57	54 (26/48)
4E	H9 and SiHa	1.18	0.23	88 (42/48)
5P	H9 and SiHa	1.89	0.20	100 (48/48)
6P	H9 and HeLa	2.05	0.43	96 (46/48)
7P	H9 and SiHa	2.97	0.45	100 (48/48)
8P	H9 and HeLa	5.67	0.61	100 (48/48)
9P	H9 and SiHa	9.91	1.63	98 (47/48)

The results indicate that specimens at 20% or more away from the CO can be expected to yield consistent results. Specimens near the CO yielded approximately equal numbers of positive and negative results. These data demonstrate that sample preparation of PreservCyt specimens using the QIAsymphony DSP AXpH DNA Kit followed by testing with the *digene* HC2 High-Risk HPV DNA Test yields reproducible results.

The results of the internal study were also used to evaluate the quantitative reproducibility of results obtained with sample preparation of PreservCyt specimens using the QIAsymphony DSP AXpH DNA Kit (see Table 48, below).

Table 48. Reproducibility of PreservCyt specimens — sample preparation using the QIAsymphony DSP AXpH DNA Kit; quantitative reproducibility

			Standard deviation				
Panel member	n	Mean RLU/CO	Within runs	Between runs	Between combinations*	Estimated total standard deviation	Estimated total CV (%)
1N	48	0.17	0.02	0.02	0.01	0.03	18.13
2E	48	1.00	0.14	0.05	0.06	0.16	16.20
3E	48	1.16	0.48	0.22	0.23	0.57	49.27
4E	48	1.18	0.16	0.14	0.10	0.23	19.63
5P	48	1.89	0.09	0.09	0.16	0.20	10.63
6P	48	2.05	0.18	0.34	0.19	0.43	20.83
7P	48	2.97	0.27	0.23	0.28	0.45	15.14
8P	48	5.67	0.35	0.44	0.24	0.61	10.85
9P	48	9.91	1.36	0.55	0.71	1.63	16.42

* Between combinations of *digene* HC2 High-Risk HPV DNA Test kits, QIAsymphony DSP AXpH DNA Kits, RCS used, QIAsymphony SP used and operator.

Reproducibility of clinical SurePath specimens

Manual testing

The reproducibility of manual testing of SurePath post-gradient cell pellet samples with the *digene* HC2 High-Risk HPV DNA Test was determined in a study using 3 different laboratories. Panel members were tested using a CO of 1.0 RLU/CO on different days and with different runs using an identical set of panel members of known positive or negative HPV status. The panel consisted of 5 positive, 2 high-negative/low-positive and 5 negative members.

Each panel member was prepared by combining unique clinical specimens collected in SurePath Preservative Fluid with a known negative and positive HPV status to obtain the desired target RLU/CO values. Each panel member was tested in duplicate, twice per day, over a period of 5 days at each of the 3 participating laboratories (see Table 49, below).

Table 49. Reproducibility of SurePath post-gradient cell pellet samples — manual testing; qualitative reproducibility

Panel member	Mean RLU/CO	Positive test result (%) (n/N)
1	0.20	0.0 (0/60)
2	0.21	0.0 (0/60)
3	0.22	0.0 (0/60)
4	0.28	3.3 (2/60)
5	0.36	3.3 (2/60)
6	0.83	21.7 (13/60)
7	1.17	43.3 (26/60)
8	19.47	100.0 (60/60)
9	25.65	100.0 (60/60)
10	81.52	100.0 (60/60)
11	154.18	100.0 (60/60)
12	765.29	100.0 (60/60)

RCS-automated testing

The reproducibility of SurePath post-gradient cell pellet sample results with RCS-automated testing were compared with the results obtained with manual testing. Two separate aliquots from the same processed SurePath post-gradient cell pellet sample (from the same specimen) were tested (see Table 50, below).

Table 50. Reproducibility of SurePath post-gradient cell pellet samples — RCS-automated testing; result agreement between RCS-automated and manual testing

Positive agreement (%) (n/N) 95% CI		Negative agreement (%) (n/N) 95% CI	
All positives	Strong-positive region (RLU/CO ≥2.5)	All negatives	Strong-negative region (RLU/CO <0.80)
99.0	100.0	97.7	98.7
(417/421)	(375/375)	(1057/1079)	(1050/1064
97.6–99.7	99.0–100.0	96.9–98.75	97.8–99.28

Sample preparation of SurePath specimens using the QIAsymphony DSP HPV Media Kit

A study was performed to evaluate the reproducibility of results using simulated SurePath specimens. Sample preparation using the QIAsymphony DSP HPV Media Kit was followed by RCS-automated testing with the *digene* HC2 High-Risk HPV DNA Test. The 4 positive panel members were prepared by adding HPV DNA-positive SiHa cells to HPV DNA-negative H-9 cells in SurePath Preservative Fluid, while the HPV DNA negative panel member contained only HPV DNA-negative H-9 cells in SurePath Preservative Fluid.

Three different operators performed testing on 6 different days using 3 different QIAsymphony SP instruments and 3 different QIAsymphony DSP HPV Media Kit lots with panel members 1N, 2E, 3P, 4P and 5P. Panel members 1N, 2E, 3P and 4P were tested with 18 replicates over 37 different runs, yielding 666 data points for panel members 2E and 3P and 665 data points for panel members 1N and 4P. Panel member 5P was tested with 16 replicates over 37 different runs, yielding 590 data points. Four data points were excluded due to insufficient volume as flagged by the QIAsymphony SP during sample preparation.

For panel members with a mean RLU/CO at 20% or more above the CO, 1921 of 1921 (100.0%) were positive. For panel members with a mean RLU/CO within 20% above or below the CO, 410 of 666 (61.6%) were positive and 256 of 666 (38.4%) were negative. For panel members with a mean RLU/CO at 20% or more below the CO, 664 of 665 (99.8%) were negative (see Table 51, below).

Table 51. Reproducibility of SurePath specimens — sample preparation using the QIAsymphony DSP HPV Media Kit; qualitative reproducibility

Panel member	Cell type	Mean RLU/CO	Standard deviation	Positive test result (%) (n/N)
1N	H-9	0.38	0.06	0.2 (1/665)
2E	SiHa and H-9	1.06	0.17	61.6 (410/666)
3P	SiHa and H-9	4.51	0.78	100.0 (666/666)
4P	SiHa and H-9	8.34	1.57	100.0 (665/665)
5P	SiHa and H-9	24.69	5.12	100.0 (590/590)

The results indicate that SurePath specimens at 20% or more away from the CO can be expected to yield consistent results. SurePath specimens near the CO yielded approximately equal numbers of positive and negative results. These data demonstrate that sample preparation of SurePath specimens using the QIAsymphony DSP HPV Media Kit followed by testing with the *digene* HC2 High-Risk HPV DNA Test yields reproducible results.

The results of the internal study were also used to evaluate the quantitative reproducibility of results obtained with sample preparation of SurePath specimens using the QIAsymphony DSP HPV Media Kit.

Three different operators performed testing on 6 different days using 3 different QIAsymphony SP instruments and 3 different QIAsymphony DSP HPV Media Kit lots with panel members 1N, 2E, 3P, 4P and 5P. Panel members 1N, 2E, 3P and 4P were tested with 18 replicates, yielding 162 data points for each panel member. Panel member 5P was tested with 16 replicates, yielding 144 data points (see Table 52, below).

Table 52. Reproducibility of SurePath specimens —sample preparation using the QIAsymphony DSP HPV Media Kit; quantitative reproducibility

			Standard deviation				
Panel member	n	Mean RLU/CO	Within runs	Between days	Between combinations*	Estimated total standard deviation	Estimated total CV (%)
1N	162	0.37	0.06	0.02	0.03	0.07	19.18
2E	162	1.05	0.14	0.07	0.10	0.18	17.41
3P	162	4.40	0.62	0.00	0.43	0.75	17.09
4P	162	8.24	1.15	1.01	1.34	1.77	21.42
5P	144	23.89	3.95	4.10	4.67	6.11	25.59

* A run consists of a combination of a QIAsymphony DSP HPV Media Kit, a QIAsymphony SP instrument and an operator on a particular day.

The quantitative reproducibility is very high as indicated by all CV values remaining below 26%. Standard deviations between runs are comparable to the corresponding value within runs, which indicates consistent results regardless of the instrument or kit lot used.

Sample preparation of SurePath post-gradient cell samples using the QIAsymphony DSP HPV Media Kit

A study was performed to evaluate the reproducibility of results using simulated SurePath post-gradient cell pellet samples. Sample preparation using the QIAsymphony DSP HPV Media Kit was followed by RCS-automated testing with the *digene* HC2 High-Risk HPV DNA Test. Cell culture material in 70% SurePath Preservative Fluid was used to mimic SurePath post-gradient cell pellet samples. The 4 positive panel members were prepared by adding HPV DNA-positive SiHa cells to HPV DNA-negative H-9 cells in SurePath Preservative Fluid, while the HPV DNA-negative panel member contained only HPV DNA-negative H-9 cells in SurePath Preservative Fluid.

Four different operators performed testing on 6 different days using 3 different QIAsymphony SP instruments and 3 different QIAsymphony DSP HPV Media Kit lots with panel members 1, 2, 3, 4 and 5. Panel members 1, 2, 3 and 4 were tested with 18 replicates over 37 different runs, yielding 666 data points for panel members 1 and 3, and 665 data points for panel members 2 and 4. Two data points were excluded due to insufficient volume as flagged by the QIAsymphony SP during sample preparation. Panel member 5 was tested with 16 replicates over 37 different runs, yielding 592 data points.

For panel members with a mean RLU/CO at 20% or more above the CO, 1923 of 1923 (100.0%) were positive. For panel members with a mean RLU/CO within 20% above or below the CO, 416 of 665 (62.6%) were positive and 249 of 665 (37.4%) were negative. For panel members with a mean RLU/CO at 20% or more below the CO, 666 of 666 (100%) were negative (see Table 53, below).

Table 53. Reproducibility of SurePath post-gradient cell pallet samples — sample preparation using the QIAsymphony DSP HPV Media Kit; qualitative reproducibility

Panel member	Cell type	Mean RLU/CO	Standard deviation	CV (%)	Positive test result (%) (n/N)
1	H-9	0.12	0.02	18.77	0.0 (0/666)
2	SiHa and H-9	0.96	0.11	11.15	62.6 (416/665)
3	SiHa and H-9	4.72	0.56	11.89	100.0 (666/666)
4	SiHa and H-9	9.34	0.98	10.46	100.0 (665/665)
5	SiHa and H-9	24.9	3.37	13.55	100.0 (592/592)

The results indicate that SurePath post-gradient cell pellet samples at 20% or more away from the CO can be expected to yield consistent results. SurePath post-gradient cell pellet samples near the CO yielded approximately equal numbers of positive and negative results. These data demonstrate that sample preparation of SurePath post-gradient cell pellet samples using the QIAsymphony DSP HPV Media Kit followed by testing with the *digene* HC2 High-Risk HPV DNA Test yields reproducible results.

The results of the internal study were also used to evaluate the quantitative reproducibility of results obtained with sample preparation of SurePath post-gradient cell pellet samples using the QIAsymphony DSP HPV Media Kit.

Four different operators performed testing on 6 different days using 3 different QIAsymphony SP instruments and 3 different QIAsymphony DSP HPV Media Kit lots with panel members 1, 2, 3, 4 and 5. Panel members 1, 2, 3 and 4 were tested with 18 replicates, yielding 162 data points for

each panel member. Panel member 5 was tested with 16 replicates, yielding 144 data points (see Table 54, below).

Table 54. Reproducibility of SurePath post-gradient cell pallet samples — sample preparation using the QIAsymphony DSP HPV Media Kit; quantitative reproducibility

			Standard deviation				
Panel member	n	Mean RLU/CO	Within runs	Between days	Between combinations*	Estimated total standard deviation	Estimated total CV (%)
1	162	0.12	0.02	0.00	0.01	0.02	19.80
2	162	1.00	0.08	0.02	0.06	0.10	10.27
3	162	4.99	0.37	0.13	0.38	0.55	11.00
4	162	9.78	0.61	0.23	0.54	0.85	8.72
5	144	26.40	2.19	0.70	1.51	2.75	10.41

* Between combinations of different days, operators, QIAsymphony DSP HPV Media Kit lots and QIAsymphony SP instruments.

The quantitative reproducibility is very high as indicated by all CV values remaining below 20%. Standard deviations between runs are comparable to the corresponding value within runs, which indicates consistent results regardless of the instrument or kit lot used.

Cross-reactivity

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

The following bacteria were tested and all tested negative in the *digene* HC2 High-Risk HPV DNA Test:

- *Acinetobacter anitratus*
- *Acinetobacter lwoffi* (ATCC® 17908)
- *Bacteroides fragilis* (ATCC 25285)
- *Bacteroides melaninogenicus*
- *Candida albicans* (ATCC 14053 or 10231)
- *Chlamydia trachomatis*
- *Enterobacter cloacae*

- *Escherichia coli* (HB101)*
- *Escherichia coli**
- *Fusobacterium nucleatum*
- *Gardnerella vaginalis*
- *Haemophilus ducreyi*
- *Klebsiella pneumoniae*
- *Lactobacillus acidophilus*
- *Mobiluncus curtisii*
- *Mobiluncus mulieris*
- *Mycoplasma hominis*
- *Mycoplasma hyorhinis*
- *Neisseria gonorrhoeae* (ATCC 19424)
- *Neisseria lactamica* (NRL 2118)
- *Neisseria meningitidis* (ATCC 13077)
- *Neisseria sicca* (ATCC 29256)
- *Peptostreptococcus anaerobius*
- *Proteus vulgaris* (ATCC 21117, 8427, 33420)
- *Serratia marcescens*
- *Staphylococcus aureus* (Cowan strain)
- *Staphylococcus epidermidis*
- *Streptococcus faecalis* (ATCC 14508)
- *Streptococcus pyogenes* (ATCC27762)
- *Treponema pallidum*
- *Trichomonas vaginalis*
- *Ureaplasma urealyticum*

The following viral or plasmid DNA or human serum were tested and all tested negative in the *digene* HC2 High-Risk HPV DNA Test:

- Adenovirus 2
- Cytomegalovirus
- Epstein-Barr virus
- Hepatitis B surface antigen-positive serum
- Herpes simplex I
- Herpes simplex II
- Human immunodeficiency virus (HIV, RT DNA)
- HPV types 1, 2, 3, 4, 5, 8, 13 and 30
- Simian virus type 40 (SV40)

* Both the *E. coli* strain used to grow plasmids (HB101) and a clinical isolate of *E. coli* were assayed.

The only plasmid that showed cross-reactivity in the *digene* HC2 High-Risk HPV DNA Test was pBR322. Cross-reactivity between pBR322 and the Probe Mix is not unexpected because it is difficult to remove all 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 the bacterial plasmid. However, 298 clinical specimens testing positive with *digene* HC2 High-Risk HPV DNA Test did not have positive results due to pBR322 when tested with a pBR322 probe. Thus, the likelihood of a *digene* HC2 High-Risk HPV DNA Test false-positive result due to homologous pBR322 sequences in clinical specimens appears to be low.

Cross-hybridization

Eighteen different HPV types (high-risk and low-risk) were tested with the *digene* HC2 High-Risk HPV DNA Test at concentrations of 4 ng/ml of HPV DNA. All of the high-risk HPV targets were positive. This study also showed that there is a small amount of cross-hybridization between HPV types 6 and 42 and the *digene* HC2 High-Risk HPV DNA Test. Patient specimens with high levels (4 ng/ml or higher) of HPV types 6 or 42 may have false-positive *digene* HC2 High-Risk HPV DNA Test results. The clinical significance of this is that patients with 4 ng/ml or higher of HPV types 6 or 42 may be unnecessarily referred to colposcopy.

The *digene* HC2 High-Risk HPV DNA Test has also been shown to cross-react with HPV types 40, 53 and 66. These types are rare, and there is insufficient evidence to establish the exact correlation between infection with these types and development of high-grade disease (15). It has also been reported in 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 (35). 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 High-Risk HPV DNA Test. Whole blood, douche, anti-fungal cream and contraceptive jelly (agents that may commonly be found in cervical specimens) were added to STM-negative and STM-positive specimens (clinical specimen pools and non-clinical specimens) at concentrations that may be found in cervical specimens.

No false-positive results were observed with any of the four agents at any concentration. However, a false-negative result may be reported in clinical specimens with HPV DNA levels close

to the CO for the test (1 pg/ml) if high concentrations of anti-fungal cream or contraceptive jelly are present. However, it is very unlikely that a clinical specimen will consist almost entirely of one of these substances because the cervix is routinely cleared prior to obtaining specimens for Pap smear and for HPV testing.

Effect of blood and other substances on PreservCyt specimens

Manual sample preparation

The effect of blood and other potentially interfering defined or undefined substances potentially present in PreservCyt specimens was evaluated in the *digene* HC2 High-Risk HPV DNA Test. Whole blood, douche, anti fungal cream and contraceptive jelly (agents that may commonly be found in cervical specimens) were added to PreservCyt 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 HPV DNA by the *digene* HC2 High-Risk HPV DNA Test.

Sample preparation using the QIAsymphony DSP HPV Media Kit

The effects of blood and other potentially interfering substances in PreservCyt specimens were evaluated using the QIAsymphony DSP HPV Media Kit for sample preparation and RCS-automated testing using the *digene* HC2 High-Risk HPV DNA Test. The effects of the following potentially interfering substances were tested:

- Anti-fungal cream
- Anti-inflammatory cream
- Blood
- Contraceptive jelly
- Douche
- Feminine deodorant suppositories
- Lubricating jelly
- Spermicide

Each substance was added to negative and positive clinical pools. No false-positive or false-negative results were observed with any of the substances at a concentration that may be found in cervical specimens. However, a false-negative result may be reported in clinical specimens with HPV DNA levels close to the CO for the test if high concentrations of anti-fungal cream, vaginal lubricating jelly or blood are present. However, it is very unlikely that a clinical specimen will consist almost entirely of one of these substances because the cervix is routinely cleared prior to obtaining specimens for Pap smear and for HPV testing.

Sample preparation using the QIAsymphony DSP AXpH DNA Kit

The effect of whole blood in PreservCyt specimens was evaluated using the QIAsymphony DSP AXpH DNA Kit for sample preparation and the *digene* HC2 High-Risk HPV DNA Test for testing. Visibly bloody clinical specimens were selected and tested using both the manual sample preparation method and the automated sample preparation method using the QIAsymphony DSP AXpH DNA Kit. Results were compared for 238 specimens and yielded a total agreement of 94.12% and a McNemar's p value of 0.2850, indicating no statistically significant difference in clinical performance between the manual sample preparation method and the automated sample preparation method using the QIAsymphony DSP AXpH DNA Kit.

The effects of the following potentially interfering substances were tested:

- Douche
- Anti-fungal cream
- Contraceptive jelly
- Peripheral blood mononuclear cells (PBMC)
- Lubricating jelly
- Feminine spray
- Spermicide
- Magnetic particles
- TopElute Fluid

Each substance was added to negative and positive cellular pools at concentrations that may be found in cervical specimens or may be added during sample preparation. No false-positive results were observed with any of the substances at any concentration. No false-negative results were observed with the exception of contraceptive jelly.

Do not collect a PreservCyt cervical specimen for automated sample preparation using the QIAsymphony DSP AXpH DNA Kit if contraceptive jelly is present.

Effect of blood and other substances on SurePath specimens

Sample preparation of SurePath samples using the QIAsymphony DSP HPV Media Kit

The effects of blood and other potentially interfering substances in SurePath samples were evaluated using the QIAsymphony DSP HPV Media Kit for sample preparation and RCS-automated testing using the *digene* HC2 High-Risk HPV DNA Test.

The effects of the following potentially interfering substances were tested:

- Anti-fungal cream
- Anti-inflammatory cream
- Blood
- Contraceptive jelly
- Douche
- Feminine deodorant suppositories
- Lubricating jelly
- Spermicide

Each substance was added to negative and positive clinical pools. No false-positive results were observed with any of the substances at a concentration that may be found in cervical specimens.

No false-negative results were observed with the exception of the following substances:

- Contraceptive jelly caused false-negative results at a very low concentration.
- If a high concentration of anti-fungal cream is present in the specimen, a false-negative result may be reported in clinical specimens with HPV DNA levels close to the CO for the test. However, it is very unlikely that a clinical specimen will consist almost entirely of anti-fungal cream because the cervix is routinely cleared prior to obtaining specimens for Pap smear and for HPV testing.

Do not collect a SurePath cervical specimen for automated sample preparation using the QIAsymphony DSP HPV Media Kit if anti-fungal cream or contraceptive jelly is present.

Sample preparation of SurePath post-gradient cell pellet samples using the QIAsymphony DSP HPV Media Kit

The effects of blood and other potentially interfering substances in SurePath post-gradient cell pellet samples were evaluated using the QIAsymphony DSP HPV Media Kit for sample preparation and RCS-automated testing using the *digene* HC2 High-Risk HPV DNA Test.

The effects of the following potentially interfering substances were tested:

- Anti-fungal cream
- Anti-inflammatory cream
- Blood
- Contraceptive jelly
- Douche
- Feminine deodorant suppositories
- Lubricating jelly
- Spermicide

Each substance was added to negative and positive clinical pools that were then processed through the BD PrepMate System to mimic a SurePath post-gradient cell pellet sample. A single false-positive result was observed for both blood and anti-fungal cream; however statistical analysis showed no significant interference. No false-positive results were observed with any of the other substances at a concentration that may be found in cervical specimens.

False-negative results were observed for anti-fungal cream, anti-inflammatory cream and contraceptive jelly. Do not collect a SurePath cervical specimen for automated sample preparation using the QIAsymphony DSP HPV Media Kit if anti-fungal cream, anti-inflammatory cream or contraceptive jelly is present.

Carryover

The RCS was designed to minimize specimen contamination or carryover of residual alkaline phosphatase through the use of disposable pipet tips for reagent and specimen aspiration. To confirm this design characteristic, QIAGEN conducted several studies to evaluate if use of the RCS increased the potential for carryover or cross-contamination of specimens compared with the manual method. Multiple RCS instruments were used to assess carryover potential from system-to-system.

In one study, 2 ng and 20 ng of HPV DNA plasmid was added to Negative Control material to prepare high-positive STM specimens. The 20 ng/ml concentration yields RLU values approximately 3–5 times higher than those of the highest positive clinical specimen expected to be observed during routine clinical testing. These simulated high-positive specimens were placed throughout the microplate in a checkerboard pattern alternating with wells containing only Negative Control (test wells). This design considers potential additive effects of sequential high-positive specimens. Microplates were then tested using both the manual and RCS-automated testing methods. After processing, the numbers of false-positive test wells were compared. RCS-automated testing did not produce more false-positive test wells than the manual testing with these simulated STM specimens, even when an extremely high sequence of positive specimens were contained on the microplate.

In a second carryover evaluation, HPV-positive patient PreservCyt specimens were combined to create a panel of specimens with differing levels of chemiluminescence to yield RLU/CO values representative of the range expected during routine clinical RCS-automated testing. The positive specimens ranged from approximately 200–1800 RLU/CO. To assess the potential for carryover, including the potential additive effects of sequential high positives, these positive panel members were placed on microplates in a checkerboard pattern next to negative control wells. These plates were then tested using the RCS-automated testing method.

The results of this carryover evaluation, using pooled patient specimens, suggests a potential false-positive rate of 0.3% due to carryover effects when performing RCS-automated testing with the *digene* HC2 High-Risk HPV DNA Test.

QIAGEN's experience conducting tests with pooled PreservCyt specimens suggests that pooling patient PreservCyt specimens creates specimens that do not exhibit characteristics similar to single patient specimens. Although the effects of this pooling on the carryover potential of RCS-automated testing are unknown, additional pre-clinical testing of RCS-automated testing indicated no increased potential for false-positive results due to carryover. These evaluations were conducted using artificial plasmid specimens with DNA concentrations nearly 5 times higher than observed in the clinical setting.

A third carryover evaluation created test specimens by adding a fluorescent dye in concentrations representative of the dynamic RLU range of the assay to background matrices that approximated the viscosity of clinical specimens and the *digene* HC2 High-Risk HPV DNA Test reagents. These test specimens were then processed using 3 separate RCS instruments, and the carryover potential of each of the following key procedural steps of the RCS was evaluated:

- Specimen transfer
- Plate-to-plate transfer
- Probe addition
- Microplate shaking
- Microplate washing

The resulting fluorescence was measured at an excitation wavelength of 485 nm and an emission wavelength of 535 nm and was sensitive enough to detect a carryover event on the order of 1:20,000, which would correspond to a false-positive result with the *digene* HC2 High-Risk HPV DNA Test (i.e., 1 pg in 20 ng). The results of this evaluation demonstrated no carryover event during any of the key procedural steps of the RCS that would lead to a false-positive *digene* HC2 High-Risk HPV DNA Test result.

On-board reagent stability

QIAGEN assessed the performance characteristics of RCS-automated testing when using reagents that remained on-board the system platform for extended periods. The reagents most likely to be subject to extended on-board placement include the Probe Mix, DR1, DR2 and the Capture Microplate.

Test performance was evaluated using both freshly prepared reagents and reagents that were allowed to age on-board the RCS instrument at room temperature for a period of 16 hours (to simulate 2 work shifts in the laboratory setting). Testing of simulated clinical specimens was

performed using 2 RCS instruments on each of 2 testing days with a defined reagents matrix (see Table 55, below).

Table 55. Study design for on-board reagent stability

RCS instrument	Day 1	Day 2
1	Aged reagents	Fresh reagents
2	Fresh reagents	Aged reagents

A plot of all RLU/CO data points is shown in Figure 3, below. The plot and regression analysis for aged versus fresh reagents indicate agreement between the aged and fresh reagents.

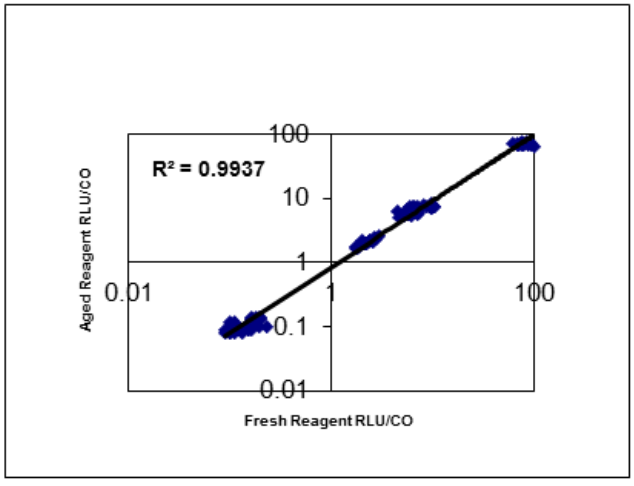


Figure 3. Scatter-plot comparing assay calibrator and control values using aged and fresh reagents.

Further examination of the agreement results shows that no qualitative results changed when using aged reagents (see Table 56, below).

Table 56. Agreement of fresh vs. aged reagents

Statistical measure	Result
Overall agreement (%) (n/N) 95% CI	100.0% (96/96) 97.97–100.0
Positive agreement (%) (n/N) 95% CI	100.0% (64/64) 97.97–100.0
Negative agreement (%) (n/N) 95% CI	100.0% (32/32) 97.97–100.0
R^2	0.9937
Slope	0.97
Intercept	0.47
Kappa	1.0

The data analysis shows the results to be statistically identical for fresh and aged reagents, indicating that the reagents are sufficiently stable when placed on board the instrument for a period of up to 16 hours.

References

1. Broker, T. R. and Botchan, M. (1986) Papillomaviruses: retrospectives and prospectives. In: Botchan, M., Grodzicker, T., and Sharp, P., eds. *DNA Tumor Viruses: Control of Gene Expression and Replication*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, p. 17 36. From the 1985 Cancer Cells Conference at Cold Spring Harbor.
2. Lorincz, A.T. and Reid, R. (1989) Association of human papillomavirus with gynecologic cancer. Curr. Opin. Oncol. **1**, 123.
3. Jenson, A.B., Kurman, R.J., and Lancaster, W.D. (1984) Human papillomaviruses. In: Belshe, R.B. *Textbook of Human Virology*. Littleton, MA: PSG Wright, p 951.
4. Becker, T.M., Stone, K.M., and Alexander, E.R. (1987) Genital human papillomavirus infection: a growing concern. Obstet. Gynecol. Clin. North Am. **14**, 389.
5. McCance, D.J., Walker, P.G., Dyson, J.L., Coleman, D.V., and Singer, A. (1983) Presence of human papillomavirus DNA sequences in cervical intraepithelial neoplasia. Br. Med. J. **287**, 784.
6. Naghashfar, Z. et. al. (1985) Identification of genital tract papillomaviruses HPV 6 and HPV 16 in warts of the oral cavity. J. Med. Virol. **17**, 313.
7. Gissmann, L., Wolnik, L., Ikenberg, H., Koldovsky, U., Schnurch, H.G., and zur Hausen, H. (1983) Human papillomavirus types 6 and 11 DNA sequences in genital and laryngeal papillomas and in some cervical cancers. Proc. Natl. Acad. Sci. U.S.A. **80**, 560.
8. Munoz, N., Bosch, F.X., Shah, K.V., and Meheus, A., eds. (1992) *IARC Scientific Publications no. 119: The Epidemiology of Cervical Cancer and Human Papillomavirus*. Lyon, France:International Agency for Research on Cancer.
9. Reid, R. et al. (1987) Sexually transmitted papillomaviral infections. I. The anatomic distribution and pathologic grade of neoplastic lesions associated with different viral types. Am. J. Obstet. Gynecol. **156**, 212.
10. Fuchs, P.G., Girardi, F., and Pfister, H. (1988) Human papillomavirus DNA in normal, metaplastic, preneoplastic and neoplastic epithelia of the cervix uteri. Int. J. Cancer **41**, 41.

11. Lorincz, A.T., Temple, G.F., Kurman, R.J., Jenson, A.B., and Lancaster, W.D. (1987) Oncogenic association of specific human papillomavirus types with cervical neoplasia. J. Natl. Cancer Inst. **79)**, 671.

12. Lorincz, A.T., Lancaster, W.D., and Temple, G.F. (1986) Cloning and characterization of the DNA of a new human papillomavirus from a woman with dysplasia of the uterine cervix. J. Virol. **58**, 225.

13. Beaudenon, S., Kremsdorf, D., Croissant, O., Jablonska, S., Wain Hobson, S., and Orth, G. (1986) A novel type of human papillomavirus associated with genital neoplasias. Nature **321**, 246.

14. Lorincz, A.T., Quinn, A.P., Lancaster, W.D., and Temple, G.F. (1987) A new type of papillomavirus associated with cancer of the uterine cervix. Virology **159**, 187.

15. Meyer, T. et al., (1998) Association of rare human papillomavirus types with genital premalignant and malignant lesions. J. Infect. Dis. **178**, 252.

16. Lorincz, A.T., Reid, R., Jenson, A.B., Greenberg, M.D., Lancaster, W., and Kurman, R.J. (1992) Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. Obstet Gynecol **79**, 328.

17. Longuet, M., Beaudenon, S., and Orth, G. (1996) Two novel genital human papillomavirus (HPV) types, HPV68 and HPV70, related to the potentially oncogenic HPV39. J. Clin. Microbiol. **34**, 738.

18. Naghashfar, Z.S., Rosenshein, N.B., Lorincz, A.T., Buscema, J., and Shah, K.V. (1987) Characterization of human papillomavirus type 45, a new type 18 related virus of the genital tract. J. Gen. Virol. **68**, 3073.

19. Nuovo, G.J., Crum, C.P., de Villiers, E.M., Levine, R.U., and Silverstein, S.J. (1988) Isolation of a novel human papillomavirus (type 51) from a cervical condyloma. J. Virol. **62**,1452.

20. Shimoda, K., Lorincz, A.T., Temple, G.F., and Lancaster, W.D. (1988) Human papillomavirus type 52: a new virus associated with cervical neoplasia. J. Gen. Virol. **69**, 2925.

21. Lorincz, A.T., Quinn, A.P., Goldsborough, M.D., McAllister, P., and Temple, G.F. (1989) Human papillomavirus type 56: a new virus detected in cervical cancers. J. Gen. Virol. **70**, 3099.

22. Lorincz, A.T., Quinn, A.P., Goldsborough, M.D., Schmidt, B.J., and Temple, G.F. (1989) Cloning and partial DNA sequencing of two new human papillomavirus types associated with condylomas and low grade cervical neoplasia. J. Virol. **63**, 2829.

23. Beaudenon, S. et al. (1987) Plurality of genital human papillomaviruses: characterization of two new types with distinct biological properties. Virology **161**, 374.

24. Schiffman, M. (1993) Latest HPV findings: some clinical implications. Contemp. Ob. Gyn. **38**, 27.

25. Volpers, C.; and Streeck, R.E. (1991) Genome organization and nucleotide sequence of human papillomavirus type 39. Virology **181**, 419.

26. Matsukura, T., and Sugase, M. (1990) Molecular cloning of a novel human papillomavirus (type 58) from an invasive cervical carcinoma. Virology **177**, 833.

27. Rho, J., Roy-Burman, A., Kim, H., de Villiers, E.M., Matsukura, T., and Choe, J. (1994) Nucleotide sequence and phylogenetic classification of human papillomavirus type 59. Virology **203**, 158.

28. Bosch, F.X. et al. (1995) International Biological Study on Cervical Cancer (IBSCC) Study Group. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. J. Natl. Cancer Inst. **87**, 796.

29. Kahn, T., Schwarz, E., and zur Hausen, H. (1986) Molecular cloning and characterization of the DNA of a new human papillomavirus (HPV 30) from a laryngeal carcinoma. Int. J. Cancer **51**, 61.

30. Koutsky, L.A. et al. (1992) A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. N. Engl. J. Med. **327**, 1272.

31. Nieminen, P., Aho, M., Vesterinen, E., Stellato, G., Vaheri, A., Soares, V.R.X., Paavonen, J. (1991) Natural history of HPV infection: preliminary results of a cohort study [abstract]. In: 1991 Papillomavirus Workshop. Seattle, WA p 77.

32. Centers for Disease Control (1987) Recommendations for prevention of HIV transmission in health-care settings. MMWR Morb. Mortal. Wkly. Rep. 36(Suppl 2), 3S.

33. Sehulster, L.M., Hollinger, F.B., Dreesman, G.R., and Melnick, J.L. (1981) Immunological and biophysical alteration of hepatitis B virus antigens by sodium hypochlorite disinfection. Appl. Environ. Microbiol. **42**, 762.

34. Martin, L.S., McDougal, J.S., and Loskoski, S.L. (1985) Disinfection and inactivation of the human T lymphotropic virus type III/lymphadenopathy associated virus. J. Infect. Dis. **152**, 400.

35. Vernon, S.D., Unger, E.R., and Williams, D. (2000) Comparison of human papillomavirus detection and typing by cycle sequencing, line blotting, and hybrid capture. J. Clin. Microbiol. **38**, 651.

36. Coleman, D. et al. (1993) European guidelines for quality assurance in cervical cancer screening. Europe against cancer programme. Eur. J. Cancer 29A(Supp. 4), S1.

37. Lorincz, A.T., Schiffman, M.H., Jaffurs, W.J., Marlow, J., Quinn, A.P., and Temple, G.F. (1990) Temporal associations of human papillomavirus infection with cervical cytologic abnormalities. Am. J. Obstet. Gynecol. **162**, 645.

38. Morrison, E.A.B. et al. (1991) Human papillomavirus infection and other risk factors for cervical neoplasia: a case control study. Int. J. Cancer **49**, 6.

39. Wheeler, C.M., Stewart, A.M., Gravitt, P.E., and Cheng, S. (1995) Generation of entire human papillomavirus genomes by long PCR: frequency of errors produced during amplification. Genome Res. **5**, 79.

40. Burk R.D. et al. (1996) Declining prevalence of cervicovaginal human papillomavirus infection with age is independent of other risk factors. Sex. Transm. Dis. **23**, 333.

41. Belinson, J. et al. (2009) Prevalence of type-specific human papillomavirus in endocervical, upper and lower vaginal, perineal and vaginal self-collected specimens: implications for vaginal self-collection. Int. J. of Cancer **127**, 1151.

42. Zhao, F. et al. (2012) Pooled analysis of a self-sampling HPV DNA Test as a cervical cancer primary screening method. J. Natl Cancer Inst. **104**, 178.

43. Lazcano-Ponce, E. et al. (2011) Self-collection of vaginal specimens for human papillomavirus testing in cervical cancer prevention (MARCH): a community-based randomized controlled trial. Lancet **378**, 1868.

44. Lazcano-Ponce, E. et al. (2014) Specimen self-collection and HPV DNA screening in a pilot study of 100,242 women. Int. J. Cancer **135**, 109.

45. Szarewski, A. et al. (2007) Human papillomavirus testing by self-sampling: assessment of accuracy in an unsupervised clinical setting. J. Med Screen **14**, 34.

46. NCCLS (1999) *Evaluation of Precision Performance of Clinical Chemistry Devices: Approved Guideline*. NCCLS document E5-A.

Symbols

The symbols in the following table are used in these instructions for use.

Symbol	Symbol definition
	Contains sufficient for 96 tests
	Contains sufficient for 384 tests
	In vitro diagnostic medical device
	Catalog number
	Manufacturer
	Authorized representative in the European Community
	Use by
	Consult instructions for use
	Global Trade Item Number

Troubleshooting Guide

		Comments and suggestions
Incorrect or no color change observed during denaturation		
a)	DNR not prepared properly	Make sure that the DNR contains the Indicator Dye and is a dark purple color.
b)	DNR not added	Make sure that the DNR was added to the specimen by measuring the specimen volume (1.5 ml is expected). If the volume indicates that DNR was not added, make the correct addition, mix and proceed with the test 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; test results should not be adversely affected.
d)	Specimen pH may be unusually acidic	If none 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 because improper specimen pH will adversely affect the test results.
Quality controls give incorrect results		
a)	Incorrect assay protocol chosen for test	If the assay protocol is incorrect for the test being performed, read the microplate again within 30 minutes after DR2 addition using the correct assay protocol.
b)	Reverse placement of QC1-LR and QC2-HR	Retest specimens.
c)	Reverse placement of HRC and QC2-HR	Retest specimens.

		Comments and suggestions
Incorrect color change observed during hybridization		
a)	Inadequate mixing of Probe Mix with denatured calibrators, quality controls and/or specimens; or Probe Mix not added; or incorrect volume of reagent added	Shake hybridization microplate or microtube rack containing microtubes for an additional 2 minutes. If there are microtubes or microplate wells that still remain purple, add an additional 25 µl of the correct Probe Mix and mix well. If upon Probe Mix 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; test results should not be adversely affected.
c)	Specimen had <1000 µl STM	Check the volume of the original specimen. Volume should be 1425 µl ± 20 µl (after removing 75 µl aliquot for testing). If volume is <1425 µl, original specimen contained <1000 µl STM. Obtain a new specimen.
Test fails assay validation; no signal observed in positive calibrators, quality controls or in specimens		
a)	No probe added to Probe Diluent	Prepare Probe Mix as described in these instructions for use. Label tubes carefully.
b)	Probe contaminated with RNase during preparation	Use aerosol-barrier pipet tips when pipetting probe and wear gloves. Prepare Probe Mix in sterile container. Only use clean, new disposable reagent reservoirs.
c)	Inadequate mixing of Probe Mix	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 Probe Mix and denatured specimen	After adding Probe Mix and specimen to each hybridization microplate well or hybridization 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 microplate well or microtube.

		Comments and suggestions
e)	Incorrect time or temperature during hybridization step	Hybridize for 60 ± 5 minutes at 65 ± 2°C. Check temperature of Microplate Heater I or waterbath. Make sure the Microplate Heater I or waterbath 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. Waterbaths should be calibrated periodically.
f)	Inadequate mixing during capture step	Shake on a Rotary Shaker I for 60 ± 5 minutes at 20–25°C as described in these instructions for use. Verify Rotary Shaker I speed by calibration. (Refer to *Rotary Shaker I User Manual*).
g)	Failure to add correct amount of DR1 or to incubate for specified time	Pipet 75 µl DR1 into each microplate well using an 8-channel pipet. Incubate at 20–25°C for 30–45 minutes.
h)	Failure to add correct amount of DR2 or to incubate for specified time	Pipet 75 µl DR2 into each microplate well using an 8-channel pipet. Incubate at 20–25°C for 15–30 minutes.
i)	DML instrument malfunction or incorrect programming	Refer to the applicable DML instrument user manual and software user manual for further instructions or contact QIAGEN Technical Services.

Elevated RLU values in calibrators, quality controls and/or specimens (≥200 RLU in many or all microplate wells); test may fail assay validation

a)	DNR not added; incorrect volume of reagent added; inadequate mixing of DNR with specimens, calibrators or quality controls	Make sure that the repeating pipet is delivering accurately prior to adding DNR. Calibrated pipets are essential. Add a half-volume of DNR 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 DNR.

		Comments and suggestions
b)	Light leak in the DML instrument; door not sealed; seal around door broken	Check background reading (raw data measurement) of the DML instrument by reading an empty microplate. A reading of greater than 50 RLU indicates that a light leak exists. Refer to the applicable DML instrument user manual for instructions or contact QIAGEN Technical Services.
c)	Contamination of DR2 or capture microplate wells by DR1 or exogenous alkaline phosphatase	See "Contamination check of DR2", page 123.
d)	Contaminated Wash Buffer	See "Contamination check of Wash Apparatus and/or water source", page 123.
e)	Contaminated Automated Plate Washer	See "Contamination check of Wash Apparatus and/or water source", page 123.
f)	Inadequate washing of capture microplate wells after DR1 incubation	Wash capture microplate wells thoroughly with Wash Buffer 6 times, either by overflowing the wells or using the Automated Plate Washer. No residual pink liquid should be visible in the microplate wells after washing. Refer to *Automated Plate Washer User Manual* for instructions on testing for contamination or malfunctions.
g)	DR1 contamination of microplate wells	Make sure all work surfaces are clean and dry. Use care when using DR1. Avoid aerosols.
h)	Blotting hybridization solution on same area of Kimtowels wipers or equivalent low-lint paper towels	Do not reblot on previously used Kimtowels wipers or equivalent low-lint paper towels.
i)	Used incorrect blotting towels	Use Kimtowels wipers or equivalent low-lint paper towels for blotting.

		Comments and suggestions
Low PC/NC ratios or high number of low-positive specimens with ratios <2.0 (>20%); test may fail assay validation		
a)	Inadequate specimen preparation	Add the correct volume of DNR and mix thoroughly by vortexing. To avoid false-positive results, make sure liquid washes entire inner surface of tube. For PreservCyt specimens, make sure that proper mixing and resuspension of the cell pellet is completed prior to denaturation incubation. A distinct color change from clear to dark purple should be seen. Incubate for 45 ± 5 minutes at 65 ± 2°C.
b)	Probe Mix inadequately mixed or insufficient Probe Mix added	Prepare Probe Mix as described. Mix thoroughly by vortexing, making sure that a visible vortex is produced. Probe Mix must be added to tubes with a positive displacement pipet or a multichannel pipet to make sure of accurate delivery.
c)	Inadequate volume of Probe Mix added to each hybridization microtube or microplate well	Make sure that the 8-channel pipet is delivering accurately prior to adding Probe Mix. Add 25 µl of Probe Mix to each microtube or microplate well containing denatured calibrators, quality controls, and specimens. Color change should be from dark purple to yellow after addition and thorough mixing. PreservCyt specimens should turn pink instead of yellow.
d)	Loss of DR1 activity	Store DR1 at 2–8°C. Use before the expiration date.
e)	Insufficient capture	The capture step should be performed using a Rotary Shaker I set at 1100 ±100 rpm. Validate shaker speed by calibration.

		Comments and suggestions
f)	Inadequate washing	Wash microplate wells thoroughly with Wash Buffer 6 times, either by overflowing the wells or using the Automated Plate Washer.
g)	Contaminated Wash Buffer	See "Contamination check of Wash Apparatus and/or water source", page 123.
Series of positive specimens with RLU values approximately the same		
a)	Contamination of capture microplate wells during test	Cover the capture microplate during all incubations. Avoid exposing tubes to aerosol contamination while performing the assay. Wear powder-free gloves during manipulations.
b)	DR2 contamination	Make sure not to contaminate the stock when pipetting DR2 into capture microplate wells. Avoid contamination of DR2 by aerosols from DR1 or from laboratory dust, etc.
c)	Automated Plate Washer malfunction	See "Contamination check of Wash Apparatus and/or water source", page 123, or refer to *Automated Plate Washer User Manual* for instructions on testing for contamination or malfunctions.
Wide CVs between replicates		
a)	Inaccurate pipetting	Check pipet to assure that reproducible volumes are being delivered. Calibrate pipets routinely.
b)	Insufficient mixing	Mix thoroughly at all steps. Vortex before and after denaturation incubation and after adding Probe Mix. Make sure that a visible vortex is produced.
c)	Incomplete transfer of liquid from hybridization microtubes or hybridization microplate wells to capture microplate wells	Make sure during the transfer step from the hybridization microplate or hybridization microtubes to the capture microplate wells that reproducible volumes are transferred.

		Comments and suggestions
d)	Improper washing conditions	Wash microplate wells thoroughly with Wash Buffer 6 times, either by overflowing the wells or using Automated Plate Washer.
e)	DR1 contamination of microplate wells	Make sure all work surfaces are clean and dry. Use care when using DR1. Avoid aerosols.
False-positive results obtained from known negative specimens		
a)	DR2 contaminated	Make sure not to cross-contaminate specimens as you aliquot DR2 between specimens. If only using part of a kit, aliquot the volume needed for that test into a clean disposable reagent reservoir prior to filling the pipet.
b)	DR1 contamination of microplate wells	Wash microplate wells thoroughly with Wash Buffer 6 times, either by overflowing the wells or using Automated Plate Washer. No residual pink liquid should be visible in the microplate wells after washing.
c)	Blotting on same area of Kimtowels wipers or equivalent low-lint paper towels over several rows	Do not blot on area that has been previously used.
d)	Inadequate specimen preparation	Add the correct volume of DNR and mix thoroughly by vortexing. To avoid false-positive results, make sure liquid washes entire inner surface of tube. For manual preparation of PreservCyt specimens, make sure that proper mixing and resuspension of the cell pellet is completed prior to denaturation incubation. Refer to the *digene* HC2 Sample Conversion Kit instructions for use. A distinct color change from clear to dark purple should be seen. Incubate for 45 ± 5 minutes at 65 ± 2°C. For manual preparation of SurePath specimens, make sure the specimens are incubated for 90 ± 5 minutes at 65 ± 2°C.

		Comments and suggestions
e)	Improper washing conditions	Wash microplate wells thoroughly with Wash Buffer 6 times, either by overflowing the wells or using the Automated Plate Washer.
f)	Contamination of pipet tip with undenatured material during transfer of denatured specimen to the hybridization microtube or hybridization microplate well	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. For PreservCyt or SurePath specimens in particular, these hybrids are likely to be present on the inside walls of the specimen denaturation tube. To prevent carryover of this non-denatured cellular material, the pipet tip must not touch the sides of the specimen denaturation tube during transfer of the denatured specimen to the hybridization microtube or hybridization microplate well.
Elevated NC RLU values (>200 RLU); remainder of test performs as expected		
a)	DR2 was incubated at a temperature greater than 20–25°C	Rerun the test and make sure that the capture and detection steps are incubated at 20–25°C.
b)	DR2 was incubated longer than 30 minutes	Read microplate after 15 minutes of incubation (and no later than 30 minutes of incubation) at 20–25°C.
c)	DR2 or Wash Buffer was contaminated with alkaline phosphatase or DR1	See "Contamination check of DR2," page 123, or "Contamination check of Wash Apparatus and/or water source", page 123.
Test fails assay validation; elevated PC$\bar{X}$/NC$\bar{X}$		
	Reverse placement of the HRC and the QC2 HR	Retest specimens. Carefully read the labels on calibrator and quality control vials to prevent reversing the placement of these reagents.

Contamination check of DR2

1. Pipet 75 µl of the aliquoted, residual or original vial of DR2 into a blank capture microplate well.

   Note: Testing the DR2 in replicates of 3 provides optimal assessment of performance.
2. Incubate 20–25°C for 15 minutes. Avoid direct sunlight.
3. Measure the microplate using a DML instrument.

   The DR2 control should be <50 RLU.

   If DR2 values are <50 RLU, the DR2 can be used to repeat the test.

   If contaminated (>50 RLU), obtain a new kit and repeat the test.

Contamination check of Wash Apparatus and/or water source

1. Label wells 1–4. Pipet 75 µl of DR2 into 4 separate capture microplate wells.

   Well 1 serves as the DR2 control.
2. Pipet 10 µl of Wash Buffer from the Wash Bottle into microplate well 2.
3. Allow Wash Buffer to flow through the washer tubing. Pipet 10 µl of the Wash Buffer from the tubing into microplate well 3.
4. Obtain an aliquot of the water used to prepare the Wash Buffer. Pipet 10 µl of the water into microplate well 4.
5. Incubate 20–25°C for 15 minutes. Avoid direct sunlight.
6. Measure the microplate using a DML instrument.

   The DR2 control (well 1) should be <50 RLU.

   Compare the RLU from wells 2, 3 and 4 to the DR2 control RLU. The individual RLU for wells 2, 3 and 4 should not exceed 50 RLU of the DR2 control RLU.

   Values exceeding 50 RLU of the DR2 control indicate contamination. See “Manual washing method”, page 52, for instructions on cleaning and maintenance of the Wash Apparatus.

Contamination check of Automated Plate Washer

1. Label wells 1–5. Pipet 75 µl of DR2 into 5 separate capture microplate wells.

   Well 1 serves as the DR2 control.
2. Pipet 10 µl of Wash Buffer from the Plate Washer Wash Bottle into microplate well 2.
3. Pipet 10 µl of the rinse liquid from the Plate Washer Rinse Bottle into microplate well 3.
4. Press the **Prime** button on the Plate Washer keypad, allowing Wash Buffer to flow through the lines. Pipet 10 µl of the Wash Buffer from the trough into microplate well 4.
5. Press the **Rinse** button on the Plate Washer keypad, allowing the rinse liquid to flow through the lines. Pipet 10 µl of the Wash Buffer from the trough into microplate well 5.
6. Cover and incubate 15 minutes at 20–25°C. Avoid direct sunlight.
7. Measure the microplate using a DML instrument.

   The DR2 control (well 1) should be <50 RLU.

   Compare the RLU from wells 2, 3, 4 and 5 to the DR2 control RLU. The individual RLU for wells 2, 3, 4 and 5 should not exceed 50 RLU of the DR2 control RLU.

   Values exceeding 50 RLU of the DR2 control indicate contamination of the Plate Washer.

   Refer to *Automated Plate Washer User Manual* for the decontamination procedure.

Contact Information

Use the QIAGEN contact information sheet provided in the test kit to contact your local QIAGEN representative.

This page intentionally left blank

This page intentionally left blank

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

Hybrid Capture technology is covered by European Patent No. 0 667 918 registered in Austria, Belgium, Switzerland, Liechtenstein, Germany, Denmark, Spain, France, United Kingdom, Greece, Ireland, Italy, Luxembourg, Netherlands and Sweden.

U.S. Hybrid Capture Patent

6,228,578B1

U.S. HPV Patents

5,876,922 • 5,952,487 • 5,958,674 • 5,981,173

1058538 08/2015