digene® HPV Genotyping PS Test
Instructions For Use

IVD

Σ 96

For the qualitative detection of human papillomavirus (HPV) types 16, 18, and 45. For use with the *digene* HC2 High-Risk HPV DNA Test.

CE

REF 613615

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Sample & Assay Technologies

Contents

Intended Use

The *digene* HPV Genotyping PS Test is a reflex test intended for the qualitative detection of high-risk HPV types 16, 18, and 45 following a positive *digene* HC2 High-Risk HPV DNA Test result (qualitative detection of 13 high-risk types). The identification of high-risk HPV types 16, 18, and 45 provides additional information to aid in the clinical management of women in cervical cancer screening programs.

Cervical specimens that may be tested with the *digene* HPV Genotyping PS Test include the following:

- Specimens collected with the *digene* HC2 DNA Collection Device
- Specimens collected using a broom-type collection device or brush/spatula combination collection device, then placed in PreservCyt® Solution

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, and cervical, vaginal, and vulvar intraepithelial neoplasia and carcinoma (1–3). It is generally accepted that these viruses are predominantly sexually transmitted and that the high-risk HPV types are the major recognized risk factor for development of cervical cancer (4–8).

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

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.

Women with normal cytology who concurrently test positive for HPV 16 or 18 have an estimated probability of 26.7% and 19.1%, respectively, of developing cervical intraepithelial neoplasia (CIN) 3 or worse within 12 years of follow-up (9). In addition, HPV types 16, 18, and 45 were the 3 most common genotypes found in squamous cell carcinoma, adenocarcinoma, and adenosquamous cell carcinoma. The 3 HPV types together accounted for 75% of the cases of squamous cell carcinoma and 94% of the cases of adenocarcinoma (10). It has also been shown that tumors containing HPV 18 or 45 are greater than 2 times more likely to cause death than tumors containing other HPV types (11).

Principle of the Procedure

The *digene* HPV Genotyping PS Test, using Hybrid Capture® 2 (HC2) technology, is an in vitro nucleic acid hybridization assay that uses type-specific oligoribonucleotides, antibody capture, and qualitative chemiluminescent signal detection. The test, performed in triplicate for each cervical specimen, genotypes for 3 high-risk types (16, 18, and 45) of HPV DNA in cervical specimens.

Denaturation

Cervical specimens are treated with a base solution, which disrupts the virus and releases the target DNA.

Hybridization

The target HPV DNA hybridizes with specific RNA probes creating DNA–RNA hybrids.

Hybrid capture

Antibodies, specific to the DNA–RNA hybrids and coated onto the surface of a microplate well, capture the DNA–RNA hybrids.

Hybrid detection

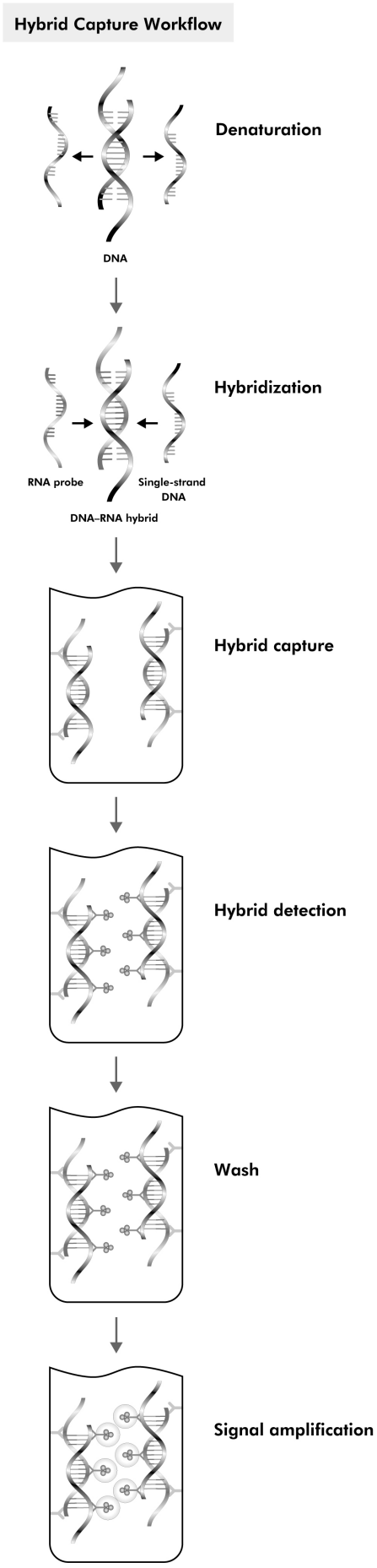
With the addition of Detection Reagent 1 (DR1), the immobilized DNA–RNA hybrids react with alkaline phosphatase-conjugated antibodies specific to the DNA–RNA hybrids. Several alkaline phosphatase molecules are conjugated to each antibody and multiple antibodies bind to each immobilized DNA-RNA hybrid, resulting in substantial signal amplification.

Wash

The DNA–RNA hybrid complex is washed to remove unbound material.

Signal amplification

With the addition of Detection Reagent 2 (DR2), a chemiluminescent reaction occurs as the substrate is cleaved by the bound alkaline phosphatase. The emitted light is measured as relative light units (RLU) on a DML instrument. The intensity of the light emitted denotes the presence of target DNA in the specimen.


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

Materials Provided

Kit contents

***digene* HPV Genotyping PS Test**	**(96)**
Catalog no.	**613615**
Number of tests*	**96**
HPV Negative Control 1	1 ml
HPV Positive Control 1	1 ml
HPV Negative Control 2	1 ml
HPV Positive Control 2	1 ml
Denaturation Reagent	12 ml
Indicator Dye	0.35 ml
Probe Diluent	5.5 ml
Detection Reagent 1	12 ml
Detection Reagent 2	12 ml
Wash Buffer x15	2 x 100 ml
HPV 16 Probe ASR	110 µl
HPV 18 Probe ASR	100 µl
HPV 45 Probe ASR	100 µl
5.5% NP-40	2 x 1.5 ml
Capture Microplate	1

* The number of test results will vary depending on the number of uses per kit as controls are required for each performance of the test. The number of allowed freeze/thaw cycles of the denatured controls may also be a factor in the number of uses per kit. See "Optional stop point," page 29, for additional information.

Materials Required but Not Provided

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDS), available from the product supplier.

Equipment and materials 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"), LumiCheck Plate Software, and digene *HC2 System Software User Manual*
- Hybrid Capture System Rotary Shaker I
- Hybrid Capture System Automated Plate Washer
- Hybrid Capture System Multi-Specimen Tube (MST) Vortexer 2 (optional)
- Hybridization microplates
- Microplate lids
- Extra-long pipet tips
- Disposable reagent reservoirs
- DuraSeal™ tube sealer film
- Plate sealers

General Laboratory-Use Equipment and Accessories

- Incubator shaker capable of maintaining a temperature of 55 ± 2°C and shaking at 1100 ± 100 rpm
- 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 (multi-channel); 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
- Alcohol wipes
- Disposable bench cover
- Powder-free, disposable gloves
- 1.5 ml and/or 5 ml round-bottom polypropylene tubes
- Floating microcentrifuge tube rack

Additional equipment and materials for PreservCyt specimen preparation

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

Warnings and Precautions

For in vitro diagnostic use.

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: 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 appropriate 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 (12, 13).
- Decontaminate and dispose of all specimens, reagents, and other potentially contaminated materials in accordance with local and national regulations.

Following denaturation and incubation, the specimens are no longer considered infectious (14); 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.

Safety and risk statements for components

The following risk and safety phrases apply to components of the *digene* HPV Genotyping PS Test:

5.5% NP-40

Contains: Ethoxylated nonylphenol. Warning! Causes mild skin irritation. 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.

Detection Reagent 1

Harmful to aquatic life with long lasting effects. Avoid release to the environment.

HPV Negative Control 1

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

advice/ attention.

HPV Positive Control 1

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

HPV Positive Control 2

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

HPV Negative Control 2

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

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.

Wash Buffer, 15x

Contains: Sodium azide. Warning! Harmful if swallowed. Causes mild skin irritation. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Dispose of contents/ container to an approved waste disposal plant. If skin irritation occurs: Get medical advice/ attention. IF SWALLOWED: Immediately call a POISON CENTER or doctor/ physician. Wear protective gloves/ protective clothing/ eye protection/ face protection.

Further information

Safety Data Sheets: www.qiagen.com/safety

Precautions

The user must always adhere to the following precautions when performing the *digene* HPV Genotyping PS 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* HPV Genotyping PS Test procedure, assay calibration, quality control, and interpretation of 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. Ensuring that the noted color changes occur will confirm that these conditions have been met.
- The kit components have been tested as a unit. Do not interchange components from other sources or from different lots.
- Nucleic acids are very sensitive to environmental nuclease degradation. Nucleases are present on human skin and on surfaces or materials handled by humans. Clean and cover work surfaces with a disposable bench cover and wear disposable, powder-free gloves when performing all test steps.
- Cervical specimens may contain blood or other biological material that may mask the color changes of the samples. Specimens that exhibit a dark color may not give the described color change. In these cases, failure to exhibit the color change will not affect the results of the test. Verify proper mixing by observing the color change of the controls.
- Make sure to prevent contamination of the capture microplate and the DR2 with exogenous alkaline phosphatase during performance of the test. Substances that may contain alkaline phosphatase include 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 positive displacement pipet in advance of reagent delivery, and check for large air bubbles periodically. Excessive amounts of large air bubbles in the 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 "Protocol 4: Hybrid detection," page 35) 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 "Protocol 5: Wash," page 37). 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 x15, Denaturation Reagent, and Indicator Dye may be stored at room temperature (15–30°C), as desired. All reagents are provided ready-to-use, except the denaturation reagent (DNR), the probe mixes, 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.

Specimen Collection and Preparation

Collect and transport cervical specimens for testing with the *digene* HPV Genotyping PS Test using one of the following specimen collection devices:

- *digene* HC2 DNA Collection Device [consisting of a cervical brush and Sample Transport Medium (STM)]
- A broom-type collection device or combination brush/spatula collection device placed in PreservCyt Solution

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

Refer to the *digene* HC2 DNA Collection Device instructions for use for additional specimen collection and handling procedures.

Cervical specimens in STM

Cervical specimens collected in STM do not require sample preparation prior to testing with the *digene* HPV Genotyping PS Test.

As the *digene* HPV Genotyping PS Test is a reflex for the *digene* HC2 High-Risk HPV DNA Test, STM specimens will have been previously denatured and are ready to proceed to "Protocol 2: Probe mix addition" of the test. At least 225 µl of each denatured STM specimen must be available to perform the *digene* HPV Genotyping PS Test.

Cervical specimens in PreservCyt Solution

PreservCyt specimens require sample preparation prior to testing with the *digene* HPV Genotyping PS Test.

Collect specimens in the routine manner, and prepare the ThinPrep® Pap Test slides according to the manufacturer-provided instructions. Following collection, store the PreservCyt specimens for up to 3 months at 2–30°C prior to sample preparation for the *digene* HPV Genotyping PS Test. PreservCyt specimens cannot be frozen.

The result of sample preparation using the *digene* HC2 Sample Conversion Kit is a denatured sample ready to proceed to "Protocol 2: Probe mix addition" of the test.

Procedure

Things to do before starting

- Allow at least 60 minutes for the incubator shaker to equilibrate to 55°C ± 2°C from a cold start. Not allowing time for this warm-up period could cause inaccurate test results.
- Make sure that the waterbath is at 65°C and the water level is adequate to immerse the entire volume of liquid in the tubes.
- Print a new "Test Data Recording Worksheet," and record the following information (see "Appendix B: Test Data Recording Worksheet", page 69):
  - Test site
  - Test date
  - Operator ID
  - Ambient temperature
  - *digene* HPV Genotyping PS Test kit lot number
- The controls and samples are run in an 8-microplate well column configuration. Complete the "Test Data Recording Worksheet" by recording the IDs of all required controls and samples in the microplate well locations. For each test, the controls must be tested in the following positions on the microplate (see Figure 1, below):
  - Negative Control 1 (NC1) replicates in microplate wells A1, B1, and C1
  - Positive Control 1 (PC1) replicates in microplate wells D1, E1, and F1
  - Negative Control 2 (NC2) with probe mix 16 in microplate well G1
  - NC2 with probe mix 18 in microplate well H1
  - NC2 with probe mix 45 in microplate well A2
  - Positive Control 2 (PC2) with probe mix 16 in microplate well B2
  - PC2 with probe mix 18 in microplate well C2
  - PC2 with probe mix 45 in microplate well D2

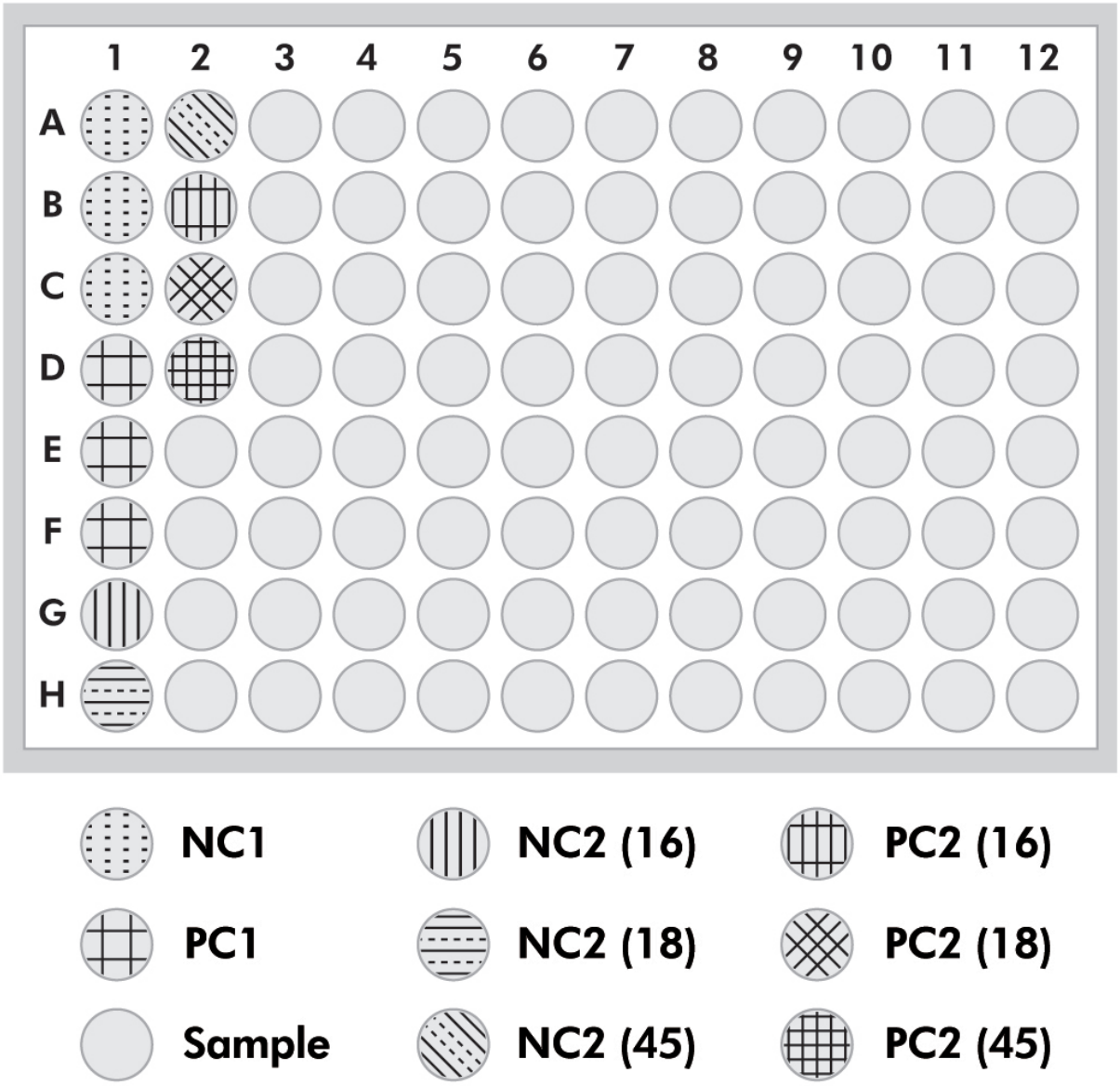


Figure 1. Position of controls and samples on microplate.

Reagent preparation

Important points before starting

- Prepare PreservCyt specimens prior to equilibrating any previously denatured specimens and reagents to room temperature.
- Remove the specimens and all required reagents from storage prior to beginning the test. Allow them to reach 20–25°C for 15–30 minutes.
- Discard all prepared reagents (unless specified differently) and reagent aliquots at the end of the test.
- Use Table 1, below, to determine the volume required for each reagent based on the number of tests.

Table 1. Required volumes of prepared and ready-to-use reagents

Number of tests/strips*	HPV 16 probe mix	HPV 18 probe mix	HPV 45 probe mix	Wash buffer	DR1	DR2
4/5	0.77 ml	0.56 ml	0.56 ml	>1 liter	2.55 ml	2.55 ml
8/5	0.91 ml	0.70 ml	0.70 ml	>1 liter	3.45 ml	3.45 ml
12/8	1.05 ml	0.84 ml	0.84 ml	>1 liter	4.35 ml	4.35 ml
16/8	1.19 ml	0.98 ml	0.98 ml	>1 liter	5.25 ml	5.25 ml
20/11	1.33 ml	1.12 ml	1.12 ml	>1 liter	6.15 ml	6.15 ml
24/11	1.47 ml	1.26 ml	1.26 ml	>1 liter	7.05 ml	7.05 ml
28/12	1.61 ml	1.40 ml	1.40 ml	>1 liter	7.95 ml	7.95 ml

* Number of tests/strips is based on testing one specimen for HPV types 16, 18, and 45 and includes the reagent amount required for the controls.

Capture Microplate

The capture microplate contains12 eight-well strips of capture wells in a plate frame. Any capture microplate wells not used during the test need to be

removed from the plate frame, returned to the foil packaging, and stored in order to be used for additional testing.

With a marker, label the capture microplate with an appropriate identifier.

Determine the number of capture microplate wells required based on the completed Test Data Recording Worksheet.

1. **Place the capture microplate upside down on a clean KimTowels wipers or**
2. **equivalent low-lint paper towel.**
3. **Using a gloved finger, or the eraser on a pencil, push the capture microplate well strips that will not be used from the plate frame.**
4. **Return the removed capture microplate well strips to the foil packaging, close, and store.**
5. **Note**: The capture microplate is stored at 2–8°C.

Denaturation reagent

Use caution when handling the Denaturation Reagent. See page 12 for warnings and precautions.

1. **Add 2 drops of Indicator Dye to the bottle of Denaturation Reagent.**
2. **Mix the DNR thoroughly.**
3. The DNR should be a uniform, dark purple color.

Label the DNR with the new expiration date.

Notes:

- Once prepared, the DNR is stable for 3 months at 2–8°C.
- If the color fades, add 1 additional drop of Indicator Dye and mix thoroughly before using.

Probe mixes

Testing each specimen for HPV types 16, 18, and 45 requires a specific probe mix for each type for a total of 3 probe mixes per specimen. An additional amount of the HPV 16 probe mix is required as it is used for 8 control microplate wells. An additional amount is required of the HPV 18 and HPV 45 probe mixes, as each is used for 2 control microplate wells.

Prepare each probe mix individually as described below.

Important points before starting

- Prepare the probe mixes during "Protocol 1: Denaturation," (see page 28).
- Take extreme care to prevent RNase contamination. Use aerosol-barrier pipet tips when pipetting the probe.
- Probe Diluent is viscous. Make sure a visible vortex is achieved when preparing the probe mixes; incomplete mixing may result in reduced signal.

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 volumes required for a 1:24:10 dilution of probe: Probe Diluent: 5.5% NP-40 to prepare the probe mix.**

   Note: 35 µl of probe mix is required per microplate well.

   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. Use the suggested volumes (see Table 1, page 22) which includes the recommended extra volume.
4. **Appropriately label a new, disposable container.**
5. Depending on the number of tests, either a 1.5 ml or 5 ml round-bottom, polypropylene tube is recommended.
6. **Pipet the required amount of Probe Diluent (see Table 2, below) into the labeled tube.**

   Pipet the required amount of the probe into the Probe Diluent (see Table 2, 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 pipet tip into the Probe Diluent.

Pipet the required amount of 5.5% NP-40 (see Table 2, below) into the labeled tube.

Table 2. Preparation of probe mix

Volume of probe mix required	Volume of Probe Diluent	Volume of probe	Volume of 5.5% NP-40
0.56 ml	384 µl	16 µl	160 µl
0.70 ml	480 µl	20 µl	200 µl
0.77 ml	528 µl	22 µl	220 µl
0.84 ml	576 µl	24 µl	240 µl
0.91 ml	624 µl	26 µl	260 µl
0.98 ml	672 µl	28 µl	280 µl
1.05 ml	720 µl	30 µl	300 µl
1.12 ml	768 µl	32 µl	320 µl
1.19 ml	816 µl	34 µl	340 µl
1.26 ml	864 µl	36 µl	360 µl
1.33 ml	912 µl	38 µl	380 µl
1.40 ml	960 µl	40 µl	400 µl
1.47 ml	1008 µl	42 µl	420 µl
1.61 ml	1056 µl	44 µl	440 µl

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

A visible vortex must be produced.

Wash buffer

Important points before starting

- Prepare the wash buffer during "Protocol 3: Hybridization and hybrid capture," (see page 33).
- For the manual microplate washing method, prepare 3 liters of 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.

1. **Mix the Wash Buffer x15 well and add the required volume of Wash Buffer x15 (see Table 3, below) to the specified container.**
2. **Add the required volume of distilled or deionized water (see Table 3, below) to the specified container.**

Table 3. Preparation of wash buffer

Volume of wash buffer required	Volume of Wash Buffer x15	Volume of distilled or deionized water
1 liter	67 ml	933 ml
1.5 liters	100 ml	1400 ml
2 liters	133 ml	1867 ml
3 liters	200 ml	2800 ml

3. **Place a clean, low-lint paper towel over any openings of the container and mix well.**

Seal the container or place on the respective instrument, as appropriate.

Label the wash buffer with the new expiration date.

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

4.
5.

Sample preparation

PreservCyt specimens require sample preparation prior to testing with the *digene* HPV Genotyping PS Test. The *digene* HPV Genotyping PS Test requires 225 µl of prepared sample to test for HPV types 16, 18, and 45. Properly stored and previously prepared PreservCyt samples may be used to perform this test. If an insufficient amount of prepared PreservCyt sample remains, a minimum of 6 ml of PreservCyt specimen must be prepared to perform the test.

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

The result of sample preparation using the *digene* HC2 Sample Conversion Kit is a denatured sample ready to proceed to "Protocol 2: Probe mix addition" of the test (see page 30). Prepare the controls separately according to "Protocol 1: Denaturation," (see page 28).

Protocol 1: Denaturation

The controls required with the *digene* HPV Genotyping PS Test are denatured according to the instructions below.

Important points before starting

- Do not denature STM specimens according to "Protocol 1: Denaturation". STM specimens were previously denatured as a result of testing with the *digene* HC2 High-Risk HPV DNA Test and are ready to proceed to "Protocol 2: Probe mix addition" of the test.
- Do not denature PreservCyt specimens or samples according to "Protocol 1: Denaturation". Denaturation of PreservCyt specimens is performed as part of the sample preparation. The prepared PreservCyt samples are ready to proceed to "Protocol 2: Probe mix addition" of the test.
- Make sure to not spill the controls or splash the DNR while performing this protocol. The addition of DNR to the control tube fills the tube such that the liquid is near the top. See page 12 for warnings and precautions.

Things to do before starting

- Make sure there is enough water in the waterbath to immerse the entire volume of the tubes.
- To avoid false-positive results, it is critical that all control material come into contact with the DNR.

1.

2. **Label the caps of the NC1, PC1, NC2, and PC2.**

   The caps will be reused to close the control tubes.

3. **Remove the caps from the NC1, PC1, NC2, and PC2, and place the caps away from possible contamination.**

   Using a single-channel pipet with a new pipet tip for each addition, pipet 500 µl of DNR into each control tube by placing the pipet tip below the surface of the liquid and dispensing.

Return the cap to each respective control and close the tube.

Make sure the caps are screwed on tightly to avoid contamination or spilling.

4. **Mix each tube thoroughly by pulse vortexing individually, at high speed, for at least 30 seconds.**

Important: Mixing after the DNR addition is a critical step.

5. **Incubate the tubes in a floating microcentrifuge tube rack in a 65 ± 2°C waterbath for 45 ± 5 minutes.**

6. Prepare the required probe mixes during this incubation (see "Reagent preparation," page 22).

Remove the tubes from the waterbath after the incubation.

7. The denatured controls may be:

- Tested immediately (proceed to "Protocol 2: Probe mix addition," page 30)
- Stored (see "Optional stop point," page 29)

Optional stop point

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

Denatured controls may be stored at 2–8°C overnight or at –20°C according to the following:

- A maximum of one freeze/thaw cycle may be performed for storage of up to 4 weeks.
- A maximum of 3 freeze/thaw cycles may be performed for storage of up to 9 days.

For each freeze/thaw cycle, a maximum of 2 hours at room temperature is allowed during the thaw cycle.

Protocol 2: Probe mix addition

Important points before starting

- Probe mix is viscous. Make sure the probe mix is thoroughly mixed and that the required amount is completely dispensed into each hybridization microplate well.
- When transferring the controls and samples to the hybridization microplate, adhere to the following:
  - Avoid touching the sides of the hybridization microplate wells 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.

Things to do before starting

- If the denatured controls or samples have been stored, allow them to equilibrate to 20–25°C.
- If the STM or PreservCyt samples have been stored in a specimen rack and the MST Vortexer 2 will be used for vortexing, remove and discard the caps from the tubes.

1.

2. **Obtain and label a hybridization microplate.**

   Vortex the control and sample tubes using one of the following methods:

   <u>Any sample type with vortexer</u>

   a. Vortex each tube individually for at least 5 seconds on the maximum speed setting.

   b. **<u>STM samples with MST Vortexer 2</u>**

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

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

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.

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

d. **<u>PreservCyt samples with MST Vortexer 2</u>**

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

a. Vortex the specimen rack for a minimum of 10 seconds on the maximum speed setting.

b. 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.

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

3. **Using a single-channel pipet with extra-long pipet tips, transfer 75 µl of each control or sample to the bottom of the empty hybridization microplate well according to Test Data Recording Worksheet.**

As applicable, store the remaining volume according to the following instructions:

- Cap the denatured controls with the original caps and store according to the limits detailed in "Optional stop point," page 29.
- Cap the STM samples with new specimen collection tube screw caps and store according to the instructions in the *digene* HC2 High-Risk HPV DNA Test instructions for use.
- Cap the PreservCyt samples with a new cap and store according to the instructions in the *digene* HC2 Sample Conversion Kit instructions for use.

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

Thoroughly vortex the probe mixes.

4. **Carefully pipet 35 µl of the appropriate probe mix into each hybridization microplate well using a single-channel or repeating positive displacement pipet and a new tip for each probe mix addition.**

5.

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

Cover the hybridization microplate with a microplate lid or plate sealer and shake for 3 ± 2 minutes on the Rotary Shaker I set at 800 ± 100 rpm.

7. **Remove the hybridization microplate from the Rotary Shaker I. Remove the microplate lid and place it on a clean surface.**

8. After shaking, the controls and STM 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 35 µl of the appropriate probe mix to the samples that remain purple and shake again for 3 ± 2 minutes on the Rotary Shaker I set at 800 ± 100 rpm.

If a sample remains purple after following this procedure, retest the specimen.

9.

Proceed to "Protocol 3: Hybridization and hybrid capture," page 33.

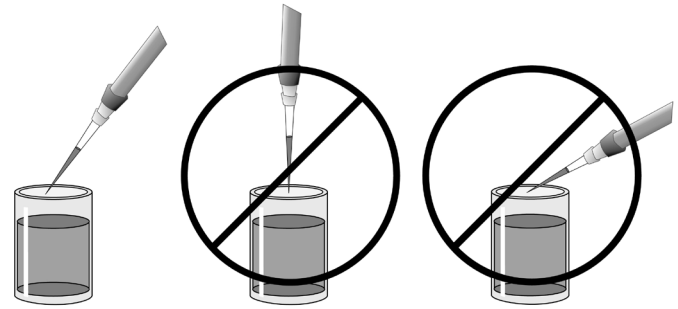
Protocol 3: Hybridization and hybrid capture

1. **Place the hybridization microplate next to the capture microplate.**

   Using an 8-channel pipet, transfer the entire contents (approximately 110 µl) of the hybridization microplate wells to the bottom of the corresponding capture microplate wells.

2. Use new pipet tips for each transfer, and allow each pipet tip to drain to ensure complete sample transfer. If desired, steady the pipet by resting the middle of the pipet tips on the top edge of the capture microplate wells (see Figure 2, below).

   Note: For a small number of samples, a single-channel pipet with 20–200 µl pipet tips may be used instead of an 8-channel pipet. Use new pipet tips for each transfer.



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

3. **Figure 2. Correct pipetting technique.**

4. **Cover the capture microplate with a new plate sealer, and incubate the capture microplate for 120 ± 5 minutes at 1100 ± 100 rpm in the incubator shaker equilibrated to 55 ± 2°C.**

   When the incubation is complete, remove the capture microplate from the incubator shaker, and carefully remove the plate sealer.

5. **Remove the liquid from the capture microplate wells by decanting into a sink; fully invert the capture microplate over the sink and shake hard with a downward motion.**

   Important: Do not reinvert the plate.

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

6. **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.

7. **Proceed to "Protocol 4: Hybrid detection," page 35.**

Protocol 4: Hybrid detection

Important points before starting

- Make reagent additions across the capture microplate in a left-to-right direction using an 8-channel pipet. Wipe the pipet tips on the disposable reagent reservoir to remove excess reagent before delivery to the capture microplate.
- If an 8-channel pipet is not used, an appropriate repeating positive displacement 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 is 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 specimens could occur (see Figure 2, page 33).

1. **Mix the DR1 thoroughly, and carefully measure the appropriate volume (see Table 1, page 22) 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 the 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 back into the reagent.
   f. Refill tips and repeat until all microplate wells are filled.

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

Cover the capture microplate with a microplate lid, clean Parafilm, or equivalent, and incubate for 30–35 minutes at 20–25°C.

Proceed to "Protocol 5: Wash," page 37.

3.

4.

Protocol 5: Wash

Wash the capture microplate using one of the methods below.

Automated Plate Washer method

Always keep the Automated Plate Washer powered ON. The Automated Plate Washer will routinely rinse the system for cleaning. Refer to *Automated Plate Washer User Manual* for further instructions.

Things to do before starting

- Verify that the wash reservoir is filled with at least 1 liter of wash buffer. If not, prepare the wash buffer (see "Reagent preparation," page 22).
- Verify that the rinse reservoir is filled with deionized or distilled water.
- Verify 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.
- The Automated Plate Washer washes only complete strips and cannot skip wells or strips. If only a partial strip of capture microplate wells is being used, place empty microplate wells in the plate frame to complete the strip prior to washing. All strips washed must have microplate wells.

1.

2. **Remove the capture microplate lid, clean Parafilm, or equivalent, and place the capture microplate on the Automated Plate Washer platform.**
3. **Verify that the Automated Plate Washer is powered ON, and that the display shows "Digene Wash Ready" or "P1".**
4. **Select the number of strips to be washed by pressing the "Rows" key and then "+" or "–" to adjust.**

   Press the "Rows" key to return to "Digene Wash Ready" or "P1".

Press "Start/Stop" to begin.

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

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

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

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

Proceed to "Protocol 6: Signal amplification," page 40.

7.

Manual Plate Washing

To do before starting: Verify that the Wash Apparatus is filled with at least 1 liter of wash buffer.

1. **Remove the capture microplate lid, clean Parafilm, or equivalent.**
2. **Place clean KimTowels wipers or equivalent low-lint paper towels on top of the capture microplate.**
3. **Make sure that the paper towels are in contact with the entire surface area of the capture microplate, and carefully invert the capture microplate.**
4. **Allow the capture microplate to drain for 1–2 minutes.**
5. **Blot the capture microplate on clean KimTowels wipers or equivalent low-lint paper towels.**
6. Carefully discard the used paper towels to avoid alkaline phosphatase contamination.

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 of the capture microplate.

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 it to drain for 5 minutes. Replace the paper towels and blot again.**
8. The capture microplate should appear white, and no pink residual liquid should remain in the capture microplate wells.

Proceed to "Protocol 6: Signal amplification," page 40.

9.

Protocol 6: Signal amplification

Important points before starting

- Use a new pair of powder-free, disposable 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, an appropriate repeating positive displacement 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 not to touch the sides of the capture microplate wells or splash reagent onto the pipet tips because cross-contamination of specimens could occur (see Figure 2, page 33).

1. **Mix the DR2 thoroughly, and measure the appropriate volume (see Table 1, page 22) into a clean, disposable reagent reservoir.**
2. **Pipet 75 µl of DR2 into each capture microplate well using the reverse pipetting technique previously described (see "Protocol 4: Hybrid detection," page 35).**
3. Verify that all the capture microplate wells have been filled accurately by observing the intensity of the yellow color; all the capture microplate wells should have a similar yellow intensity.
4. **Cover the capture microplate with a microplate lid and incubate at 20–25°C for 15 minutes (and no longer than 30 minutes of incubation).**

   Avoid direct sunlight.

Proceed to "Protocol 7: Measuring the capture microplate and generating results," page 41.

Protocol 7: Measuring the capture microplate and generating results

1. **After incubation is complete, measure the capture microplate using the raw data measurement function of a DML instrument.**

   Refer to the respective software user manual for details about measuring a capture microplate.

2. **Print the raw data report.**

   Important: The raw data report must be printed after measurement. The raw data report cannot be saved using the *digene* assay analysis software.

3. **Appropriately label the raw data report with the information specified on the Test Data Recording Worksheet.**

4. **If a full capture microplate was not used, remove the used capture microplate wells from the plate frame, rinse the plate frame thoroughly with distilled or deionized water, dry, and reserve for the next test.**

5. **Discard all reagent aliquots and prepared reagents, unless otherwise specified.**

Interpretation of Results

The *digene* HPV Genotyping PS Test cutoff (CO) corresponds to 5,000 copies of HPV 16, 18, 45 plasmids.

Important: Before interpreting the test results, complete all quality control and assay calibration verification requirements (see "Quality Control," page 52).

Results of testing

The CO for determining positive specimens is the PC1 mean. Using the calculated PC1 mean during assay calibration verification, determine the RLU/CO for each specimen. The test results are determined as follows:

- Samples with a RLU/CO value ≥2.0 are considered "positive" for that HPV type.
- Samples with a RLU/CO value <2.0 are considered "negative" or "no HPV DNA detected" for that HPV type. HPV DNA sequences are either absent or the HPV DNA levels are below the detection limit of the test.

Troubleshooting guide

		Comments and suggestions
Improper or no color change observed during the denaturation of the controls		
a)	Incorrect preparation of the DNR	Verify that the DNR contains the Indicator Dye and is a dark purple color.
b)	The DNR was not added to the controls	Verify that the DNR was added to the controls by measuring the volume in the microplate. The volume should be 75 µl. If the volume indicates that the DNR was not added, make the appropriate addition of DNR, mix, and proceed with the test if the proper color change is observed.

	Comments and suggestions
Quality controls give incorrect results	
Incorrect placement of controls on microplate	Adjust the assay calibration verification procedure so that it matches the "Test Data Recording Worksheet." Make sure to match the results with the appropriate assay calibration verification criteria.
Improper color change observed during probe mix addition	
a) Inadequate mixing of the probe mix with the controls and/or samples or the incorrect volume of probe mix was added	Shake the hybridization microplate for an additional 2 minutes. If there are samples that still remain purple, add an additional 35 µl of the appropriate probe mix and mix well by shaking for 3 minutes at 800 ± 100 rpm. 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) The specimen contains blood or other materials that mask the color change	The described color change is not expected with these specimens; test results should not be adversely affected.
Test fails assay calibration verification; no signal in controls or in samples	
a) The probe mix was prepared without the probe	See "Reagent preparation," page 22, for instructions on preparing the probe mix.

		Comments and suggestions
b)	Contamination of the probe mix with RNase during preparation	Wear disposable, powder-free gloves and use aerosol-barrier pipet tips when pipetting the probe and probe mix. Prepare the probe mix in sterile containers.
c)	Inadequate mixing during the preparation of the probe mix	After adding the probe to the probe mix, vortex thoroughly at high speed for at least 5 seconds. A visible vortex must be produced.
d)	Incorrect time or temperature during "Protocol 3: Hybridization and hybrid capture"	Retest the specimens.
e)	Failure to add the correct amount of DR1 or incubate for the specified time	Pipet 75 µl of the DR1 into each microplate well using an 8-channel pipet. Incubate at 20–25°C for 30–35 minutes.
f)	Failure to add the correct amount of DR2 or to incubate for the specified time	Pipet 75 µl of DR2 into each microplate well using an 8-channel pipet. Incubate at 20–25°C for 15–30 minutes.
g)	The DML instrument is malfunctioning	Refer to the maintenance, service, and troubleshooting sections in the appropriate user manual for further instructions, or contact QIAGEN Technical Services.

	Comments and suggestions
Elevated RLU (≥200 RLU) of controls and/or samples; test may fail assay calibration verification criteria	
a) Incorrect volume of the DNR added or inadequate mixing	Verify that the repeating positive displacement pipet is delivering accurately prior to adding the DNR. Calibrated pipets are essential. Add a half-volume of DNR to each microplate well and mix well. To avoid false-positive results, make sure the liquid washes the entire inner surface of the microplate well. Controls should turn purple after the addition of the DNR.
b) Light leak in the DML instrument	Perform a 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 may exist. Refer to the maintenance, service, and troubleshooting sections in the appropriate user manual for further instructions, or contact QIAGEN Technical Services.
c) Contamination of the DR2 or negative controls with DR1 or exogenous alkaline phosphatase	See "Appendix A: Contamination Evaluation Procedures", page 66.
d) Contamination of the wash buffer	See "Appendix A: Contamination Evaluation Procedures", page 66.
e) Contamination of the Automated Plate Washer	See "Appendix A: Contamination Evaluation Procedures," page 66.

		Comments and suggestions
f)	Inadequate washing of the capture microplate wells after the DR1 incubation	Wash the microplate wells thoroughly with the wash buffer 6 times, overflowing the microplate wells each time, or use 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)	Contamination of the negative controls with DR1	Make sure all work surfaces are clean and dry. Use care when using the DR1. Avoid creating aerosols.
h)	Contamination of the of the capture microplate during decanting and blotting	Always blot the capture microplate on new, clean KimTowels wipers or equivalent low-lint paper towels. Do not blot the capture microplate on previously used paper towels, as cross-contamination could occur.
i)	Use of incorrect blotting paper towels	Use KimTowels wipers or equivalent low-lint paper towels for blotting.
j)	PC2 material used as PC1 or test fails assay verification of PC1/NC1 ratio.	Repeat the test and make sure that the appropriate controls are used.

	Comments and suggestions
Low PC/NC ratio or high number (>20%) of low-positive specimens; test may fail assay calibration verification criteria	
a) Inadequate sample preparation	During denaturation, make sure to add the correct volume of the DNR to each sample. Mix thoroughly by vortexing. To avoid false-positive results, make sure the liquid washes the entire inner surface of the tube. During the sample preparation of PreservCyt specimens, make sure the sample is properly mixed and the resuspension of the cell pellet is complete prior to the denaturation incubation. Refer to the *digene* HC2 Sample Conversion Kit instructions for use for additional information. The sample should undergo a distinct color change to dark purple. Incubate for 45 ± 5 minutes at 65 ± 2°C.
b) Inadequate denaturation of the controls	Make sure that the temperature of the waterbath is accurate and there is enough water in the waterbath to immerse the entire volume of the tubes. Make sure the controls are thoroughly mixed after the addition of the DNR.
c) Inadequate mixing of the probe mix or insufficient probe mix added to the controls and/or samples	During probe mix preparation, vortex the probe mix thoroughly and make sure a vortex is visible. The probe mix must be added to the microplate wells with a repeating positive displacement pipet or a multi-channel pipet to make sure of accurate delivery. See "Reagent preparation," page 22, for additional instructions.

		Comments and suggestions
d)	Inadequate volume of the probe mix added to the controls and/or samples	Verify that the pipet is delivering accurately prior to adding the probe mix to the controls and/or samples.
e)	Loss of DR1 activity	Makes sure to store the DR1 at the specified temperature (see "Reagent Storage and Handling," page 17).
f)	Insufficient capture of the DNA–RNA hybrids	Make sure the incubator shaker is functioning properly and is within calibration.
g)	Inadequate washing	Wash the microplate wells thoroughly with the wash buffer 6 times, overflowing the microplate wells each time, or use the Automated Plate Washer.
h)	Contamination of the wash buffer	See "Appendix A: Contamination Evaluation Procedures," page 66.
Series of positive samples with RLU values approximately the same		
a)	Contamination of the capture microplate wells	Cover the capture microplate during all incubations. Avoid exposing the reagents and the capture microplate to aerosol contamination while performing the test. Wear powder-free gloves while performing the test.
b)	Contamination of the DR2	Make sure not to contaminate the DR2 when pipetting the DR2 into the capture microplate wells. Avoid contamination of the DR2 from aerosols.
c)	Malfunction of the Automated Plate Washer	Refer to *Automated Plate Washer User Manual* for instructions on testing for contamination or malfunctions.

	Comments and suggestions	
Large CV of control replicates		
a)	Inaccurate pipetting during performance of the test	Make sure that the pipet is delivering accurately. Calibrate the pipets used routinely.
b)	Insufficient mixing during performance of the test	Make sure to mix thoroughly at all steps, especially during the denaturation and after adding the probe mix.
c)	Incomplete transfer of the liquid from the hybridization microplate wells to the capture microplate wells	Make sure a complete liquid transfer from the hybridization microplate wells to the capture microplate wells occurs.
d)	Improper washing of the microplate wells	Wash the microplate wells 6 times thoroughly with wash buffer, overflowing the microplate wells each time, or use the Automated Plate Washer. No residual pink liquid should be visible in the microplate wells after washing.
e)	Contamination of the microplate wells with DR1	Make sure all work surfaces are clean and dry. Use care when using the DR1. Avoid creating aerosols.

	Comments and suggestions
False-positive results obtained from known negative specimens	
a) Inadequate sample preparation	Make sure to add the correct volume of the DNR to each control and sample. Mix thoroughly by vortexing. To avoid false-positive results, make sure the liquid washes the entire inner surface of the tube. During the sample preparation of PreservCyt specimens, make sure the sample is properly mixed and the resuspension of the cell pellet is complete prior to the denaturation incubation. Refer to the *digene* HC2 Sample Conversion Kit instructions for use for additional information. A distinct color change to dark purple should be observed. Incubate for 45 ± 5 minutes at 65 ± 2°C.
b) Contamination of the pipet tip with undenatured material during transfer of the sample to the hybridization microplate well	Insufficient mixing can result in incomplete denaturation of non-specific DNA–RNA hybrids endogenous to cervical specimens. PreservCyt specimens, in particular, are likely to have these hybrids present on the walls of the specimen tube. In order to prevent possible carryover of this undenatured cellular material, do not allow the pipet tip to touch the sides of the specimen tube during transfer of the sample to the hybridization microplate well.

		Comments and suggestions
c)	Contamination of the microplate wells with DR1	Wash the microplate wells thoroughly 6 times with wash buffer, overflowing the microplate wells each time, or use the Automated Plate Washer. No residual pink liquid should be visible in the microplate wells after washing.
d)	Contamination of the of the capture microplate during decanting and blotting	Always blot the capture microplate on new, clean KimTowels wipers or equivalent low-lint paper towels. Do not blot the capture microplate on previously used paper towels, as cross-contamination could occur.
e)	Improper wash of the capture microplate wells	Wash the microplate wells thoroughly 6 times with wash buffer, overflowing the microplate wells each time, or use the Automated Plate Washer. No residual pink liquid should be visible in the microplate wells after washing.
f)	Contamination of the DR2	Make sure not to cross-contaminate samples while adding the DR2 to the samples. If using part of a kit, aliquot the volume of DR2 needed for that test into a clean, disposable reagent reservoir prior to filling the pipet.

		Comments and suggestions
Elevated NC1 RLU values (>150 RLU) with the test performing as expected		
a)	Incubation of the capture microplate with DR2 performed outside the specified duration or specified temperature range	The test results are invalid. Repeat the test, and make sure the capture microplate is incubated for the proper duration at the specified temperature.
b)	Contamination of the DR2 or the wash buffer with alkaline phosphatase or DR1	See "Appendix A: Contamination Evaluation Procedures," page 66.

Quality Control

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* HPV Genotyping PS Test requires assay calibration with each test; therefore, it is necessary to verify each test. This assay calibration verification procedure is not intended as a substitute for internal quality control testing. Acceptable ranges for the verification of the assay calibration have been established only for QIAGEN-approved DML instruments.

The test must meet the specified assay calibration verification criteria. If any of the following criteria are invalid, the test results are invalid.

Negative Control 1

The NC1 must be tested in triplicate with each test using the HPV 16 probe mix. Perform the following steps to verify the assay calibration of the NC1 replicates.

Calculate the mean and the coefficient of variation (CV) of the 3 NC1 replicates.

A valid NC1 mean is ≥10 and ≤185 RLU and the CV is ≤35%. Proceed as

1. follows:

- If the required assay calibration verification criteria are met for the NC1, proceed to the assay calibration verification of the PC1.
- If the required assay calibration verification criteria are not met for the NC1, continue with this procedure.

Note: The formula for calculating the CV is: (standard deviation/mean) x 100 = CV%

2. **Remove the NC1 replicate with an RLU value farthest from the previously calculated mean as an outlier.**

3. **Calculate the mean and CV with the 2 remaining NC1 replicates.**

If the mean and CV meet the required assay calibration verification criteria for the NC1, proceed to the assay calibration verification of the PC1.

If the required assay calibration verification criteria are not met for the NC1, the assay calibration is invalid, and the test must be repeated for all specimens. Accordingly, do not report the *digene* HPV Genotyping PS Test results.

Positive Control 1

1. The PC1 must be tested in triplicate with each test using the HPV 16 probe mix. Perform the following steps to verify the assay calibration of the PC1 replicates.

Calculate the mean and CV of the 3 PC1 replicates.

A valid PC1 CV is ≤25%. Proceed as follows:

- If the required assay calibration verification criterion is met for the PC1, proceed to the assay calibration verification of the PC1/NC1 mean.
- If the required assay calibration verification criterion is not met for the PC1, continue with this procedure.

Remove the PC1 replicate with an RLU value farthest from the previously calculated mean as an outlier.

Calculate the mean and CV with the 2 remaining PC1 replicates.

2. If the CV meets the required assay calibration verification criterion for the PC1, proceed to the assay calibration verification of the PC1/NC1 mean.

3. If the required assay calibration verification criterion is not met for the PC1, the assay calibration is invalid, and the test must be repeated for all specimens. Accordingly, do not report the *digene* HPV Genotyping PS Test results.

Positive control 1 mean / negative control 1 mean

Using the previously calculated PC1 mean and NC1 mean, calculate the PC1/NC1 ratio.

A valid PC1/NC1 ratio is ≥2.0 and ≤15.0. Proceed as follows:

- If the required assay calibration verification criterion is met for the PC1/NC1 ratio, proceed to the assay calibration verification of the probe-specific quality controls.
- If the required assay calibration verification criterion is not met for the PC1/NC1 ratio, the assay calibration is invalid and the test must be repeated for all specimens. Accordingly, do not report the *digene* HPV Genotyping PS Test results.

Probe-specific quality controls

Quality control samples are supplied with the *digene* HPV Genotyping PS Test and must be used for internal quality control for each performance of the test. 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 NC1 and PC1. 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.

For an assay to be valid, the RLU/CO of each quality control must fall within the defined criteria as specified in Table 4, below.

If any of the quality controls are not within these ranges, the test is invalid and must be repeated for all specimens. Accordingly, do not report the *digene* HPV Genotyping PS Test results.

Table 4. Quality control assay validity criteria

Quality control	Minimum (RLU/CO)	Maximum (RLU/CO)
NC2	0.001	0.999
PC2	2.0	10.0

Limitations

- The detection of HPV using the *digene* HPV Genotyping PS Test does not preclude infection with more than one type of HPV.
- The assay only detects high-risk HPV types 16, 18, and 45. Other high-risk and low-risk HPV types may be present in the specimen.
- Specimens containing greater than 1.25% (v/v) of blood may have a false-negative test result.
- 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.

Performance Characteristics

Agreement between the *digene* HPV Genotyping PS Test and a validated quantitative PCR HPV genotyping test

A performance evaluation was conducted at QIAGEN to determine the test result agreement for the identification of HPV types 16, 18, and 45 using the *digene* HPV Genotyping PS Test in comparison to a validated qPCR test.

A total of 287 PreservCyt and 290 STM archived cervical specimens obtained from a routine screening population. The *digene* HC2 High-Risk HPV DNA Test result was determined for each specimen prior to inclusion in the study. The results of the *digene* HC2 High-Risk HPV DNA Test were as follows:

- 238 positive STM specimens
- 52 negative STM specimens
- 237 positive PreservCyt specimens
- 50 negative PreservCyt specimens

For each HPV type (16, 18, and 45), each specimen was tested in triplicate with both the *digene* HPV Genotyping PS Test and the qPCR test method for the detection of the respective HPV type for a total of 1731 test results for each testing method. After discordant resolution, 11 discordant results remained (see Table 5, below).

The agreement of the *digene* HPV Genotyping PS Test with qPCR was 99.4% (1720/1731) with a 95% confidence interval (CI) of 98.9–99.6%.

Table 5. Test result agreement of the *digene* HPV Genotyping PS Test result with the qPCR test result

		***digene* HPV Genotyping PS Test result**	
		+	**–**
qPCR test result	**+**	110	4
	–	7	1610

Agreement in the detection of specific HPV genotypes

The test result agreement of the *digene* HPV Genotyping PS Test to the qPCR test was determined based on the HPV genotype (see Table 6, below).

Table 6. Test result agreement of the *digene* HPV Genotyping PS Test and the qPCR test for HPV genotype

Genotype	Agreement (%) (n/N) 95% CI
HPV 16	99.1 (572/577) 98.0–99.6
HPV 18	99.7 (575/577) 98.7–99.9
HPV 45	99.3 (573/577) 98.2–99.7

Agreement in test results based on specimen type

The test result agreement of the *digene* HPV Genotyping PS Test to the qPCR test was determined based on the specimen type (see Table 7, below).

Table 7. Test result agreement of the *digene* HPV Genotyping PS Test and the qPCR test for specimen type

Specimen type	Agreement (%) (n/N) 95% CI
STM specimens	99.8 (868/870) 99.2–99.9
PreservCyt specimens	99.0 (852/861) 98.0–99.4%

Analytical specificity

A nonclinical panel of cloned HPV plasmid DNA at a concentration of 1E+7 copies per assay was tested with the each of the 3 HPV probe mixes (16, 18, and 45) used in the *digene* HPV Genotyping PS Test. The testing determined that no cross-reactivity existed between the 3 HPV probe mixes and the following HPV plasmid DNA:

- High-risk HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82
- Low-risk HPV types 1, 2, 3, 4, 5, 6, 8, 11, 13, 30, 34, 34, 40, 42, 43, 44, 67, 69, 70, and 71

Reproducibility

The overall reproducibility of the *digene* HPV Genotyping PS Test was determined. Testing was performed at QIAGEN facilities using 3 different kit lots

and 3 different operators performing the test in 3 different laboratories. Each operator tested the same panel members in triplicate over a minimum of 5 days.

STM specimen reproducibility

The panel for STM specimen reproducibility consisted of plasmid solutions for each HPV type tested (HPV 16, 18, and 45) at the following concentrations:

- 0 pg/ml (negative)
- 0.5 pg/ml (high-negative)
- 1.5 pg/ml (low-positive)
- 10 pg/ml (high-positive)

The observed test results agreed 100% with the expected test results. Based on the results of STM reproducibility testing, the *digene* HPV Genotyping PS Test is very reproducible with STM specimens.

PreservCyt specimen reproducibility

The panel for PreservCyt specimen reproducibility consisted of SiHa cells positive for HPV 16 at the following concentrations:

- 0 cells/assay (negative)
- 250–750 cells/assay (high-negative)
- 1000–5000 cells/assay (low-positive)
- >5000 cells/assay (high-positive)

The observed test results agreed 95.3% (103/108) with the expected test results. Based on the results of the PreservCyt specimen reproducibility testing, the *digene* HPV Genotyping PS Test is very reproducible with PreservCyt specimens.

Recovery of DNA for STM and PreservCyt specimens

STM and PreservCyt specimens were examined for recovery of HPV 16 DNA. SiHa cells were spiked into STM and PreservCyt Solution at the following concentrations:

- 500 cells/assay (high-negative)
- 2000 cells/assay (low-positive)
- 5000 cells/assay (high-positive)

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* HPV Genotyping PS Test using the HPV 16 probe mix.

The results demonstrated that recovery of HPV 16 DNA from human carcinoma cells is equivalent for the 2 media and that the PreservCyt sample preparation does not affect the analytical sensitivity of the *digene* HPV Genotyping PS Test.

Cross-reactivity

Cross-reactivity panel

A panel of organisms commonly found in the female anogenital tract was tested to determine if cross-reactivity would occur with the *digene* HPV Genotyping PS Test. All the microorganisms were assayed at concentrations between 10^5 and 10^7 organisms per ml.

The following organisms were tested and did not cross-react with the *digene* HPV Genotyping PS Test:

- Acinetobacter anitratus (ATCC 49139)
- *Acinetobacter lwoffi* (ATCC 17925)
- *Bacteroides fragilis* (ATCC 25285)
- *Bacteroides melaninogenicus* (ATCC 25845)
- *Candida albicans* (ATCC 10231)
- *Chlamydia trachomatis* (ATCC VR-878)
- *Enterobacter cloacae* (ATCC 13047)

- *Escherichia coli* HB101 (ATCC 33694)
- *Escherichia coli* (ATCC 25922)
- *Fusobacterium nucleatum* (ATCC 25586)
- *Gardnerella vaginalis* (ATCC 49145)
- *Haemophilus ducreyi* (ATCC 700724)
- *Klebsiella pneumonia* (ATCC 13883)
- *Lactobacillus acidophilus* (ATCC 4356)
- *Mobiluncus curtisii* (ATCC 35241)
- *Mobiluncus mulieris* (ATCC 35243)

Cross-reactivity as determined by sequence analysis

To make sure that no overlapping sequences would cross-react with the oligonucleotides used in the *digene* HPV Genotyping PS Test probe mixes, a sequence analysis (blast) was performed against the following viruses:

- Adenovirus 2
- Cytomegalovirus
- Epstein-Barr Virus
- Hepatitis B surface antigen-positive serum
- Herpes simplex 1
- Herpes simplex 2
- Human immunodeficiency virus (HIV, RT DNA)
- pBR322
- Simian virus type 40 (SV40)

The sequence analysis results indicated that it would be unlikely that the *digene* HPV Genotyping PS Test probe mixes would cross-hybridize with the listed viruses and cause a false-positive test result.

Effect of blood and other substances on STM specimens

The effect of blood and other potentially interfering defined substances was evaluated in the *digene* HPV Genotyping PS Test. Whole blood, contraceptive jelly, spermicide, moisturizer, hemorrhoid anesthetic, body oil, anti-fungal cream, and vaginal lubricant (agents that may commonly be found in cervical specimens) were added to STM at concentrations that may be found in cervical specimens.

No false-positive results were observed with any of the agents at any concentration. However, false-negative results were observed when testing with blood at the lowest target input concentration of 2 pg/ml. With a 2 pg/ml target concentration, interference of detection was observed with concentrations of blood equal to or greater than 17.5 µl/ml (1.75% v/v). No interference was observed with blood concentrations of 12.5 µl/ml (1.25% v/v). No false-negative results were observed with any of the other agents tested.

Effect of blood and other substances on PreservCyt specimens

The effect of blood and other potentially interfering defined substances was evaluated in the *digene* HPV Genotyping PS Test. Whole blood, contraceptive jelly, spermicide, moisturizer, hemorrhoid anesthetic, body oil, anti-fungal cream, and vaginal lubricant (agents that may commonly be found in cervical specimens) were added to PreservCyt Solution at concentrations that may be found in cervical specimens. No false-positive or false-negative results were observed with any of the agents at any of the concentrations tested.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen. com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

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Symbols

The following symbols are used in these instructions for use:

Symbol	Symbol definition
	Contains sufficient for < n > tests
	In vitro diagnostic medical device
	Catalog number
	Global Trade Item Number
	Manufacturer
	Authorized representative in the European Community

Symbol	Symbol definition
	Use by
	Consult instructions for use

Contact Information

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Appendix A: Contamination Evaluation Procedures

In the event that the test does not perform as expected, contamination may be the cause. To evaluate if contamination is a possibility, perform the following evaluation process for each reagent.

Detection Reagent 2

Notes:

- To avoid contaminating the DR2, wear disposable, powder-free gloves and do not touch the pipet tips on any work surfaces.
- When working with the DR2, avoid working in direct sunlight.

Obtain a clean capture microplate well strip and place in a plate frame.

1. **Pipet 75 µl of the aliquoted, residual, or original vial of DR2 into one**
2. **capture microplate well.**

Note: Test the DR2 in a replicate of 3 to provide the optimal assessment of performance.

3. **Incubate at 20–25°C for 15 minutes. Avoid direct sunlight.**
4. **Measure the capture 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 the DR2 is contaminated (>50 RLU), obtain a new kit and repeat the test.

1.
2. ## Wash Apparatus and/or water source

Obtain a clean capture microplate well strip and place in a plate frame.

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

Well 1 serves as the DR2 control.

Pipet 10 µl of the wash buffer from the Wash Bottle into microplate well 2.

Allow the wash buffer to flow through the washer tubing. Pipet 10 µl of the wash buffer from the tubing into capture microplate well 3.

Obtain an aliquot of the water used to prepare the wash buffer. Pipet 10 µl of the water into capture microplate well 4.

Incubate at 20–25°C for 15 minutes. Avoid direct sunlight.

Measure the capture microplate using a DML instrument.

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

6. Compare the RLU from wells 2, 3, and 4 to the DR2 control RLU. The
7. 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 "Wash buffer", page 26, for instructions on cleaning and maintaining the Wash Apparatus.

Automated Plate Washer

Obtain a clean capture microplate well strip and place in a plate frame.

1. **Label wells 1–5. Pipet 75 µl of DR2 into 5 capture microplate wells.**
2. Well 1 serves as the DR2 control.

3. **Pipet 10 µl of the wash buffer from the Plate Washer Wash Bottle into microplate well 2.**

4. **Pipet 10 µl of the rinse liquid from the Plate Washer Rinse Bottle into**
5. **microplate well 3.**

Press the "Prime" key on the Plate Washer keypad, allowing the wash
6. **buffer to flow through the lines. Pipet 10 µl of the wash buffer from the trough into microplate well 4.**

Press the "Rinse" key on the Plate Washer keypad, allowing the rinse liquid
7. **to flow through the lines. Pipet 10 µl of the wash buffer from the trough into**
8. **microplate well 5.**

Cover and incubate for 15 minutes at 20–25°C. Avoid direct sunlight.

Measure the capture 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.

Appendix B: Test Data Recording Worksheet

Test Site:						Test Date:					Operator ID:	
Ambient temperature:		°C				*digene* HPV Genotyping PS Test lot number:						
	1	2	3	4	5	6	7	8	9	10	11	12
A	NC1	NC2 (45)										
B	NC1	PC2 (16)										
C	NC1	PC2 (18)										
D	PC1	PC2 (45)										
E	PC1											
F	PC1											
G	NC2 (16)											
H	NC2 (18)											

Ordering Information

Product	Contents	Cat. no.
digene HPV Genotyping PS Test	For 96 reactions: Reagents, Buffers, 4 Controls, and Capture Microplate	613615
Hybrid Capture 2 Modular System		
DML 2000	Microplate luminometer, 240 V	5000-1020
DML 3000	Microplate luminometer, 120V/240 V	5000-00031
LumiCheck Plate User Package	Plate and software for use with the DML 2000 or DML 3000	6000-5023
Rotary Shaker I	Rotary shaker, 240 V	6000-2240E
Automated Plate Washer	96-well plate washer, 240 V	6000-00175
MST Vortexer 2	Multi- specimen tube vortexer, 240 V	6000-5022
Accessories		
Hybridization Microplates	Clear polystyrene 96-well microplates (100)	6000-1203
Microplate Lids	Clear polystyrene microplate lids (100)	6000-5001
Extra-long Pipet Tips	Pipet tips, 6 box/case	5075-1011

Disposable Reagent Reservoirs	Plastic reagent storage units with 25 ml capacity (100)	5090-1010
DuraSeal tube sealer film	Sealant film	6000-5003
Plate sealers	Adhesive plate sealers (100)	5070-1010
Sample preparation		
digene HC2 Sample Conversion Kit	For the sample conversion of up to 250 PreservCyt specimens: Sample Conversion Buffer, Specimen Transport Medium, Denaturation Reagent, and Indicator Dye	5127-1220

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Sample & Assay Technologies