Hybrid Capture® 2 High-Risk HPV DNA Test™

DNA with PAP†

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

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

THIS ABBREVIATED VERSION OF THE DIGENE HC2 HR HPV DNA TEST PRODUCT INSERT IS PROVIDED FOR CONSUMER EDUCATIONAL INFORMATION ONLY. *THE PROCEDURAL DIRECTIONS AND ASSOCIATED WARNINGS AND PRECAUTIONS NECESSARY TO PERFORM THE TEST IN A QUALIFIED LABORATORY HAVE BEEN REMOVED; THEREFORE THIS DOCUMENT IS INSUFFICIENT FOR THIS PURPOSE. PLEASE REFER TO THE PRODUCT INSERT INCLUDED IN THE HC2 HR HPV DNA TEST KIT FOR THE COMPLETE LABELING AND INSTRUCTIONS THAT ARE REQUIRED TO PERFORM THE TEST.*

REF 5199-1220

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

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NAME AND INTENDED USE

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

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

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

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

The use of this test is indicated:

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

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

WARNING

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

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

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

Another Digene kit, the hc2 HPV DNA Test (Catalog Number: 5198-1220), which detects both high-risk (using the kit's High-Risk HPV Probe) and some low-risk HPV types (using the kit's Low-Risk HPV Probe) **should not** be used

as an adjunct for screening, because low-risk types are not associated with risk of cervical cancer. Only the hc2 High-Risk HPV DNA Test (DNAwithPap) should be used as an adjunct for screening.

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

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

SUMMARY AND EXPLANATION

In women, human papillomaviruses (HPVs) can infect the cervix, vagina, vulva, urethra, or the area around the anus. More than 70 types of HPV have been identified, and are generally classified as high-risk or low-risk depending on their known association or lack of association with cancer and its precursor lesion, high-grade cervical intraepithelial neoplasia (CIN 2-3). The presence of certain HPV types in the female genital tract is associated with a number of diseases, including condyloma, Bowenoid papulosis, cervical, vaginal, and vulvar intraepithelial neoplasia and cancer.[1,2] It is generally accepted that these viruses are predominantly sexually transmitted and that high-risk HPV types are a major recognized risk factor for development of cervical cancer.[2-6]Infection of the cervix with high-risk HPV types can be associated with cytological and histological changes that are detected by Pap screening, colposcopy, or biopsy. The natural history of how HPV infection progresses to cancer, however, is not completely understood. Low-risk HPV types 6 and 11 have been associated with the presence of genital warts, or condylomas, but have been linked infrequently with precancerous or cancerous cervical changes. There are many other low-risk HPV types that are not associated with genital warts or cervical cancer.[7,8]

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

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

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

HPV infection is common in adults who have had more than one sexual partner (or a single partner who has had multiple partners) and can persist for years with no symptoms. Infection with some HPV types is an important risk factor for cervical cancer; however, most women with HPV infection do not develop cervical cancer or CIN 2-3, and infections regress. Most infections cause mild cytologic changes that resolve. HPV DNA has been shown to be

present in approximately 10% of women with normal cervical epithelium but the actual prevalence in specific groups of women is strongly influenced by age and other demographic variables.[2,13,21] Prospective studies (age 16-60 years) have shown that 15-28% of HPV DNA positive women developed squamous intraepithelial lesions (SIL) suggestive of CIN 1-3 or cancer within 2 years compared to only 1-3% of HPV DNA negative women.[4,22,23] In particular, the risk of progression for HPV types 16 and 18 was greater (approximately 40%) than for other HPV types.[4,6,10,23,24] Most SIL was low-grade.

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

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

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

PRINCIPLE OF THE PROCEDURE

The hc2 High-Risk HPV DNA Test using Hybrid Capture 2 technology is a nucleic acid hybridization assay with signal amplification that utilizes microplate chemiluminescent detection. Specimens containing the target DNA hybridize with a specific HPV RNA probe cocktail. The resultant RNA:DNA hybrids are captured onto the surface of a microplate well coated with antibodies specific for RNA:DNA hybrids. Immobilized hybrids are then reacted with alkaline phosphatase conjugated antibodies specific for the RNA:DNA hybrids, and detected with a chemiluminescent substrate. Several alkaline phosphatase molecules are conjugated to each antibody. Multiple conjugated antibodies bind to each captured hybrid resulting in substantial signal amplification. As the substrate is cleaved by the bound alkaline phosphatase, light is emitted that is measured as relative light units (RLUs) on a luminometer. The intensity of the light emitted denotes the presence or absence of target DNA in the specimen.

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

SPECIMEN COLLECTION AND HANDLING

The types of cervical specimens recommended for use in the hc2 High-Risk HPV DNA Test are listed below. Specimens taken with other sampling devices or transported in other transport media have not been qualified for use with this assay. **The hc2 High-Risk HPV DNA Test's performance characteristics with other specimen types and collection devices are unknown**. Cervical specimens must be collected prior to the application of acetic acid or iodine if colposcopic examination is being performed. See the HC Cervical Sampler or hc2 DNA Collection Device package insert for additional specimen collection and handling procedures.

CERVICAL BRUSHES*

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

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

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

CERVICAL BIOPSIES*

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

SPECIMENS IN CYTYC PRESERVCYT SOLUTION

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

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

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

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

1. *Cover caps with Parafilm® or equivalent prior to shipping specimens previously frozen. Specimens may be shipped frozen or 20-25°C.*

2. *When removing specimens from the freezer for testing, replace caps immediately with specimen collection tube screw caps.*

INTERPRETATION OF SPECIMEN RESULTS

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

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

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

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

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

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

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

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

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

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

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

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

DIAGNOSTIC ALGORITHM

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

Table 2
Diagnostic Algorithm

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

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

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

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

LIMITATIONS OF THE PROCEDURE

- The hc2 High-Risk HPV DNA Test for human papillomavirus types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 is not recommended for evaluation of suspected sexual abuse.
- Prevalence of HPV infection in a population may affect performance. Positive predictive values decrease when testing populations with low prevalence or individuals with no risk of infection.
- A negative result does not exclude the possibility of HPV infection because very low levels of infection or sampling error may cause a false-negative result. Also, this test does not detect DNA of HPV low-risk types (6, 11, 42, 43, 44 and many other low-risk types).
- The hc2 High-Risk HPV DNA Test should only be used with cervical specimens collected using the hc2 DNA Collection Device or the HC Cervical Sampler with Digene Specimen Transport Medium (STM) or cervical cytologic specimens collected using a broom-type collection device and placed in PreservCyt Solution. Biopsy specimens may be assayed only if they are placed immediately in STM and stored at -20°C until assayed.
- The hc2 DNA Collection Device or HC Cervical Sampler should not be used for collection of specimens from pregnant women.
- Infection with HPV is not an indicator of cytologic HSIL or underlying high-grade CIN, nor does it imply that CIN 2-3 or cancer will develop. Most women infected with one or more high-risk HPV types do not develop CIN 2-3 or cancer.
- A negative High-Risk HPV result does not exclude the possibility of future cytologic HSIL or underlying CIN 2-3 or cancer. A small proportion of high-grade lesions occur in women who are High-Risk HPV negative by existing technologies.[6]
- A small amount of cross-hybridization between HPV types 6 and 42 (low-risk HPV types) and the High-Risk HPV Probe exists. Specimens with high levels (4 ng/ml or higher) of HPV 6 or HPV 42 DNA may be positive. It has also been reported in the literature that complex probe cocktails similar to that used in this test may cause false-positive results due to cross-hybridization with HPV types 11, 53, 54, 55, 66, MM4, MM7, MM8, or MM9.[39] Although several of these HPV types are rare or novel types not often encountered with high-grade disease, patients whose specimens contain high levels of these HPV DNA types may incorrectly be reported as positive in the hc2 High-Risk HPV DNA Test.[12,40]
- The hc2 High-Risk HPV DNA Test is designed to detect high-risk HPV types including 39, 58, 59, and 68. Analytical studies conducted by Digene, using cloned HPV plasmid DNA, demonstrate that the assay detects these types at levels ranging from 0.62 pg/ml to 1.39 pg/ml. This is equivalent to the detection characteristics of the other HPV types targeted by the hc2 High-Risk HPV DNA Test. Digene was able to validate the detection of these HPV types in only a limited number of clinical specimens. Due to the low prevalence of these types in the general population (as demonstrated by Bosch et. al.), the performance characteristics of the hc2 High-Risk HPV DNA Test for the detection of HPV types 39, 58, 59, and 68 has not been statistically confirmed.
- If high concentrations of anti-fungal cream, contraceptive jelly, or douche are present at the time a specimen is collected for HPV testing, there is a likelihood of obtaining a false-negative result should these specimens contain HPV DNA levels that yield RLU/CO values near the assay cutoff.
- Cross-reactivity between the hc2 high-risk HPV DNA Test probe and the plasmid pBR322 is possible. The presence of pBR322 homologous sequences has been reported in human genital samples and false-positive results could occur in the presence of high levels of bacterial plasmid.

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

EXPECTED RESULTS

HIGH-RISK HPV PREVALENCE

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

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

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

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

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

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

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

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

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

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

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

PERFORMANCE CHARACTERISTICS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

IN WOMEN 30 YEARS AND OLDER, SCREENING PERFORMANCE OF THE HC2 HIGH-RISK HPV DNA TEST AS AN ADJUNCT TO THE PAP TEST TO HELP GUIDE PATIENT MANAGEMENT

Test Performance in Clinical Samples

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

ANALYTICAL SENSITIVITY

Internal Study using Plasmid DNA

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

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

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

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

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

External Study Using Clinical Specimens

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

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

methods and a positive and negative agreement of 96.7% and 44.6%, respectively (95% CI =92.5-98.9 and 31.3-58.5).

Table 10
Analytical Detection of HPV DNA Comparing the hc2 HPV DNA Test to HPV Type-specific PCR

		PCR		
		Positive	*Negative*	*Total*
hc2 HPV	*Positive*	148	31	179
	Negative	5	25	30
	Total	153	56	209

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

EQUIVALENCE BETWEEN STM AND PRESERVCYT SOLUTION SPECIMENS

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

REPRODUCIBILITY

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

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

Table 11
Summary of Overall Statistics for Multicenter Reproducibility of the hc2 High-Risk HPV DNA Test

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Table 15
Clinical HPV DNA Paired Specimen Test Reproducibility

Specimen Type	HPV Prevalence	Positive Agreement (n/N) 95% CI	Negative Agreement (n/N) 95% CI	Overall Agreement (n/N) 95% CI
STM (initial)	29.2%	92.15 (270/293) 88.45 - 94.96	97.89 (695/710) 96.54 - 98.81	96.21 (965/1003) 94.84 - 97.31
PC (with Retest algorithm)	19.0%	88.37 (190/215) 83.31 - 92.33	97.53 (909/933) 96.20 - 98.35	95.82 (109/1148) 94.40 - 96.83

These results reflect variability associated with sample collection, in addition to the variability due to sample processing and the assay procedure. Further inspection of these results revealed that result variability was concentrated in the assay cutoff region. When only

specimens yielding results outside the cutoff region are included in the analysis, positive agreement for PC specimens increases to 94.0% while negative agreement increases to 99.4%; while similarly for STM specimens, the positive and negative agreement values increase to 97.5% and 99.6% respectively.

CROSS-REACTIVITY

CROSS-REACTIVITY PANEL

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

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

Acinetobacter anitratus
Acinetobacter lwoffi (ATCC 17908)
Bacteroides fragilis (ATCC 25285)
Bacteroides melaninogenicus
Candida albicans (ATCC 14053 or 10231)
Chlamydia trachomatis
Enterobacter cloacae
Escherichia coli (HB101)*
Escherichia coli
Fusobacterium nucleatum
Gardnerella vaginalis
Haemophilus ducreyi
Klebsiella pneumoniae
Lactobacillus acidophilus
Mobiluncus curtisii
Mobiluncus mulieris
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 (ATCC 27762)
Treponema pallidum
Trichomonas vaginalis
Ureaplasma urealyticum

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

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

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

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

CROSS-HYBRIDIZATION

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

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

EFFECT OF BLOOD AND OTHER SUBSTANCES ON STM SPECIMENS

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

EFFECT OF BLOOD AND OTHER SUBSTANCES ON PRESERVCYT SOLUTION SPECIMENS

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

REPRODUCIBILITY OF HC2 HIGH-RISK HPV DNA TEST WITH CLINICAL SPECIMENS COLLECTED IN STM

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

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

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

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

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

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

REPRODUCIBILITY OF PRESERVCYT SOLUTION SPECIMENS IN THE HC2 HIGH-RISK HPV DNA TEST

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

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

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

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

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

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

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