

**Digene High-Risk HPV DNA Test
HYBRID CAPTURE[®] 2**

P890064/S009

Section III: Summary of Safety and Effectiveness Data

I. General Information

- A. Device Generic Name:
Human Papillomavirus DNA Detection Kit
- B. Device Trade Name:
Digene Hybrid Capture[®] 2 High-Risk HPV DNA Test
Note: Device has also been sold under the Trade Name *Hybrid Capture[®]*

II

- C. Applicant's Name and Address:
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- D. PMA Number: P890064 Supplement S009
- E. Date of Panel recommendation: _____
- F. Date of notice of approval to the applicant: _____

II. Indications for Use

The *Digene High-Risk HPV DNA Test* using *Hybrid Capture[®] 2* (HC2) technology is a nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of thirteen high-risk types of human papillomavirus (HPV) DNA in cervical specimens. The HPV types detected by the assay are the high-risk types 16/18/31/33/35/39/45/51/52/56/58/59/68. The *Digene High-Risk HPV DNA Test* cannot determine the specific HPV type present.

The use of this test is indicated:

1. As a general population screening test in conjunction with the Pap smear, for women 30 years of age and older, as an aid to determine the absence of high-grade cervical disease or cancer. In women with a concurrent normal Pap smear and a negative HC2 HPV result, the probability of detecting evidence of high-grade cervical disease upon colposcopy is reduced relative to a normal Pap result alone, based on the increased negative predictive value of the combined use of both methods. This result is not intended to deter the patient from proceeding to colposcopy, should other clinical indicators warrant such action.

When screening women in the general population in conjunction with the Pap, this test provides a single time point assessment of the risk of having developed cervical disease and the probability of detecting evidence of high-grade disease upon colposcopy. Detection of HPV has been shown to identify a greater number of women with severe cervical disease in the absence of cytologic abnormalities; therefore, the probability of observing high-grade disease upon colposcopy is increased. However, the presence of HPV in the absence of cytologic abnormalities is not a definitive indicator that high-grade cervical disease exists or will develop; the prognostic value of the test has not been fully validated in clinical studies.

2. To screen patients with ASCUS (atypical squamous cells of undetermined significance) Pap smear results independent of age to determine the need for referral to colposcopy. The results of this test are not intended to deter women from proceeding to colposcopy.
3. In women with LSIL or HSIL Pap smear results prior to colposcopy, an HC2 HPV result will aid the physician in patient management by assisting with risk assessment of women to determine absence of high-grade disease. This result is not intended to deter the patient from proceeding to colposcopy.

The *Digene High-Risk HPV DNA Test* is not intended for use as a screening test in the general population for women under 30 years of age.

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

Cervical specimens that may be tested with the Digene HPV Test include the following:

- Specimens collected with the Digene Cervical Sampler™.
- Biopsies collected in Digene Specimen Transport Medium (STM).
- Specimens collected using a broom-type collection device and placed in Cytoc PreservCyt® Solution*.

*PreservCyt is a registered trademark of Cytoc Corporation, Boxborough, MA.

III. Device Description

The *Digene High-Risk HPV DNA Test* using *Hybrid Capture⁰ 2* technology is a nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of human papillomavirus (HPV) DNA in cervical specimens. The HPV types detected by the *Digene High-Risk HPV DNA Test* (catalog no. 5101-1296) are high and intermediate risk types 16/18/31/33/35/39/45/51/52/56/58/59 and 68.

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 which is measured as relative light units (RLU) on the *Digene DML 2000™ Microplate Luminometer*. The intensity of the light emitted denotes the presence or absence of target DNA in the specimen. An RLU measurement equal to or greater than the Cutoff Value indicates the presence of HPV DNA sequences in the specimen. An RLU measurement less than the Cutoff Value indicates the absence of the intermediate/high risk HPV DNA sequences tested or that HPV DNA levels are below the detection limit of the assay.

Specimens that may be tested with the *HC2 High-Risk HPV DNA Test* include exfoliated cervical cells collected with the *Digene Cervical Sampler*, cervical biopsies collected in *Digene Specimen Transport Medium (STM)*, exfoliated cervical cells collected using a broom-type collection device and placed in Cytoc ThinPrep® Pap Test™ PreservCyt® Solution.*

IV. Contraindications, Warnings and Precautions

1. The Digene Hybrid Capture 2 *High-Risk HPV DNA Test* for human papilloma-virus types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 is not recom-mended for evaluation of suspected sexual abuse.
2. 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.
3. A negative result does not exclude the possibility of infection with high-risk HPV since very low levels of infection or sampling error may cause a false negative result.
4. The *Digene High-Risk HPV DNA Test* can only be used with cervical specimens collected using the *Digene Cervical Sampler* or *Digene Specimen Transport Medium* or cervical specimens collected using a broom-type collection device and placed in Cytoc ThinPrep Pap Test PreservCyt Solution. Biopsy specimens may be assayed only if they are placed immediately in *Digene Specimen Transport Medium* and stored at -20°C until assayed.
5. Infection with HPV is not a definitive indicator of the presence of high-grade cervical disease, nor does it imply in all cases that high-grade disease or cancer will develop. Most women infected with one or more high-risk HPV types do not develop high-grade lesions or cancer.
6. A small amount of crosshybridization between HPV types 6 and 42 (low risk HPV types) and the high-risk 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 crosshybridization with HPV types 11, 53, 54, 55, 66, MM4, MM7, MM8, or MM9. Although several of these HPV types are rare or novel types not often encountered with high-grade disease, patients whose specimens contain high levels of these HPV DNA types may incorrectly be referred to colposcopy.

7. The *Digene 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 this assay detects these types at levels ranging from 0.62 pg/ml to 1.39 pg/ml. This is equivalent to the detection characteristics of the other HPV types targeted by the Digene 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, the performance characteristics of the Digene High-Risk HPV DNA Test for the detection of HPV types 39, 58, 59, and 68 has not been statistically confirmed.
8. 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.
9. 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.

10. Safety Precautions

- a. HANDLE ALL ASSAY SPECIMENS AND DISPOSED MATERIALS AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS. Patient specimens should be handled at the BSL 2 level as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories*, 3rd Edition, 1993, pp. 10 – 13 and NCCLS Approved Guideline M29-A, *Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*.
- b. Do not pipette by mouth.
- c. Do not smoke, eat, or drink in areas where reagents or specimens are handled.
- d. Wear disposable powder-free gloves while handling reagents or specimens. Wash hands thoroughly after performing the test.
- e. All materials used in this assay, including reagents and specimens, should be disposed of in a manner that will inactivate infectious agents.

- f. **Solid Wastes:** Autoclave.
- g. **Liquid Wastes:** Add sodium hypochlorite to a final concentration of 1.0% (1:5 dilution of household
- h. bleach). Allow 30 minutes for decontamination before disposal^{24,25}.

SPILLS: Non-base-containing spills should be wiped thoroughly with a 5% sodium hypochlorite solution (full-strength household bleach). Base-containing spills should be neutralized, wiped dry, and then the spill areas should be wiped with a 5% sodium hypochlorite solution.

The wiped area should be covered with absorbent material, saturated with a 5% sodium hypochlorite solution and allowed to stand for at least 10 minutes. A glass or plastic cover or tray can be used to reduce exposure to fumes.

All wiping materials should be treated as hazardous waste.

11. Safety and Health Risk Information

The materials below have been assessed according to the requirements of the EC Directives 67/548/EEC and 88/379/EEC as amended and CHIP2 and 96 as amended.

- | | | |
|-----------|------------------|---|
| C | Corrosive | Denaturation Reagent contains Sodium Hydroxide.
R35: Causes severe burns.
S26: In case of contact with eyes rinse immediately with plenty of water and seek medical advice.
S45: In case of accident or if you feel unwell, seek medical advice immediately.
S36/37/39: Wear suitable protective clothing, gloves, eye and face protection. |
| Xi | Irritant | Probe Diluent contains Acetic Acid, Acrylic Acid.
R36/38: Irritating to eyes and skin.
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S45: In case of accident or if you feel unwell, seek medical advice immediately.
S36/37/39: Wear suitable protective clothing, gloves, eye and face protection. |
| T | Toxic | Wash Buffer contains Sodium Azide.
R25: Toxic if swallowed.
R32: Contact with acids liberates toxic gas.
S45: In case of accident or if you feel unwell, seek medical advice immediately.
S36/37/39: Wear suitable protective clothing, gloves, eye and face protection. |

12. Handling Precautions

- a. Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
- b. Do not use the reagents beyond the expiration date on the outer box label.
- c. The *Digene High-Risk HPV DNA Test Procedure, Quality Control and the Interpretation of Specimen Results* must be followed closely to obtain reliable test results.
- d. It is important to pipette the exact reagent volume indicated and to mix well after each reagent addition. Failure to do so could result in erroneous test results. Ensuring that the noted color changes occur will help confirm that these conditions have been met.
- e. These components have been tested as a unit. **Do not** interchange components from other sources or from different lots.
- f. Nucleic acids are very sensitive to environmental nuclease degradation. Nucleases are present on human skin and on surfaces or materials handled by humans. Clean and cover work surfaces with disposable pads **and wear powder-free gloves when performing all assay steps.**
- g. Care should be taken to prevent contamination of the Capture Microplate and Detection Reagent 2 with exogenous alkaline phosphatase during performance of the assay. Substances which may contain alkaline phosphatase include Detection Reagent 1, bacteria, saliva, hair and oils from the skin. Covering the Capture Microplate after the wash step and during Detection Reagent 2 incubation is especially important, since exogenous alkaline phosphatase may react with Detection Reagent 2 producing false positive results.
- h. Protect Detection Reagent 2 from prolonged exposure to direct light. Use reagent immediately after aliquoting and avoid direct sunlight.
- i. Care should be taken to deliver the correct volumes of reagents to the reaction tubes and microplates at all steps and to mix well after each reagent addition. The repeating pipettor should be primed in advance of reagent delivery and checked for large air bubbles periodically. Excessive amounts of large air bubbles in the repeating pipettor tip may cause inaccurate delivery and can be avoided by filling the pipettor, dispensing all the liquid, and refilling. See pipettor instruction manuals for specific directions for use.
- j. Multichannel pipetting should be performed using the reverse pipetting technique for dispensing Detection Reagents 1 and 2. Check each pipette tip on the multichannel pipettor for proper fit and filling. See specific directions for use.

- k. Care should be taken during washing to ensure that each microwell is washed thoroughly. Inadequate washing will result in increased background and may cause false positive results. Residual wash buffer in wells may result in reduced signal or poor reproducibility.

V. Alternative Practices and Procedures

Alternative practices and procedures for the detection and typing of human papillomavirus include filter hybridization, Southern blot hybridization, filter *in situ* hybridization, *in situ* hybridization, and polymerase chain reaction (PCR) methods. None of these procedures are approved for clinical use in the United States.

VI. Marketing History

The previous generation *Hybrid Capture™ System HPV DNA Test* was first distributed in the United States in November 1995. This version has been discontinued in the United States, with the last kit sold in October 2000.

The *Digene Hybrid Capture 2 HPV DNA Test* is registered for sale in the following countries: USA, Canada, Mexico, Argentina, Brazil, Uruguay, China, India, Korea, France, Hungary, Lithuania, Portugal, Switzerland, Turkey, and the United Kingdom. Additional countries where the test is sold include the Benelux Region of Europe, Germany, Italy, Spain, Australia, Taiwan, Chile, Colombia, and Venezuela. This test was first marketed in Europe, Canada and Australia in mid-1997 and introduced in the United States shortly after approval of P890064/S006 on March 17, 1999. The first *High-Risk* version of the test was sold in the United States in December 2000, and is currently sold in the United States and Canada.

Since the introduction of the *HC2 HPV DNA Test*, there has been one recall related to the effectiveness of the device, catalog no. 5101-1096. Recall No. Z-0097-1 was initiated in September 2000. The Low-Risk probe had degraded prior to expiration. There have been no market withdrawals for any reason related to the safety or effectiveness of the *HC2 HPV High-Risk DNA Test*.

VII. Potential Adverse Effects of the Device on Health

No adverse effects of the *Digene HC2 HPV DNA Test* on health have been reported to Digene Corporation. When used as indicated, there are no known potential adverse effects on the health of patients evaluated for human papillomavirus infection associated with this *in vitro* diagnostic device. Potential adverse health effects for laboratory personnel performing the test include those inherent in working with human body fluids and with chemical corrosives and irritants, as contained in the Warnings and Precautions section of the Product Instructions. No additional potential adverse health effects are known or anticipated.

VIII. Summary of Pre-clinical Laboratory Studies

A. Analytical Sensitivity

A non-clinical panel of cloned HPV plasmid DNA samples was tested to determine if each of the 13 HPV types are detectable by the Digene HC2 High-Risk HPV DNA Test and to determine the analytical sensitivity of the assay for each of the HPV types. 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.

B. Equivalence between STM and PreservCyt[®] Solution (PC) Specimens

Equivalence between STM and PC specimens was examined for equal recovery of HPV 18 DNA from approximately 10⁶ positive HeLa cells containing integrated HPV 18 genomes spiked into STM and into a negative cell pool in PC. The results demonstrated that recovery of HPV 18 DNA from human carcinoma cells is equivalent for the two media and that the PC preparation procedure does not affect the analytical sensitivity of the HC2 High-Risk HPV DNA Test.

C. 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 in triplicate. The results are shown in Table 1.

Table 1
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

^aNumbers in parentheses indicate 95% confidence intervals.

These results illustrate the excellent reproducibility of the HC2 High-Risk HPV DNA Test.

D. HC2 High-Risk HPV DNA Test Cross-Reactivity

A battery of bacteria, viruses and plasmids commonly found in the female anogenital tract, as well as a collection of cutaneotropic HPV types for which clones were available, were assayed to determine if cross-reactivity would occur with the HPV probes used in the HC2 High-Risk HPV DNA Test. The only plasmid that showed cross-reactivity in the HC2 High-Risk HPV DNA Test was pBR322. 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.

E. Cross-Hybridization Studies

Eighteen different HPV types (high and low risk) were tested with the High-Risk HPV HC2 assay 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 cross-hybridization between Low-Risk 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.

F. Effect of Blood and Other Substances on STM and PreservCyt 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 HPV negative and positive samples (clinical specimen pools and non-clinical samples) in both STM and PC at concentrations that may be found in cervical specimens.

No false positive results in STM were observed with any of the four agents at any concentration. However, a false negative result in STM may be reported in clinical specimens with HPV DNA levels close to that of the positive cutoff for the assay (1.0 pg/ml) if a high level of anti-fungal cream or contraceptive jelly is present. No false positive or false negative results were observed in PreservCyt with any of the four agents at any concentration.

IX. Summary of Clinical Studies

A. Study Design

For the purposes of this submission, Digene prospectively analyzed the results from eight independent clinical studies conducted by prominent medical, academic and government institutions located in the United States, Western Europe, Latin America, Africa and Asia. The hypothesis tested here is that women who are not infected with the HPV virus are not at risk for developing cervical cancer. Conversely, women who are infected with a high-risk HPV virus type are at increased risk for having or developing cervical disease.

Since the vast majority of HPV infections resolve spontaneously and do not result in cervical disease, infection with HPV alone is not a definitive indication of cervical disease. However, by combining the information provided by a Pap smear and an HPV test, the physician can better determine the relative risk and therefore the appropriate course of treatment for the patient. The objective of using these clinical investigation results is to demonstrate that testing with the *HC2 High-Risk HPV Test* in conjunction with the Pap smear offers an advantage to patient management not available with the current practice of Pap

alone for the determination of relative risk of cervical disease and cancer in individual patients.

B. Patient Assessment

The studies utilized the established Pap methods in use in the countries in which the study was conducted. In all but two cases, the Bethesda Grading System was utilized to interpret the Pap results. In addition, high-grade cervical disease was diagnosed through the use of colposcopy-directed biopsy for each study. These studies assessed the clinical usefulness of the *HC2 High-risk HPV DNA Test* in comparison to the Pap smear for women over 30 years of age. All but two studies also performed prospective HPV testing using the *HC2 High-Risk HPV DNA Test*. One of the eight studies (identified as “US 2” in the following result tables) was unique from the others in that this study was a ten-year longitudinal natural history study evaluating HPV and its relationship to cervical disease. This study was also one of the two studies that did not utilize prospective HC2 HPV testing. Archived cervicovaginal (CVL) specimens collected during this study were, however, tested retrospectively using the *HC2 High-Risk HPV DNA Test*, and a cross-sectional analysis was performed. The remaining studies were cross-sectional general population screening studies utilizing the *Hybrid Capture HC2 HPV Test*. As previously indicated, one of the remaining seven screening studies was conducted in the United States, six were conducted in six countries in Europe, Latin America, Africa and Asia.

C. Demographic Data

Digene prospectively analyzed the results from eight independent clinical studies conducted by prominent medical, academic and government institutions located in the United States, Western Europe, Latin America, Africa and Asia. These studies included more than 44,000 women that had ages of 30 years or older with diverse racial composition.

D. Data Analysis and Results

The performance of the HC2 High-Risk HPV DNA Test observed from these eight clinical studies is summarized in the following tables 2 and 3, for women aged 30 years and over and diagnosed with histologically-confirmed high-grade cervical neoplasia (defined as CIN3 or more severe).

Table 2
Performance Estimates of HC2 HPV Test: Sensitivity and Specificity

<i>Population</i>	<i>n</i>		<i>Sensitivity(%)</i>			<i>Specificity(%)</i>		
			<i>PAP Alone</i>	<i>HPV Alone</i>	<i>HPV + PAP</i>	<i>PAP Alone</i>	<i>HPV Alone</i>	<i>HPV + PAP</i>
Western Europe 1	7592		51.6 (14/27)	96.3 (26/27)	100 (27/27)	98.5 (7453/7565)	96.2 (7275/7565)	95.1 (7193/7565)
		95% <i>CI</i>	32.0-71.3	81.0-99.9	87.2-100	98.2- 98.8	95.7-96.6	94.6-95.6
Western Europe 2	9761		90.2 (46/51)	94.1 (48/51)	98.0 (50/51)	97.2 (9447/9710)	97.0 (9419/9710)	95.7 (9291/9710)
		95% <i>CI</i>	78.6-96.7	83.8-98.8	89.6-100	97.0-97.6	96.6-97.3	95.3-96.1
Latin America 1	6115		58.4 (45/77)	94.8 (73/77)	97.4 (75/77)	98.7 (5962/6038)	93.9 (5669/6038)	93.4 (5637/6038)
		95% <i>CI</i>	46.68-69.6	87.2-98.6	90.9-99.7	98.4-99.0	93.3-94.5	92.7-94.0
Latin America 2 [†]	6176		82.3 (51/62)	93.6 (58/62)	96.8 (60/62)	94.03 (5749/6114)	93.96 (5745/6114)	89.82 (5492/6114)
		95% <i>CI</i>	70.5-90.8	84.3-98.2	88.8-99.6	93.4-94.6	93.3-94.6	89.1-90.6
Africa	2925		84.1 (90/107)	89.7 (96/107)	92.5 (99/107)	86.4 (2436/2818)	80.0 (2253/2818)	76.4 (2152/2818)
		95% <i>CI</i>	75.8-90.5	82.4-94.8	85.8-96.7	85.1-87.7	78.4-81.4	74.8-77.9
Asia	1936		97.6 (41/42)	100 (42/42)	100 (42/42)	76.3 (1445/1894)	83.0 (1572/1894)	68.0 (1287/1894)
		95% <i>CI</i>	87.4-99.9	91.6-100.0	91.6-100.0	74.3-78.2	81.2-85.0	65.8-70.1
U.S. 1	1040		50.0 (1/2)	100 (2/2)	100 (2/2)	97.6 (1013/1038)	96.2 (999/1038)	95.5 (991/1038)
		95% <i>CI</i>	1.26-98.7	15.8-100.0	15.8-100.0	96.5-98.4	94.9-97.3	94.0-96.7
U.S. 2 [‡]	10,031		51.7 (30/58)	70.7 (41/58)	81.0 (47/58)	98.2 (9791/9973)	91.9 (9167/9973)	90.8 (9053/9973)
		95% <i>CI</i>	38.2-65.1	57.3-81.9	68.6-90.1	97.9-98	91.4-92.5	90.2-91.3

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

[‡] Single Pap and HPV reading at baseline, disease evaluation at 3 years

Table 3
Performance Estimates of HC2 HPV Test: Positive and Negative Predictive Value

Population	n		Prevalence(%)	Positive Predictive Value (%)			Negative Predictive Value (%)		
				CIN 3	PAP Alone	HPV Alone	HPV + PAP	PAP Alone	HPV Alone
Western Europe 1	7592		0.36 (27/7592)	11.1 (14/126)	8.23 (26/316)	6.77 (27/399)	99.83 (7453/7466)	99.99 (7275/7276)	100.0 (7193/7193)
		95% CI	0.23-0.52	6.21-17.9	5.45-11.8	4.51-9.69	99.70-99.91	99.92-100.0	99.95-100.0
Western Europe 2	9761		0.52 (51/9761)	14.9 (46/309)	14.2 (48/339)	10.7 (50/469)	99.95 (9447/9452)	99.97 (9419/9422)	99.99 (9291/9292)
		95% CI	0.39-0.69	11.1-19.4	10.6-18.3	8.02-13.8	99.88-99.98	99.91-99.99	99.94-100.00
Latin America 1	6115		1.26 (77/6115)	37.2 (45/121)	16.5 (73/442)	15.8 (75/476)	99.47 (5962/5994)	99.93 (5669/5673)	99.96 (5637/5639)
		95% CI	0.99-1.57	28.6-46.4	13.2-20.3	12.6-19.4	99.25-99.63	99.82-99.98	99.87-100.0
Latin America 2 [†]	6176		1.10 (68/6176)	12.3 (51/416)	13.6 (58/427)	8.8 (60/682)	99.81 (5749/5760)	99.93 (5745/5749)	99.96 (5492/5494)
		95% CI	0.86-1.39	9.3-15.8	10.5-17.2	6.8-11.2	99.7-99.9	99.8-100.0	99.9-100.0
Africa	2925		3.66 (107/2925)	19.1 (90/472)	14.5 (96/661)	12.9 (99/765)	99.31 (2436/2453)	99.51 (2253/2264)	99.63 (2152/2160)
		95% CI	3.01-4.40	15.6-22.9	11.9-17.4	10.6-15.5	98.89-99.60	99.13-99.76	99.27-99.84
Asia	1936		2.17 (42/1936)	8.37 (41/490)	11.5 (42/364)	6.47 (42/649)	99.93 (1445/1446)	100.0 (1572/1572)	100.0 (1287/1287)
		95% CI	1.57-2.92	6.07-11.2	8.44-15.3	4.70-8.65	99.62-100.0	99.77-100.0	99.71-100.0
U.S. 1	1040		0.19 (2/1040)	3.85 (1/26)	4.88 (2/41)	4.08 (2/49)	99.90 (1013/1014)	100.0 (999/999)	100.0 (991/991)
		95% CI	0.02-0.69	0.10-19.6	0.60-16.5	0.50-14.0	99.45-100.0	99.63-100.0	99.63-100.0
U.S. 2 [‡]	10,031		0.58 (58/10031)	14.2 (30/212)	4.8 (41/847)	4.9 (47/967)	99.71 (9791/9819)	99.81 (9167/9184)	99.88 (9053/9064)
		95% CI	0.44-0.75	9.76-19.6	3.50-6.51	3.59-6.41	99.59-99.81	99.70-99.89	99.78-99.94

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

[‡] Single Pap and HPV reading at baseline, disease evaluation at 3 years

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

E. Device Failures and Replacements

There were no device failures or product recalls during the course of these clinical studies that would have adversely impacted study results.

X. Conclusions Drawn from the Studies

A. Risk/benefit Analysis

The HC2 High-Risk HPV DNA Test used in conjunction with Pap poses minimal risk of an adverse effect on public health. This relatively small risk is outweighed by the beneficial effects of combination HPV/Pap testing that include (1) identification of cases of underlying cervical disease that otherwise may be missed by cytology alone and (2) more accurate identification of women

at decreased or no risk of having or developing cervical disease in the short term.

The risk associated with a positive HC2 High-Risk HPV DNA Test result is an unnecessary colposcopy procedure for women infected with HPV, but with no disease present. A minor complication occasionally associated with colposcopy is bleeding from the biopsy site.

As stated in the Indications for Use, the *Digene High-Risk HPV DNA Test* is designed to augment existing methods for the detection of cervical disease and should be used only 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. The use by medical professionals of the full complement of clinical information is expected to mitigate the increase in colposcopy referrals that might otherwise be anticipated based solely on the HPV and Pap results.

B. Safety

No unanticipated adverse device effects have been reported during these clinical studies as a result of performing the *HC2 High-Risk HPV DNA Test* in conjunction with Pap for general population screening.

C. Effectiveness

The eight primary clinical studies for which the prospective data analysis was performed each demonstrate that for cytologically normal women, the relative risk for high-grade cervical disease or cancer is moderately higher for those who are positive for the high-risk HPV types when compared to women who have no evidence of HPV infection. The addition of the *HC2 High Risk HPV DNA Test* to the current screening program would therefore allow the physician to determine, 1) the likelihood of the presence of high-grade cervical disease and cervical cancer, 2) the relative risk of development of disease for the patient, and, 3) the need for appropriate follow-up.

The increased sensitivity of the assay compared to Pap alone for identifying women with underlying high-grade neoplasia has been demonstrated by these eight independent clinical studies. A positive *HC2 High Risk HPV DNA Test*, in conjunction with a negative Pap, has been shown by these results to identify women at increased risk for cervical disease. By identifying HPV infection, the primary causal factor for the development of cervical disease and cancer, the physician can inform a Pap normal patient that she must be diligent in returning for her regular screening visits. Likewise, when the results are negative for both tests, this should provide the physician and the patient with an increased level of confidence that the risk of cervical disease is reduced at that point in time. Therefore, the combination of negative Pap smear results and negative HPV DNA results can identify women at little or no risk for cervical disease more accurately than the Pap smear alone.

In conclusion, the combination of the Digene High-Risk HPV DNA Test with the Pap smear can identify cases of underlying cervical disease in women that otherwise may be missed by cytology alone. The combination of HPV and Pap can also identify women at decreased or no risk of having or developing cervical disease in the short term.

The FDA will complete the following sections:

XI. Panel Recommendations

XII. CDRH Decision

XIII. Approval Specifications