CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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HPV High Risk Screening with Genotyping

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Effective Date:
August 19, 2013

CPT Code: **– 87621** **If Result is NOT DETECTED**
– 87621 (x3) **If Results is DETECTED (Genotype reported)**

LIS Information:

HPV Test Code	Description	LOINC
7000420	HPV Genotype Group	
7000430	HPVHR 16 DNA	61372-9
7000440	HPVHR 18 DNA	61737-7
7000450	OTHER HPVHR DNA	71431-1

Summary:
CPAL is proud to announce the availability of the real-time PCR, FDA-approved cobas® HPV Test from Roche Diagnostics. The test is performed on the fully automated cobas® 4800 System. The cobas® HPV Test individually identifies genotypes 16 and 18, the two highest-risk HPV genotypes responsible for more than 70 percent of cervical cancer cases, while simultaneously detecting 12 other high risk HPV genotypes (HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). **One test, in one run, from one patient sample delivers 3 results, eliminating the need**

for reflex testing. Only 2 ml of patient sample is required, reducing the risk of QNS. The performance of the cobas® HPV test is clinically validated in the landmark Athena study which shows proven equivalency in performance against HC2 in the ASC-US population.

Intended Use:

The cobas® HPV Test is a qualitative in vitro test for the detection of Human Papillomavirus in patient specimens. The test utilizes amplification of target DNA by the Polymerase Chain Reaction (PCR) and nucleic acid hybridization for the detection of 14 high-risk (HR) HPV types in a single analysis. The test specifically identifies types HPV16 and HPV18 while concurrently detecting the rest of the high risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). The cobas® HPV Test is indicated:

(a) To screen patients 21 years and older with ASC-US (Atypical squamous cells of undetermined significance) cervical cytology test results to determine the need for referral to colposcopy.

(b) To be used in patients 21 years and older with ASC-US cervical cytology results, to assess the presence or absence of high-risk HPV genotypes 16 and 18. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management. The results of this test are not intended to prevent women from proceeding to colposcopy.

(c) In women 30 years and older, the cobas® HPV Test can be used with cervical cytology 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.

(d) In women 30 years and older, the cobas® HPV Test can be used to assess the presence or absence of HPV genotypes 16 and 18. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.

Testing Schedule:

Monday through Friday – Day Shift

Method:

Real Time PCR

Specimen (IMPORTANT):

ThinPrep® Pap Test™ PreservCyt® Solution. Cervical specimens collected in PreservCyt solution using an endocervical brush/spatula have been validated for use with the **cobas®** HPV Test. Follow the manufacturer's instructions for collecting cervical specimens.

NOTE: Cytology sampling and slide preparation should be performed prior to HPV testing. Alternatively, an aliquot of ThinPrep® Pap Test™ PreservCyt® Solution may be removed and submitted for HPV testing.

Cervical specimens collected in PreservCyt solution can be transported at 2-30ºC.

Cervical specimens collected in PreservCyt solution may be stored at 2-30°C for up to 6 months after the date of collection prior to performing the **cobas®** HPV test. See PreservCyt solution labeling for storage requirements prior to cytology processing. PreservCyt specimens should not be frozen.

Alternative Specimen Type:

Cervical specimens collected in SurePath solution using cervical sampling devices with detachable heads, which include either a broom-type device or a combination brush/plastic spatula have been subjected to an analytical validation in-house at CPAL for use with the ***cobas®*** *HPV test. Follow the manufacturer's instructions for collecting cervical specimens. Samples submitted in SurePath media are subjected to a modification of the manufacturer's recommendations. This test modification (for specimens submitted in SurePath media) was developed and its performance characteristics determined by The Central Pennsylvania Alliance Laboratory, LLC. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical laboratory testing.*

Minimum Specimen Volumes:

2mls PreservCyt media
1mL for SurePath Media

Note about unacceptable specimens:
The specimen types listed above are the ***ONLY*** acceptable specimens. Urethral swabs, biopsies, anal swabs, specimens in Digene HC Cervical Sampler collection devices, vaginal swabs, etc. will be rejected.

Reference Ranges: Not Detected

Clinical Background:

Persistent infection with human papillomavirus (HPV) is the principal cause of cervical cancer and its precursor cervical intraepithelial neoplasia (CIN)[1-3]. The presence of HPV has been implicated in greater than 99% of cervical cancers, worldwide[3]. HPV is a small, non-enveloped, double-stranded DNA virus, with a genome of approximately 8000 nucleotides. There are more than 118 different types of HPV[4,5] and approximately 40 different HPVs that can infect the human anogenital mucosa.[6,7] However, only a subset of approximately 14 of these types is considered high-risk for the development of cervical cancer and its precursor lesions.[3,8-13]

Although persistent infection with high-risk (HR) HPV is a necessary cause of cervical cancer and its precursor lesions, a very small percentage of infections progress to these disease states. Sexually transmitted infection with HPV is extremely common, with estimates of up to 75% of all women experiencing exposure to HPV at some point.[14] However, almost all of infected women will mount an effective immune response and clear the infection within 2 years without any long term health consequences.[15-20] An infection with any HPV type can produce cervical intraepithelial neoplasia (CIN) although this also usually resolves once the HPV infection has been cleared.[21]

In developed countries with cervical cancer screening programs, the Pap smear has been used since the mid-1950s as the primary tool to detect early precursors to cervical cancer. Although it has decreased the death rates due to cervical cancer dramatically in those countries, the Pap smear and subsequent liquid based cytology methods require interpretation by highly trained cytopathologists and have a high rate of false negatives. Cytological abnormalities are primarily due to infection with HPV; however, various inflammatory or sampling variations can result in false positive cytology results. Triage of an abnormal cytology result involves repeat testing, colposcopy and biopsy. A histologically confirmed high-grade lesion must be surgically removed in order to prevent the development of invasive cervical cancer.

Papillomavirus is extremely difficult to culture *in vitro*, and not all patients infected with HPV have a demonstrable antibody response. Nucleic acid (DNA) testing by PCR is a non-invasive method for determining the presence of a cervical HPV

infection. Proper implementation of nucleic acid testing for HPV may increase the sensitivity of cervical cancer screening programs by detecting high risk lesions earlier in women 30 years and older with NILM cytology and reducing the need for unnecessary colposcopy and treatment in patients 21 and older with ASC-US cytology.

Assay Performance:

Performance characteristics of the cobas HPV Test are summarized below.

Performance of the cobas® HPV test in ASC-US population

The **cobas**® HPV test demonstrated sensitivity, specificity and positive and negative predictive values comparable with the hc2 test, but also offers unique individual identification of 16 and 18 genotypes, all in a single test.

- Estimated disease prevalence of 5.1% ≥ CIN2 (80/1,578)
- Estimated disease prevalence of 2.9% ≥ CIN3 (46/1,578)

		cobas® HPV test		QIAGEN® hc2 test	
		Point Estimate	**95% CI**	**Point Estimate**	**95% CI**
≥ CIN2 (n = 80)	Sensitivity (%)	90.0 (72/80)	82, 95	87.2 (68/78)	78, 93
	Specificity (%)	70.5 (1,056/1,498)	68, 73	71.1 (1,056/1,485)	69, 73
	Positive Predictive Value (%)	14.0 (72/514)	13, 15	13.7 (1,056/1,485)	8, 9
	Negative Predictive Value (%)	99.2 (1,056/1,064)	99, 99	99.1 (1,056/1,066)	98, 100
≥ CIN3 (n = 46)	Sensitivity (%)	93.5 (43/46)	83, 98	91.3 (42/46)	80, 97
	Specificity (%)	69.3 (1,061/1,532)	67, 72	70.0 (1,062/1,517)	68, 72
	Positive Predictive Value (%)	8.4 (43/514)	8, 9	8.5 (42/4979)	8, 9
	Negative Predictive Value (%)	99.7 (1,061/1,064)	99, 100	99.6 (1,062/1,066)	99, 100

- Absolute risk for ≥ CIN2 in ASC-US population was 14.0% (72/514) and for ≥ CIN3 in ASC-US was 8.4%[2]

Performance of the cobas HPV test in the NILM population (≥ 30 years)

	Negative Predictive Value (%)[3]	**Absolute Risk (%)[4]**
≥ CIN2	99.2 (98.5-99.7)	11.4 (8.3, 14.7)
≥ CIN3	99.7 (99.3-100.0)	9.7 (6.9, 12.6)

- Women ≥ 30 in the Negative Cytology population who are HPV 16 and/or 18 positive are at similar risk for disease progression as are those women with ASC-US cytology and pooled hrHPV positive.

Extensive in house method comparisons between the current HCII (Qiagen) method and the cobas HPV Test were performed prior to implementation of this assay. In addition, analytic validation of SurePath samples was performed to confirm comparable results between the HCII method and the cobas HPV test. SurePath samples for HPV testing are not FDA approved for use with the cobas HPV Test (*see note above*). The method comparison studies verified the expected performance characteristics of the cobas® HPV Test.

Limitations of Procedure:

- ✓ The cobas® HPV Test detects DNA of the high-risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. This test does not detect DNA of HPV low-risk types (e.g. 6, 11, 42, 43, 44) since there is no clinical utility for testing of low-risk HPV types.

- ✓ The cobas® HPV Test is not recommended for evaluation of suspected sexual abuse.

- ✓ The performance of the cobas® HPV Test has not been adequately established for HPV vaccinated individuals.

- ✓ Detection of high-risk HPV is dependent on the number of copies present in the specimen and may be affected by specimen collection methods, patient factors, stage of infection and the presence of interfering substances.

- ✓ 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.

- ✓ Infection with HPV is not an indicator of cytologic HSIL or underlying high-grade CIN, nor does it imply that CIN2-3 or cancer will develop. Most women infected with one or more high-risk HPV types do not develop CIN2-3 or cancer.

- ✓ A negative high-risk HPV result does not exclude the possibility of future cytologic HSIL or underlying CIN2-3 or cancer.

- ✓ Though rare, mutations within the highly conserved regions of the genomic DNA of Human papillomavirus covered by the cobas® HPV Test's primers and/or probes may result in failure to detect the presence of the viral DNA.

- ✓ Cervical specimens often show visibly detectable levels of whole blood as a pink or light brown coloration. These specimens are processed normally on the cobas® 4800 System. If concentrations of whole blood exceed 1.5% (dark red or brown coloration) in PreservCyt solution, there is a likelihood of obtaining a false-negative result. The cobas® HPV Test performance has not been validated with PreservCyt specimens which have been treated with glacial acetic acid for removal of red blood cells. Any such processing of PreservCyt specimens prior to HPV testing would invalidate the cobas® HPV Test results.

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See also **www.CPALmolecular.com** for information about this and other test offerings at CPAL.

If you are interested in HPV Test brochures for clinicians and physician offices, please contact the CPAL laboratory (717) 851-1416.