

# SECTION 2: SUMMARY OF COMMERCIALY AVAILABLE HPV TESTS

## KEY MESSAGES

- HPV tests vary and use different methods to detect the HPV: some HPV tests will detect the DNA and other HPV tests will detect E6/E7 mRNA.
- Tests that are commercially available at this time, and being used in some countries for cervical cancer screening include: Hybrid Capture 2 (Qiagen), CareHPV (Qiagen), Cobas HPV Test (Roche), Cervista (Hologic), Aptima HPV Assay (Hologic), BD HPV Assay (BD) and Xpert HPV (Cepheid).
- In choosing which HPV test will be used in the screening program, consideration needs to be given to results of clinical trials, clinical validation of the test, and other operational and logistical aspects of the test and its requirements.

## GENERAL ASPECTS OF HPV TESTS

Understanding the technical and operational aspects of available HPV tests is an important part of planning an HPV test-based screening program. There are numerous HPV tests available commercially, although only those that have been clinically validated should be used for cervical cancer screening programs.

HPV can be detected through tests that identify high-risk HPV types, either by amplification of a viral DNA fragment (with or without genotyping), or through mRNA detection (Table 1). HPV DNA tests identify the DNA of one or more oncogenic HPV types<sup>2</sup> without prior DNA amplification. Other detection tests amplify a viral DNA fragment using polymerase chain reaction (PCR) to obtain copies, both conventionally and in real-time. HPV genotyping identifies specific viral types (usually HPV 16 and 18). The mRNA tests identify expression of HPV E6 and E7 oncoproteins.

<sup>2</sup> At present, over 150 HPV types have been described, of which approximately 50 cause infections of the genital epithelium. HPV types considered oncogenic are 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.

**Table 1. HPV Tests Used for Cervical Cancer Screening**

TEST	TECHNIQUE	NAME
DNA	Direct: Genome detection	Hybrid Capture 2
		CareHPV test
	Amplification	GP5+/GP6+ bio PCR-EIA
		Cervista HPV HR
	Amplification and genotyping of HPV-16 and HPV-18	Cervista HPV 16/18
		Cobas HPV test
		Xpert HPV
		Abbott RealTime High Risk (HR) HPV assay
		PapilloCheck
RNA	Amplification of E6/E7 proteins	Aptima HPV Assay
		PreTect HPV-Proofer HV
	Monoclonal antibodies	AVantage HPV E6 Test

The clinical sensitivity of an HPV test is an important consideration for the use of the test in screening programs. A description of the various HPV tests and their performance in screening programs is summarized below.

## Direct genome detection tests

### Hybrid Capture 2

Hybrid Capture 2 (HC2) is used for cervical cancer screening, and has been clinically validated. This test detects high-risk HPV types (HR-HPV) by means of a probe cocktail for 13 HR-HPV. It is a technique in which DNA hybrids are identified with RNA probes. The Hybrid Capture 2 (HC2) technique was originally developed by the Digene Corporation (Maryland, U.S.A) and is currently produced by Qiagen (Maryland, U.S.A). Since 2000, this kit has the approval of the United States Food and Drug Administration (FDA) for routine use in early detection activities in combination with cytology. In Latin America, this test has been approved and used in public health screening programs in Colombia, Argentina, and Mexico.

Sample collection can be carried out by trained physicians, nurses, or nursing auxiliaries using a brush that is introduced into the endocervical canal, and then placed in a tube that contains a medium for transport to the laboratory.

In the laboratory, cervical cells are subjected to an alkaline denaturation solution that exposes the genetic material. Subsequently, through the use of an RNA probe cocktail (with 13 types of HR-HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) a viral-RNA: DNA hybrid is formed in the presence of any of these viruses. Hybridization is identified through specific antibodies and a chemiluminescent solution that emits light in the presence of hybrids. A luminometer is required to detect hybrids.

The test is reported as positive when light is emitted and negative when it is not, according to the final reading of the chemiluminescence signal. A positive test means that the woman has been infected by one or more of the 13 HR-HPV types. This test cannot identify the HPV type or whether one or more HPV types are present. The HC2 test is not designed to give a quantitative result; some studies have used the relative light unit (RLU) value as a quantitative evaluation of viral load.

Tests can be processed manually, semi-automatically, or be automated through use of a robot. The semiautomatic processing system has the capacity for 88 samples. Therefore, delivery of results depends on the time needed to fill the machine, which can be approximately 15 days, based on some reports from demonstration areas in Latin America. With the automated method, delivery time is approximately five hours for 352 samples per run.

As with all HPV tests, the advantages include high sensitivity and high negative predictive value. Therefore, a woman with a negative HPV test result has an extremely low probability of developing any cervical lesions in the next 5 years. The disadvantages include lower specificity and cross-reactions with low-risk probes.

### **CareHPV**

The CareHPV test uses the same principle as the Qiagen HC2 technique and detects 14 high-risk HPV types in an automated, faster process. The test has been clinically validated. The sample collection is done the same way as HC2, which was previously described. It includes DNA denaturation, hybridization with an RNA probe, hybrid capture and detection, and signal amplification. This technique has limitations similar to those of the HC2 test. CareHPV is a rapid test that requires 2.5 hours to process the 90 samples that fit in the well. It is commercially available in China and India and it is currently being used in several implementation studies in Latin America and other countries.

## **DNA Amplification Tests**

PCR gene amplification is another important molecular technique that makes it possible to obtain millions of copies from a specific DNA fragment. Different primer sets have been designed, which for the most part target region L1 and make it possible to differentiate, through specific probes, the most frequent types of high, intermediate, and low risk HPV, by doing plate hybridization of the biotinylated products previously amplified by PCR. This technique is very sensitive with a detection level down to one viral copy. However, due to its high sensitivity, this method is very susceptible to contamination. At present, in addition to generic PCR, there are specific tests that report certain viral HPV types and multiple PCR that identify several genome fragments. Tests include, among others, GP5+/GP6+, Cervista, Cobas Test, and Abbott RealTime High Risk (HR) HPV assay.

### **GP5+/bio-GP6+ PCR-EIA**

This technique was developed using GP5+/bio-GP6+ primers that amplify a fragment from the HPV L1 region; it has been clinically validated. This technique detects 37 viral types: 14 HR-HPV and 23 low-risk HPV (LR-HPV). This test is used in trials and has the advantage that the PCR products for the specific high-risk HPV types can be genotyped by reverse line blot analysis. It is one of the most frequently used techniques in research studies around the world. This technique is NOT for commercial use, nor for cervical cancer screening programs.

PCR products hybridize with a mixture of specific oligonucleotides. An enzyme immunoassay (EIA) is used for detection. Use of the GP5+/6+ PCR-EIA technique on raw extracts has high analytical sensitivity.

It has the advantages that in a simple format, up to 42 PCR products can be simultaneously typed per membrane per day, and that membranes can be easily rehybridized at least 15 times without loss of specificity or sensitivity. Its limitation is that it is not available commercially.

### ***Cervista HPV HR and Cervista HPV 16/18***

The Cervista HPV HR test is an analytically and clinically validated in vitro diagnostic test for the qualitative detection of 14 HR-HPV types in cervical specimens. Cervista cannot determine the specific HPV type. Cervista HPV 16/18 detects HPV 16 and 18. The test was approved by the FDA in 2009 to be used together with cervical cytology in women aged  $\geq 30$  years.

Cervista uses Invader chemistry, a signal amplification method for detection of specific nucleic acid sequences. This method uses two types of isothermal reactions that occur simultaneously: a primary reaction that occurs on the targeted DNA sequence and a secondary reaction that produces a fluorescent signal. The instrument has an internal control that reduces false negatives produced by a low number of cells. Cervista HPV 16/18 uses the same technology as Cervista HPV HR for genotyping.

One of the advantages is that this technique is highly reproducible and sensitive. The internal quality control to confirm sample quality is one of its greatest advantages in the market. However, according to the manufacturer it has certain limitations, including cross-reactivity to two HPV types of unknown risk; more specifically is positive for HPV-67 with 5,000 copies/reaction and positive for HPV-70 with 50,000 copies/reaction. Furthermore, low levels of infection or sampling error may cause false negatives.

### ***Cobas HPV Test***

The Cobas HPV test detects 12 high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), and specifically reports on HPV 16 and 18. This is a clinically validated in vitro qualitative test. The system uses the  $\beta$ -globin gene as an internal control for specimen integrity, extraction, and amplification. The system is totally automated, facilitating laboratory workflow. It consists of a Cobas Z thermocycler and the necessary software for real-time PCR, using primers for the HPV L1 region. The procedure includes processing of DNA extraction samples and real-time PCR analysis. The technique does not cross-react with non-carcinogenic genotypes. Furthermore, the operator has minimal contact with the sample, preventing contamination. This system can carry out 96 tests in approximately five hours.

The advantages of this system are reduction in processing and work time; reduction in repetitive motions; reduction in the risk of errors due to fatigue; reduction in the production of biohazard waste; and reduction in costs by eliminating the need for additional reagents.

Limitations mentioned by the manufacturer include that testing needs to be done by personnel with experience in PCR techniques and with the Cobas HPV test system. Furthermore, only the Cobas x 480 instrument and the Cobas z 480 analyzer have been validated for use with this product. No other sample preparation instrument

or PCR system can be used with this product. The presence of PCR inhibitors, as well as a low number of virus copies in the sample, may cause false negatives or invalid results.

### ***Abbott RealTime High Risk (HR) HPV assay***

The Abbott RealTime High Risk (HR) HPV assay test detects 14 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). This test reports on HPV 16 and 18 separately from the other high-risk HPV types.

This is a completely automated in vitro qualitative process that is clinically validated according to international requirements for use in screening in women starting at age 30 years.

The system consists of an m2000sp instrument that prepares the nucleic acid and an m2000rt analyzer that carries out real-time PCR using a mixture of multiple primers and probes for amplification and detection of HR-HPV DNA and for the  $\beta$ -globin gene, as an internal quality control of cervical cells collected in liquid-based cytology.

The response time of the process is from six to eight hours for 96 samples and depends on the DNA extraction method used.

The advantages of this technique are the automation of the multiple steps—reducing personnel—time used, and risk of contamination. Subjective interpretation is one of the test's limitations. In recent years, real-time PCR has been introduced in molecular HPV diagnosis as a tool for quantitative determination of viral load as well as for diagnosis of infection. Real-time detection of amplified products can be carried out using fluorescent molecules that are inserted in double chain DNA, such as SYBR Green, or through hybridization with different types of probes, such as Taqman probes, fluorescent primers, or molecular beacons and hydrolysis probes. Probe use increases reaction specificity.

### ***BD HPV Assay***

The BD HPV test is a real-time PCR that amplifies the region that codes HR-HPV E6/E7 oncoproteins. It has been clinically validated. These regions are present throughout the stages of the disease's progression and the assay has been designed to detect specific regions according to virus type, instead of amplification of gene regions detected with L1 primer sets. The test provides individual information for six HPV types (16, 18, 31, 45, 51, and 52), as well as detection of all 14 HR-HPV. The BD HPV test performs as well as other tests approved by the FDA and those with European Commission CE (Conformité Européenne) marking—including HC2—and using cervical specimens collected in PreservCyt medium (Hologic, Marlborough, MA, U.S.A.).

The samples are processed in the BD Viper system, which has an internal quality control. This technique achieved CE approval in January 2015 and is available for commercial use. The system is totally automated and can process 1-30 samples per run and 120 results per patient per day, including genotyping.

### ***Xpert HPV***

The Xpert HPV test is a real-time PCR that simultaneously detects DNA encoding for E6/E7 oncoproteins of 14 HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). The samples are processed as individual

cartridges in the GeneXpert platform from Cepheid (Sunnyvale, CA, U.S.A.). This is a molecular diagnostic platform with a capacity to process one test at a time, to 80 tests, in one hour. Test results are reported for overall high-risk HPV status, as well as the presence of high-risk HPV genotypes.

## E6/E7 mRNA Detection

The carcinogenic process is regulated by HPV E6 and E7 oncoproteins and, as a result, excessive expression of these genes is a risk marker for cervical cancer. It has been postulated that detection of E6/E7 oncogene expression could be more specific and be a better cancer risk predictor than the HPV-DNA test. At least two methods use RNA detection: the Aptima HPV Assay test of E6/E7 messenger RNA (Gen-Probe), which detects 13 HR-HPV types and HPV-66; and the PreTect HPV-Proofer (NorChip) test, which detects RNA of HPV types 16, 18, 31, 33, and 45.

### **APTIMA HPV Assay**

This qualitative test is based on direct detection of the expression of E6 and E7 mRNA oncoproteins, from the 14 types of HR-HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) through real-time amplification (48, 49). The APTIMA HPV Assay does not discriminate among the 14 types. The test can analyze cervical samples collected in tubes for ThinPrep cytology with PreservCyt solution. The assay includes an internal control to oversee nucleic acid capture, amplification, detection, as well as user or APTIMA HPV E6/E7 instrument errors. This system can carry out up to 250 tests in approximately five hours.

This technique was approved by the FDA in 2011 for screening women starting at age 30 years, in combination with Pap smears.

It has several limitations, such as, that the test has not been evaluated in HPV-vaccinated individuals; that detection of high-risk HPV mRNA depends on the number of copies in the specimen and that, in addition and according to the literature, false positives can occur with low-risk HPV.

## Arbor Vita OncoE6 Cervical Test

The OncoE6 test is a lateral flow strip test that detects high levels of viral E6 oncogene as a biological marker and it could be used as a triage test in women positive for the HPV test. The product has been evaluated in a large-scale study in China. It has CE approval and is ready for marketing.

Sample collection is done using a Dacron swab and PreservCyt media. It does not require complex equipment for processing. The equipment costs around US\$2,000 and can process 45 specimens per operator per day, a volume that can be processed in a clinic within 2-2.5 hours.

## Performance of HPV tests

The scientific evidence to demonstrate that HPV tests are effective in reducing cervical cancer mortality is available from cross-sectional studies in which the HPV tests are evaluated alongside cervical cytology to evaluate test sensitivity and specificity. Evidence is obtained from randomized clinical trials in which women are assigned to two groups: an HPV test intervention group and a Pap smear control group. This makes it possible to evaluate test sensitivity and specificity as well as reduction in cancer mortality. Table 2 shows the HPV test sensitivity and specificity of the tests described above.

**Table 2. Performance of HPV tests used for cervical cancer screening**

TEST	SENSITIVITY (%)	SPECIFICITY (%)
Hybrid Capture 2	97.5	84.3
CareHPV	90.0	84.2
Cervista HPV	100	
Cobas HPV Test	97.3	84.5
Abbott RealTime High Risk (HR) HPV assay	95.0	87.2
Aptima HPV Assay	97.6	90.2
Xpert HPV	100	81.5

Source: Cuzick J et al. 2013.

## Selection of the HPV test for the cervical cancer screening program

Once the decision has been made to introduce HPV testing into the screening program, the most suitable HPV test can be chosen from the options available on the market. The selection needs to be based on clinical validation of the test, operational and logistical aspects, and the test's costs and benefits. Some questions to consider are as follows:

- What HPV types are detected by the test?
- How are the results presented: by HPV type or as HPV positive/negative?
- What are the manufacturer requirements and costs of the HPV test, equipment and supplies?
- What is the appropriate lot size to process samples?
- How long does specimen processing take?
- What type of training is needed to process the tests?
- What in-country support is available for equipment installation and maintenance?
- How is the quality of the test result controlled?
- Can self-sampling be used with the HPV test?
- What are the requirements for storage, and other supply chain management issues?
- With the local distributor of the HPV test, are there any conditions, arrangements and additional costs to consider?

### ***Considerations for choosing an HPV Test***

- Before selecting an HPV test from among the wide range available on the market, conduct a cost-benefit analysis and consider the feasibility of implementing the HPV test in the context of the screening program.
- Choose an HPV test that has been clinically validated.
- HPV tests authorized by regulatory agencies, such as the U.S.A. Food and Drug Administration and/or the European Medicines Agency (EMA) would be prudent options.
- Introducing a specific HPV test into the screening program, and then later changing to another HPV test may be logistically difficult and will have cost implications.
- HPV tests have expiration dates—for example, 9 months or 12 months—and supply chain management aspects need to be considered when choosing the HPV test for use in the conditions and context of the screening program.