Cumhuriyet Medical Journal, 45(1): 1-11, 2023
DOI: http://dx.doi.org/10.7197/cmj.1187503

Cumhuriyet Medical Journal

| cmj.cumhuriyet.edu.tr | Founded: 2004 Available online, ISSN:1305-0028 Publisher: Sivas Cumhuriyet Üniversitesi

FDA-Approved Molecular Tests Used to Define Human Papillomavirus (HPV) Infections which Cause Cervix Cancer

Abdulhamit Çalı[1,2,A*], Rukiye Aslan[1,3,B], Cem Çelik[1,C], Ahmet Alim[1,d]

[1] *Sivas Cumhuriyet University, Faculty of Medicine, Department of Medical Microbiology, Sivas, Turkey*

[2] *Lokman Hekim University, Vocational School of Health Services, Medical Laboratory Techniques Program, Ankara, Turkey*

[3] *Sivas Cumhuriyet University, Vocational School of Health Services, Department of Medical Services and Techniques, Sivas, Turkey*

**Corresponding author*

Review Article

History

Received: 12/10/2022
Accepted: 26/03/2023

ABSTRACT

Human papillomavirus (HPV) is a non-enveloped, commonly sexually transmitted virus with icosahedral symmetry and double-stranded circular DNA. Its genome, which is about 8 kb in size, encodes early genes (E1-8) and two late structural capsid genes (L1 and L2). Among the genes that play a role in viral pathogenesis, L1, E6, and E7 genes frequently exist. The E6 and E7 viral genes have a significant role in apoptosis inhibition, viral spread, development of squamous intraepithelial lesion (SIL), cell immortalization, neoplastic transformation, and invasive cancer.

Demonstrating the relationship between cervical cancer and HPV infections has led to increased interest in this subject and the classification of some HPV genotypes in the high-risk group (HR-HPV) for cervical cancer. Numerous commercial molecular tests have been developed to identify HPV genotypes involving different approaches. HPV molecular tests approved by the US Food and Drug Administration (FDA) include Hybrid Capture® 2 (HC2), Cervista™, cobas®, Aptima®, and BD Onclarity™. This article reviews five FDA-approved tests' methodologies, limitations, and commonalities. The HC2 and Cervista™ tests use non-PCR-based signal amplification methods, while the cobas® and BD Onclarity™ tests use PCR-based target amplification methods. On the other hand, the Aptima® test uses the mRNA transcriptional mediated amplification (TMA) method.

Each of these methods used in the diagnosis and follow-up of HPV has its strengths and weaknesses. These HPV molecular tests have high sensitivity and specificity. They are also more automated and repeatable than cytological methods. In addition to these advantages, there are also several limitations. Because of these limitations, molecular tests are no more perfect than cytological tests. This situation shows that these tests should not be used alone in the evaluation of HPV infections and cancer identification. On the contrary, HPV test results should be correlated with cytology or biopsy findings.

Keywords: Cervical cancer; Human papillomavirus; HPV; Molecular diagnosis

Rahim Ağzı Kanserine Neden Olan Human Papillomavirüs (HPV) Enfeksiyonlarının Tanımlanması için Kullanılan FDA Onaylı Moleküler Testler

Süreç

Geliş: 12/10/2022
Kabul: 26/03/2023

ÖZ

İnsan papilloma virüsleri (HPV), zarfsız, genellikle cinsel yolla bulaşan, ikozahedral simetriye ve çift zincirli halkasal DNA'ya sahip bir virüstür. Yaklaşık 8 kb büyüklüğündeki genomu, erken genleri (E1-8) ve iki geç yapısal kapsid genini (L1 ve L2) kodlamaktadır. Viral patogenezde rol oynayan genler arasında sıklıkla L1, E6 ve E7 genleri bulunmaktadır. E6 ve E7 viral genleri, apoptoz inhibisyonu, viral yayılım, skuamöz intraepitelyal lezyon (SIL) gelişimi, hücre ölümsüzleşmesi, neoplastik transformasyon ve invaziv kanserde önemli bir role sahiptir.

Rahim ağzı kanseri ve HPV enfeksiyonları arasındaki ilişkinin gösterilmesi, bu konuya olan ilginin artmasına ve bazı HPV genotiplerinin rahim ağzı kanseri için yüksek riskli grup (HR-HPV) içerisinde sınıflandırılmasına neden olmuştur. HPV genotiplerinin tanımlanması için farklı yaklaşımları içeren çok sayıda ticari moleküler testler geliştirilmiştir. ABD Gıda ve İlaç Dairesi (FDA) tarafından onaylanmış HPV moleküler testleri arasında Hybrid Capture® 2 (HC2), Cervista™, cobas®, Aptima® ve BD Onclarity™ bulunmaktadır. Bu makalede FDA onaylı beş testin metodolojileri, sınırlamaları ve ortak özellikleri gözden geçirilmektedir. HC2 ve Cervista™ testleri PCR tabanlı olmayan sinyal amplifikasyon yöntemlerini kullanırken, cobas® ve BD Onclarity™ testleri PCR tabanlı hedef amplifikasyon yöntemlerini kullanır. Öte yandan Aptima® testi, mRNA transkripsiyonel aracılı amplifikasyon (TMA) yöntemini kullanır.

HPV tanı ve takibinde kullanılan bu yöntemlerin her birinin kendilerine özgü güçlü ve zayıf yönleri bulunmaktadır. Bu HPV moleküler testleri yüksek duyarlılık ve özgüllüğe sahiptirler. Ayrıca sitolojik yöntemlerden daha otomatik ve tekrarlanabilirlerdir. Bu avantajlara ek olarak, çeşitli sınırlamaları da vardır. Bu sınırlamalar nedeniyle moleküler testler sitolojik testlerden daha mükemmel değildir. Bu durum, HPV enfeksiyonlarının değerlendirilmesinde ve kanser teşhisinde bu testlerin tek başına kullanılmaması gerektiğini göstermektedir. Aksine, HPV test sonuçları sitoloji veya biyopsi bulguları ile ilişkilendirilmelidir.

Anahtar sözcükler: Rahim ağzı kanseri; İnsan papillomavirüs; HPV; Moleküler tanı

[a] *abdulhamit.cali@lokmanhekim.edu.tr* *https://orcid.org/0000-0001-6423-9609* [b] *raslan@cumhuriyet.edu.tr* *https://orcid.org/0000-0001-5843-626X*
[c] *ccelik@cumhuriyet.edu.tr* *https://orcid.org/0000-0002-7141-5874* [d] *ahmetalim@cumhuriyet.edu.tr* *https://orcid.org/0000-0001-9577-5965*

How to Cite: Çalı A, Aslan R, Çelik C, Alim A (2023) Fda-Approved Molecular Tests Used To Define Human Papillomavirus (HPV) Infections Which Cause Cervix Cancer, March 2023, 45(1): 1-11

Human papillomaviruses (HPV); are commonly sexually transmitted, non-enveloped, icosahedral symmetry, double-stranded circular DNA viruses [1]. The icosahedral structure consists of structural proteins, the major capsid protein (L1) and the minor capsid protein (L2) [2]. HPV has a genome of about 8 kb. This genome encodes early genes (E1-8) and two late structural capsid genes (L1 and L2). The genome also has a 0.9 kb non-coding region[3,4].

More than 100 HPV genotypes that differ in their oncogenic potential have been identified in [5]. Antigenically different genotype groups cause the formation of specific lesions. These groups are classified as "low risk (low risk, LR); probable high risk (PrHR); and high risk (high risk, HR) groups" according to their cancer responsibility. Members of the LR-HPV group may cause benign lesions (papilloma and condyloma), while members of the HR-HPV group may cause cancers such as cervical cancer [6]. Clinically, there are 14 HPV genotypes (HPV-16, 18, 58, 33, 45, 31, 52, 35, 59, 39, 51, 56, 66, and 68, respectively) worldwide within the HR-HPV group [7].

HPV-16 and 18 are the two genotypes most associated with cervical cancer. The main functions of each gene of the HPV-16 virus are summarized in Figure 1. Among the genes that play a role in viral pathogenesis are frequently found in L1, E6, and E7 genes. E6 and E7 genes increase viral spread by inhibiting apoptosis. In addition, the expression of E6 and E7 oncogenes are associated with the development of squamous intraepithelial lesion (SIL), as well as having a prominent place in cell immortalization, neoplastic transformation, and invasive cancer development [7,8].

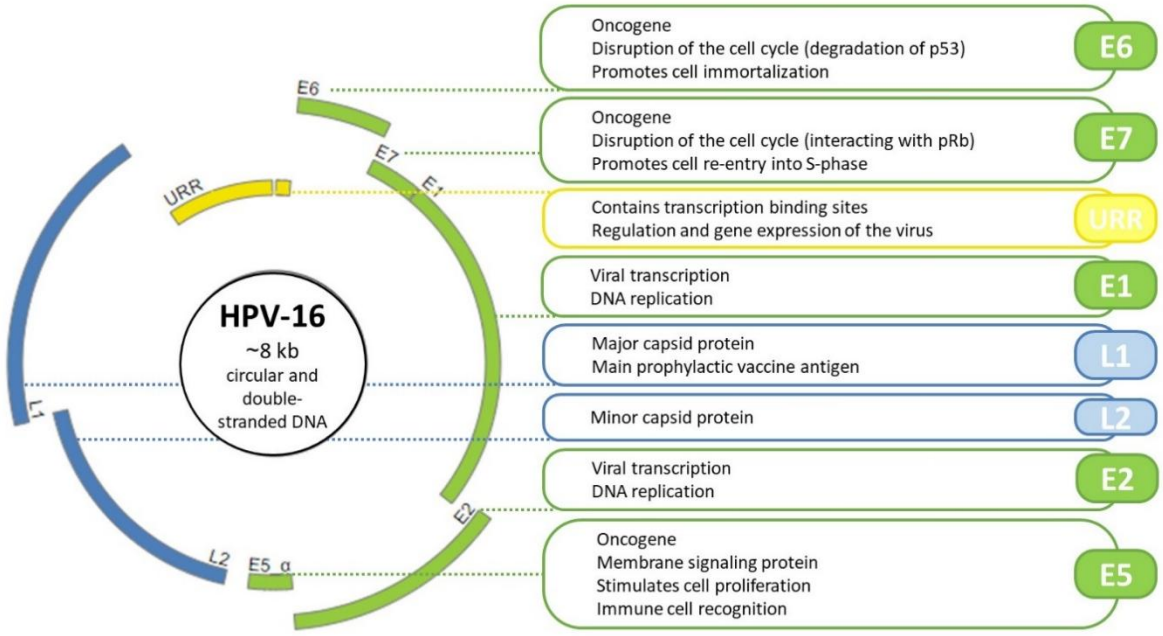

Figure 1. The genome of HPV-16 virus and functions of genes. The HPV genome consists of three main regions: the URR region containing the transcription binding region, the early gene (E1-8) regions, and the L1-2 late structural capsid gene regions [3,9]

HPV is the main cause of cervical cancer, one of the most common types of cancer in women worldwide [2,10]. Screening tests can identify this type of cancer. This situation has increased the importance of histopathological and molecular methods. Molecular tests are more sensitive than Papanicolaou (Pap) smear [2]. However, since most HPV infections are transient, it is not recommended to use molecular tests as direct screening tests. Because this case, patients may be exposed to unnecessary invasive procedures. Therefore, algorithms based on the combined use of Pap smear and molecular tests have been improved, which predict cervical cancer screening to be carried out within certain programs to avoid or minimize such situations. In addition, various methods are being developed to diagnose HPV by detecting biomarkers related to HPV[6]. In addition, the United States Food and Drug Administration (FDA) has approved that some commercial tests can be used as an adjunct screening test for HPV infections and for genotyping for certain genotypes [10]. This review aims to provide information about FDA-approved molecular tests that can be used to differentiate lesions at risk of progression into cervical cancer and discuss the limitations of these tests.

Molecular Tests Used in the Diagnosis of HPV

While HPV cannot be replicated in standard tissue culture, it can only be reproduced in special media such as xenograft or organotypic raft cultures. However, these methods are not used in routine virological diagnosis because their application is difficult and time-consuming. These methods are used only for vaccine development and research purposes [4]. For this reason, accurate identification is made by molecular biological techniques as well as cytological examinations [11]. There are three basic molecular methods used for HPV identification. These are nucleic acid-hybridization methods, signal amplification methods, and target amplification methods [6]. The main features of these methods are given in Table 1.

Table 1. Comparison of molecular methods used in the diagnosis of HPV

Method	Strengths	Weaknesses
Nucleic acid- hybridization	The Southern blotting method is the gold standard The presence of HPV is associated with morphology	Large amounts of purified DNA low sensitivity Time-consuming
Signal amplification	Commercial kits Quantitative method Lower false positive rate High sensitivity to genotyping	Certified licensed products Cocktail approach to genotyping
Target amplification	Flexible technology (viral load and genotype) Very high sensitivity Multiplex analysis	Contamination with previously amplified material can lead to false positives No standardization Low amplification signals for some HPV genotypes

Nucleic Acid-Hybridization Method

In nucleic acid-hybridization methods, radioactively labeled substances are used detection of HPV from cervical specimens [13,14]. These methods are Southern blotting, in situ hybridization, and dot-blot hybridization [13]. Among them, the Southern blotting method is the gold-standard method for HPV genomic analysis [15]. Although significant knowledge has been gained from these methods, the use of these methods is finite by low sensitivity, time-consuming procedures, and the need for relatively large amounts of purified DNA [13–15].

Signal Amplification Method

Signal amplification methods are an extension of direct probe techniques with increased sensitivity with innovations in detection methods [12]. In signal amplification systems, nucleic acids are first hybridized with specific probes. Then the degree of signals generated from these hybrid complexes is increased [4]. Hybrid Capture® 2 (HC2) method is the first signal amplification-based method approved by the FDA and used diagnosis of HPV. In addition, Cervista™ HPV HR and Cervista™ HPV 16/18 tests are commercial tests approved by the FDA and use the signal amplification method [11,17].

Target Amplification Method

The target amplification method is the most flexible and sensitive of all DNA analysis methods [17]. This method can be carried out for detection, viral load analysis, DNA sequencing, and mutation analysis [19,20]. Polymerase Chain Reaction (PCR) is one of these methods. PCR is a method that provides in vitro amplification and diagnosis of unique DNA regions in HPV infections, as in most viral infections. In addition, multiplex analyses, which allow simultaneous analysis of multiple target DNA sequences, can be performed to diagnose HPV infections [20].

Environmental contamination is very significant for PCR tests. Previously amplified materials may result in a false positive result for a negative sample [22]. There is an inherent risk of false-negative results when there is no competition between reagents, or there are multiple infections in low-copy samples.

Therefore, the detection of HPV genotypes may be adversely affected. In addition, recurrent infections constitute the disadvantage of this method [21].

The target amplification tests approved by the FDA are the cobas® HPV, APTIMA® HPV, and BD Onclarity™ HPV tests. Of these, the cobas® HPV and BD Onclarity™ HPV tests are based on DNA target amplification, while the APTIMA® HPV test is based on mRNA transcription-mediated amplification [8].

Some features of HPV molecular tests classified by signal amplification and target amplification methods and approved by the FDA are summarized in Table 2

Table 2. Comparison of FDA-approved HPV molecular tests[8]

Feature	Digene HC2 HR-HPV	Cervista™ HPV HR and HPV 16/18	cobas® HPV	APTIMA® HPV	BD Onclarity™ HPV
Manufacturer	Qiagen	Hologic	Roche	Hologic	Becton Dickinson
FDA approval date	2001	2009	2011	2011	2018
Method	Non-PCR-based DNA signal amplification	Non-PCR-based DNA signal amplification (Fluorescent resonance energy transfer FRET)	PCR-based DNA target amplification	mRNA transcription-mediated amplification (TMA)	PCR-based DNA target amplification
Target gene	Complete genome	L1, E6, and E7	L1	E6 and E7	E6 and E7
Detectable genotypes	13 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68)	Cervista® HPV HR test; 14 HR- HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) Cervista® HPV 16/18 test detects HPV-16 and 18 genotypes	14 HR-HPV genotypes Channel 1: HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 Channel 2: HPV 16 Channel 3: HPV 18	APTIMA® HPV test; 14 HR-HPV genotypes (6, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) APTIMA HPV 16 18/45 test; HPV-16, 18/45 genotypes	14 HR- HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68)
Internal control	No	Human HIST2H2BE	β - globin	An internal control transcript (HPV16 E6/7 transcript) is added to each reaction	β - globin
Collection medium	HC2 Specimen Collection Device (cervical brush and STM). PreservCyt Solution or BD SurePath	ThinPrep	PreservCyt	ThinPrep (2000 processor) Pap Test vials (containing PreservCyt Solution)	LBC media (BD SurePath ™, Hologic PreservCyt) BD Onclarity cervical brush
Test reading method	Automatic (chemiluminescence)	Fluorescence	Automatic (fluorescence)	Luminometer	Automatic (fluorescence)

FDA: US Food and Drug Administration, PCR: Polymerase Chain Reaction, HC2: Hybrid Capture 2, HPV: Human Papillomavirus, mRNA: messenger RNA, HR: High Risk.

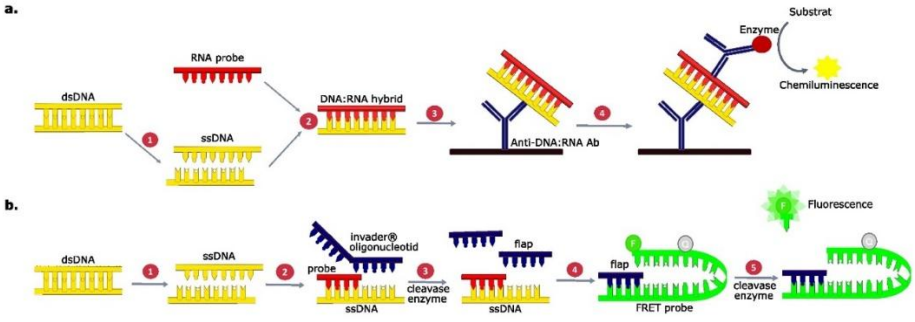

Figure 2. HPV signal amplification methods. A) Hybrid Capture® 2 method used in HC2 HR-HPV test. B) FRET probe method used in Cervista™ HPV HR and Cervista™ HPV 16/18 assays (inspired by resource 22).

HC2 HR-HPV Test

Hybrid Capture® 2 (Digene/Qiagen, Gaithersburg, MD, USA) is a signal amplification method used for the detection of HPV-DNA from endocervical and liquid cytological samples. It is used to identify 13 HR-HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and 5 LR-HPV genotypes. It is a reliable and reproducible method[23,24]. The patient sample collected in the test solution (PreservCyt) is prepared with the test kit (Digene). The test is a semi-quantitative molecular hybridization test that works by detecting radiation from a chemiluminescent substrate. That is, it is nonradioactive signal amplification based on the hybridization of target DNA to labeled RNA probes in solution. DNA-RNA hybrid formed in a consequence of the hybridization of HPV DNA and RNA probe generates a signal by enzyme-linked immune sorbent assay (ELISA) with alkaline phosphatase-conjugated monoclonal antibodies (Figure 2a). Results are appraised as the ratio of the positive control sample to relative light units (RLU). Luminous intensity is measured with a luminometer. The recommended 1 RLU/PC (positive cutoff value) of 1 pg/mL is equivalent to 5000 viral copies. Samples with an RLU/PC ratio ≥1.0 are considered positive [25]. While this test categorizes LR and HR viruses, it is not specific for HPV genotyping. That is, it cannot identify specific HPV types. If more than one genotype is positive, the distinction cannot be made. With this grouping, it can be determined that there is a precancerous lesion risk of 15% for HR- HPV -16/18 and below 3% for other HR types. HPV genotyping is important in determining the oncogenic types of HPV 6.

The HC2 test can only test for the presence of 13 HR-HPV genotypes [26]. It can cross-react with the HPV-66 genotype [27]. It is thought that the test results should be compared with the results of another method. Since the HC2 test can give false positive results (10%), HC2 negative samples should also be included in studies for analysis accuracy [26,27].

Cervista™ HPV HR Test

The Cervista™ HPV HR test (Hologic, Inc; Marlborough, MA) was approved by the FDA in 2009. It is a qualitative signal amplification assay that detects specific nucleic acid sequences (DNA) for 14 HR-HPV strains (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). It uses the Invader® oligonucleotide (Hologic™, Inc) [27]. This method is based on the enzymatic degradation by the structure-specific 5' nuclease enzyme Cleavase® (Hologic™, Inc). Sequence-specific probes and Invader® oligonucleotides bind to the target DNA sequence to form a substrate for the enzyme. The probes in the target sequence open and close, and multiple 5' wings are formed in the target sequence. These multiple split wings bind to the universal hairpin fluorescent energy transfer (FRET) oligonucleotide, creating a different substrate for the enzyme. The enzyme breaks down FRET and creates fluorescent signals as the wings open and close (Figure 2b) [28]. The Cervista HPV HR test consists of two isothermal reactions, a primary reaction in the target DNA sequence and a secondary reaction that generates a fluorescent signal, and an internal DNA probe [27]. A mixture of 3 oligonucleotides is used as a reagent to test the 14 HR HPV types in total. The mix 1 (A5/16) is used for HPV types 51, 56, and 66; the mix 2 (A7) is used for HPV types 18, and the mix 3 (A9) is used for HPV types 16, 31, 33, 35, 39, 45, 52, 58, 59 and 68 [29]. The Cervista HPV HR is a method designed to reduce false-positive results caused by the cross-reactivity of low-risk HPV types. However, there is an internal control system that confirms that a sufficient amount of DNA has been tested [26]. It does not cross-activity with LR-HPV genotypes and non-oncogenic genotypes. It avoids most unnecessary colposcopy practices and can operate with a small amount of patient sample (2 mL). Thus, it increases the reliability of the test by reducing the number of insufficient DNA samples, and it prevents false-negative HPV results. The Cervista HPV HR test is among the most recommended molecular methods for HPV diagnosis. It reliably detects 14 HR HPV genotypes, including the HPV-66 genotype. The test has high specificity, sensitivity, and accuracy [27].

Cervista™ HPV 16/18 Test

The Cervista HPV 16/18 (Hologic, Inc; Marlborough, MA) test was approved by the FDA in 2011. It is a genotyping test used for the detection of HPV 16 and 18. The Invader® oligonucleotide (Hologic TM, Inc), which is the signal amplification method, is used to detect specific nucleic acid sequences [27]. It works in the same way as the Cervista HPV HR [28]. Besides the L1 gene region, it also targets other gene regions. There is a low rate of positivity among genotypes. The sensitivity of the test is 98% in cervical intraepithelial neoplasia (CIN 2) lesions and 100% in CIN 3 lesions. The Cervista is a qualitative signal amplification method using specific nucleic acid sequences and signal amplification method of 14 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). It is a test with high analytical

sensitivity and specificity against HPV-16/18 genotypes. However, this test cannot identify HPV genotype specifically [6]. For both the analytical and clinical performance of an HPV diagnostic test, the specificity of the test is so significant [27]. The Cervista HPV 16/18 test consists of a primary reaction at the target DNA sequence and a secondary reaction that generates a fluorescent signal [30]. The Cervista HPV 16/18 uses a mixture of 2 oligonucleotides, each containing a probe specific to the L1, E6, and E7 genomic regions. The Cervista 16 and 18 both contain two genes for the detection of cellular DNA in oligonucleotide mixtures targeting human histone protein. The "signal-to-noise" value for each test is evaluated above zero (FOZ). The FOZ threshold value for a positive result is considered 2.13 [29].

The Cervista 16/18 test has high specificity, sensitivity, accuracy, and interlaboratory repeatability. Because of the increased incidence of HPV-16 and 18 infections and cervical cancers, the data on the presence of these genotypes is significant for screening strategies for cervical cancers [31]. The Cervista tests are more useful for patients who do not have a sample of primary oropharyngeal carcinoma or for whom head-neck carcinoma is not identified. Because HPV-16 is the predominant, high-risk HPV genotype in oropharyngeal carcinoma, the Cervista 16/18 test provides an assessment of the health status of patients accurately with oropharyngeal carcinoma[32].

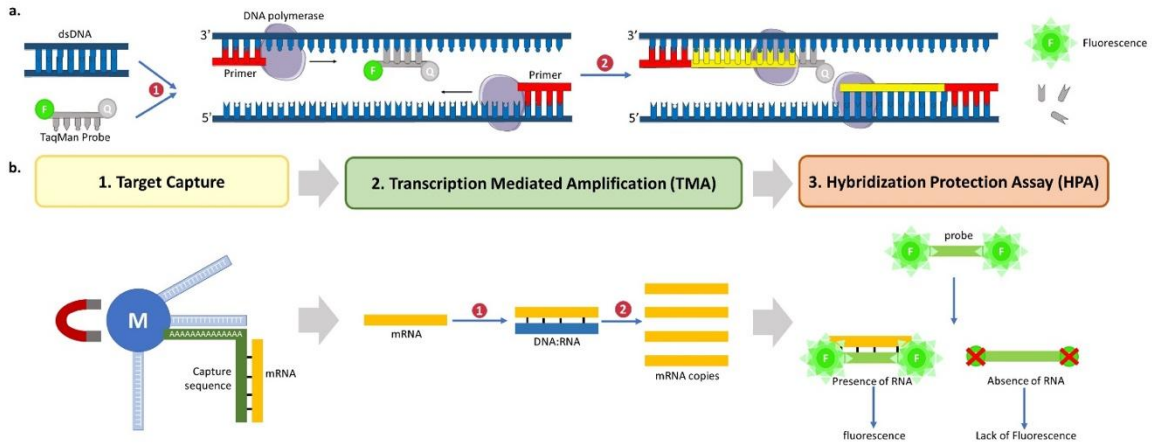

Figure 3. HPV target amplification methods. A) TaqMan probe method used in cobas® HPV and BD Onclarity™ HPV tests. B) Transcription-mediated amplification used in the APTIMA® HPV test (inspired by reference no: 22).

cobas® HPV Test

The cobas® HPV test, produced by Roche (Branchburg, NJ), was first approved for use in 2011 [33]. Liquid-based cervical swab sample is used as the test material [34]. This test makes automatic HPV identification using a real-time PCR technique. This test is a qualitative test that amplifies the DNA of the target gene L1 [35]. The reaction happens in a single PCR tube. There are four fluorescent probes for HPV-16, HPV-18, the other 12 genotypes, and each of the internal control (β-globin) [34]. Thus, the test can identify HPV-16 and HPV-18 types specifically [35].

The reaction occurs when the sample contains the L1 gene sequence of the HR-HPV genotypes. The specific primers bind to complementary sequences, and amplification occurs. One end of the TaqMan probes is labeled with a fluorophore and the other end with a quencher. The fluorescent emission does not occur due to the extinguisher. Once the probe is attached to an identifier sequence, it will degrade because of the continued qPCR reaction due to the 5'→3' exonuclease activity of the polymerase (Figure 3a). When the probe breaks down, fluorescent radiation occurs because the fluorophore is separated from the quencher. When the probe breaks down, fluorescent radiation occurs because the fluorophore is separated from

the quencher. The detection process is performed by detecting the fluorescent beam [36].

There are studies in the evaluation results of the cobas® HPV test that show that the test's repeatability is very consistent [37]. This fully automated test is easy to apply. It can provide reliable results in as little as 4 hours [38]. It has higher sensitivity compared to the HC2 HR-HPV test [39]. According to the study of Stoler et al. with 1578 cervical samples, the sensitivity of the HC2 HR-HPV test was 87.2%, while the sensitivity of the cobas® HPV test was 90% [40]. This test does not cross-react with other microorganisms. This test also does not interact with lubricants or antifungals [34]. This test can only differentiate between HPV-16 and HPV-18 genotypes. Because other HPV types have the same signal, their detection is not possible.

APTIMA® HPV Tests

The APTIMA® HPV test, produced by Hologic Gene-Probe (San Diego, CA) was approved for use by the FDA in 2011 [8]. As the second test in 2012, APTIMA® 16 18/45 was approved. ThinPrep cervical samples are used as test material [41]. All the tests approved so far detect the viral genome of DNA. However, the APTIMA® HPV test is the first FDA-approved test to detect mRNAs of the E6 and E7 gene regions. Detection of mRNAs of E6/E7 genes makes this test method more specific and sensitive than other DNA detection methods [42]. While the APTIMA® HPV test can identify 14 HR-HPV E6/E7 gene region mRNAs, only three genotypes (HPV-16, -18, and -45) can be detected with the APTIMA® 16 18/45 test [43].

The APTIMA® test consists of three steps, which take place in a single tube. These include target capture, transcription-mediated amplification, and detection of amplification products [41]. The samples are transferred to the sample transport medium, firstly. Thanks to this medium, cell lysis takes place, and mRNAs are released. Then, the target mRNAs bind to complementary oligonucleotides with a poly-deoxyadenosine (polyA) tail. Subsequently, these hybrids are linked by a poly-deoxythymidine (PoliT) tail attached to magnetic microparticles. This targeted mRNA is separated by a magnet. The complementary DNAs (cDNA) are built using reverse transcriptase and T7 polymerase enzymes (Figure 3B-1). The composed cDNAs allow the formation of new RNA multiplexes. The Hybridization Protection Test (HPA) is used to detect the resulting amplicons. With this method, the hybridization of the duplicated sequences with fluorescent-labeled oligonucleotide probes takes place. This results in fluorescent radiation. This emitted light is measured with a luminometer. If hybridization does not occur, the probe is degraded with the borate buffered solution (Figure 3b) [41].

The HPV E6/E7 mRNA marker is a better indicator of advanced disease than the commonly used HC2 method. According to the study conducted by Ratnam et al. (2011), the sensitivities of APTIMA® and HC2 HR-HPV tests were detected at 96.3% and 94.3%, respectively [44]. In addition, this test was determined to have a higher specificity compared to the cobas® HPV test. In a study of 1000 samples by Castle et al., they found that the APTIMA® HPV test was more specific than the cobas® HPV test [45]. It does not cross-react with LR-HPV strains and can be automatically processed. In addition to many advantages, it is stated as a disadvantage that it has a lower detection limit compared to other tests [43].

The APTIMA® HPV test, which emerged more recently than the HC2 method, is likely to have some limitations because it is less tested than the HC2 method. Preisler et al. detected cross-reactivity in 26, 61, 62, 67, 70, 82, and 83 strains for the APTIMA® test in their investigations with HC2 HR-HPV, cobas® HPV, and APTIMA® HPV tests [46]. In the studies conducted during the development of this test, it was determined that 26, 67, 70, and 82 strains were cross-reacted [41].

BD Onclarity™ HPV

The BD Onclarity™ HPV test, produced by Becton Dickinson (Sparks, MD), was FDA approved in 2018. After cervical samples are collected with swabs, they are collected in the BD SurePath™ and run from the solution. The DNA target amplification is performed using the Real-time PCR method, with this fully automated test. The E6/E7 oncogenes of 14 HR-HPV genotypes can be detected by this test[47]. The test consists of two stages. The first step consists of cell lysis and DNA isolation. The second stage is based on the TaqMan probe method, as is the method of the cobas® HPV test (Figure 3a) [47,48]. However, the reaction is performed in three separate tubes in this test, unlike the cobas® HPV test. A total of 15 probes and 4 fluorescent dyes are used, 14 for viral sequences and 1 for internal control. HPV-16, 18, and 45 genotypes are detected in the first of three PCR tubes, HPV-31, 33/58, and 56/59/66 are detected in the second PCR tube, and the HPV-51, 52, and 35/39/68 are detected in the third PCR tube. There is also a human β-globin gene region in each tube for internal control [47].

According to the results of studies conducted during the development of this test, the performance of the BD Onclarity™ HPV test to detect high-grade cervical disease was found to be higher compared to other FDA-approved HPV tests [47]. In addition, according to the study of Bottari et al., this test was found to be more specific and sensitive compared to the HC2 method [49]. Unlike other FDA-approved tests, this test has also been evaluated for people who have been vaccinated. Compared to unvaccinated women, this test has low sensitivity (80%) and high specificity (52.1%) in vaccinated women [47]. In addition, this test can distinguish between different genotypes[47,49]. It is easy to use because it is a fully automated system [47]. In addition to its advantages, the high probability of false negative results in the use of chemical drugs such as mucin, acyclovir, and clindamycin and the expensive test system is among the disadvantages of the test.

Conclusion

The molecular HPV tests, which are approved by the FDA, have some limitations. The cross-reactivity, false-positive, and false-negative results are among these limitations. It is also known that some drug use affects the tests. The studies were conducted to evaluate the analytical specificity of HPV molecular tests with the microorganisms (bacteria, fungi, viruses, etc.) that can be found in the female urogenital system microbiota and unclassified HPV types in an undetermined risk group. The studies were conducted to evaluate the analytical specificity of HPV molecular tests with the microorganisms (bacteria, fungi, viruses, etc.) that can be found in the female urogenital system microbiota and unclassified HPV types in an undetermined risk group. According to these studies, cross-reactivity was observed in the HC2 HR-HPV, Cervista™ HPV, Cervista™ HPV 16/18, and APTIMA® HPV tests, while no cross-reactivity was observed in the cobas® HPV and BD Onclarity™ HPV tests [34,41,47,50–52]. It was determined that there was cross-reactivity between the DNA test probe and the plasmid-pBR322, in the HC2 HR-HPV test. The presence of homologous sequences of this plasmid in genital samples has been reported. Therefore, false positive results may occur if there is a high level of bacterial plasmid in sample 50. The cross-reactivity was detected in the HPV-67 and 70 genotypes in the Cervista™ HPV HR test. The cross-reactivity was detected in the HPV-31 genotype in the Cervista™ HPV 16/18 test [51,52]. In the APTIMA® test, cross-reactivity was observed with low-risk HPV genotypes 26, 67, 70, and 8241. When the tests were examined in terms of cross-reactivities, it was determined that the reliability of cobas® HPV and BD Onclarity™ HPV tests were higher than the others. False positive results may occur in all molecular HPV tests due to cross-contamination. For this reason, it is necessary to use nuclease-free, disposable sterile materials to avoid cross-contamination. In the case of a low level of infection, there is a possibility that some tests will result in false negatives. These tests are the HC2 HR-HPV, Cervista™ HR HPV, and Cervista™ HPV 16/18 tests. When working with these tests, the possibility of HPV infection in negative results should not be ignored.

If the test samples are contaminated with certain drugs, creams, and/or gels, the possibility of false results increases. This affects the results of all tests negatively. In this context, attention should be paid to the use of antifungal cream and birth control gel in the HC2 HR-HPV test, the use of contraceptive jelly and/or antifungal cream in Cervista™ HR HPV and Cervista™ HPV 16/18 tests [51,52], the use of vaginal moisturizer in cobas® HPV test[34], use of antifungal medication in APTIMA® test [41], and the BD Onclarity™ HPV test, the use of vaginal creams containing mucin, acyclovir, and/or clindamycin [47]. People who will have these tests should stop using these drugs and/or creams for a certain period.

In the BD Onclarity™ and cobas® HPV tests, the presence of blood that causes the samples to discolor (red/brown samples) also affects the test's operation. The blood levels exceeding the 4% concentration for the BD Onclarity™ test and 2% for the cobas® HPV test increase the likelihood of false-negative results.

In conclusion, FDA-approved molecular tests have high sensitivity and specificity in the diagnosis of HPV. In addition, these tests are more automatic and reproducible than cytological methods. In addition to these advantages of the tests, the existence of limitations described in this review should not be overlooked. This indicates that molecular tests should not be used alone in the evaluation of HPV infections. Therefore, molecular HPV test results need to be correlated with cytological test results.

Acknowledgments

A part of this study was held in Sivas (Türkiye) between 15-17 September 2022. Presented as an oral presentation at the 3rd International Cancer Days congress.

The figures were quite generated using server Medical Art, provided by the server, licensed under

Conflict Of Interest

There is no conflict of interest.

References

1. Ursu RG, Damian C, Porumb-Andrese E, et al. Merkel Cell Polyoma Virus and Cutaneous Human Papillomavirus Types in Skin Cancers: Optimal Detection Assays, Pathogenic Mechanisms, and Therapeutic Vaccination. Pathogens. 2022; 11(4).
2. Ryan KJ. Sherris Tibbi Mikrobiyoloji. Başustaoğlu A, eds. Hipokrat Yayınevi; 2019.
3. Taberna M, Mena M, Pavón MA, Alemany L, Gillison ML, Mesía R. Human papillomavirus-related oropharyngeal cancer. Ann Oncol. 2017; 28(10): 2386-2398.
4. Şahiner F. Genital insan papillomavirus enfeksiyonlarının moleküler tanısında karşılaşılan sorunlar ve yeni gelişmeler. Mikrobiyol Bul. 2014; 48(4).
5. Faridi R, Zahra A, Khan K, Idrees M. Oncogenic potential of human papillomavirus (HPV) and its relation with cervical cancer. Virol J. 2011; 8: 1-8.
6. Abreu ALP, Souza RP, Gimenes F, Consolaro MEL. A review of methods for detect human Papillomavirus infection. Virol J. 2012; 9(1): 1-9.
7. Zur Hausen H. Molecular pathogenesis of cancer of the cervix and its causation by specific human papillomavirus types. Curr Top Microbiol Immunol. 1994; 186: 131-156.
8. Salazar KL, Duhon DJ, Olsen R, Thrall M. A review of the FDA-approved molecular testing platforms for human papillomavirus. J Am Soc Cytopathol. 2019; 8(5): 284-292.
9. The Papillomavirus Episteme. Accessed August 19, 2022.
10. Okunade KS. Human Papillomavirus and Cervical Cancer. J Obs Gynaecol. 2020; 40(5): 602-608.
11. Lenhoff A. Five FDA-approved HPV assays. MLO Med Lab Obs. 2012; 44(7): 14,16,18.
12. Shen-Gunther J, Yu X. HPV molecular assays: Defining analytical and clinical performance characteristics for cervical cytology specimens. Gynecol Oncol. 2011; 123(2): 263-271.
13. Hubbard RA. Human papillomavirus testing methods. Arch Pathol Lab Med. 2003; 127(8): 940-945.
14. Villa LL, Denny L. CHAPTER 7 Methods for detection of HPV infection and its clinical utility. Int J Gynecol Obstet. 2006;94(SUPPL. 1).
15. Wei ZT, Chen HL, Wang CF, Yang GL, Han SM, Zhang SL. Depiction of Vaginal Microbiota in Women With High-Risk Human Papillomavirus Infection. Front Public Heal. 2021; 8(January):1-10.
16. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol. 1975; 98(3): 503-517.
17. Hwang SJ, Shroyer KR. Biomarkers of cervical dysplasia and carcinoma. J Oncol. 2012; 2012.
18. Meyer T, Arndt R, Stockfleth E, Flammann HT, Wolf H, Reischl U. Strategy for typing human papillomaviruses by RFLP analysis of PCR products and subsequent hybridization with a generic probe. Biotechniques. 1995; 19(4): 632—639.
19. Takács T, Jeney C, Kovács L, Mózes J, Benczik M, Sebe A. Molecular beacon-based real-time PCR method for detection of 15 high-risk and 5 low-risk HPV types. J Virol Methods. 2008; 149(1): 153-162.
20. Gibson UEM, Heid CA, Williams PM. A novel method for real time quantitative RT-PCR. Genome Res. 1996; 6(10): 995-1001.
21. Ilhan ZE, Łaniewski P, Thomas N, Roe DJ, Chase DM, Herbst-Kralovetz MM. Deciphering the complex interplay between microbiota, HPV, inflammation and cancer through cervicovaginal metabolic profiling. EBioMedicine. 2019; 44: 675-690.
22. Magaji YG, Baba J, Suleiman KO. An overview of the general characteristics , pathogenicity , transmission , and diagnosis of human papilloma virus (HPV). 2022; 1(1): 27-39.
23. Castle PE, Lorincz AT, Mielzynska-Lohnas I, et al. Results of human papillomavirus DNA testing with the Hybrid Capture 2 assay are reproducible. J Clin Microbiol. 2002; 40(3): 1088-1090.
24. Castle PE, Wheeler CM, Solomon D, Schiffman M, Peyton CL. Interlaboratory reliability of hybrid capture 2. Am J Clin Pathol. 2004; 122(2): 238-245.

25. Kurian EM, Caporelli ML, Baker S, Woda B, Cosar EF, Hutchinson L. Cervista HR and HPV 16/18 assays vs hybrid capture 2 assay: Outcome comparison in women with negative cervical cytology. Am J Clin Pathol. 2011; 136(5): 808-816.
26. Min KJ, So KA, Lee J, et al. Comparison of the Seeplex HPV4A ACE and the Cervista HPV assays for the detection of HPV in hybrid capture 2 positive media. J Gynecol Oncol. 2012; 23(1): 5-10.
27. Day SP, Hudson A, Mast A, et al. Analytical performance of the Investigational Use Only CervistaTM HPV HR test as determined by a multi-center study. J Clin Virol. 2009; 45(SUPPL. 1): S63-S72.
28. Einstein MH, Martens MG, Garcia FAR, et al. Clinical validation of the Cervista® HPV HR and 16/18 genotyping tests for use in women with ASC-US cytology. Gynecol Oncol. 2010; 118(2): 116-122.
29. Guo M, Khanna A, Dawlett M, Patel S, Gong Y, Staerkel G. Efficacy of reflex HPV16/18 genotyping in predicting CIN3/VAIN3 in women with HPV+/Pap– results. J Am Soc Cytopathol. 2016; 5(1): 31-36.
30. Einstein MH, Garcia FAR, Mitchell AL, Day SP. Age-stratified performance of the cervista HPV 16/18 genotyping test in women with ASC-US cytology. Cancer Epidemiol Biomarkers Prev. 2011; 20(6): 1185-1189.
31. Bartholomew DA, Luff RD, Quigley NB, Curtis M, Olson MC. Analytical performance of Cervista ® HPV 16/18 genotyping test for cervical cytology samples. J Clin Virol. 2011; 51(1): 38-43.
32. Guo M, Khanna A, Dhillon J, et al. Cervista HPV assays for fine-needle aspiration specimens are a valid option for human papillomavirus testing in patients with oropharyngeal carcinoma. Cancer Cytopathol. 2014; 122(2): 96-103.
33. Luiza M, Dias N, Martins TR, et al. Multiple HPV genotype infection impact on invasive cervical cancer presentation and survival. PLoS One. 2017; 12(8): 1-10.
34. cobas HPV Test: South Branchburg: Roche Molecular Systems. Accessed August 4, 2022.
35. Chao X, Sun T, Wang S, et al. Research of the potential biomarkers in vaginal microbiome for persistent high-risk human papillomavirus infection. Ann Transl Med. 2020; 8(4): 100-100.
36. Rao A, Young S, Erlich H, et al. Development and characterization of the cobas human papillomavirus test. J Clin Microbiol. 2013; 51(5): 1478-1484.
37. Motevaseli E, Azam R, Akrami SM, et al. The Effect of Lactobacillus crispatus and Lactobacillus rhamnosus Culture Supernatants on Expression of Autophagy Genes and HPV E6 and E7 Oncogenes in The HeLa Cell Line. Cell J. 2016; 17(4): 601-607.
38. Komatsu A, Igimi S, Kawana K. Optimization of human papillomavirus (HPV) type 16 E7-expressing lactobacillus-based vaccine for induction of mucosal E7-specific IFNγ-producing cells. Vaccine. 2018; 36(24): 3423-3426.
39. Hesselink AT, Berkhof J, Van Der Salm ML, et al. Clinical validation of the HPV-Risk assay, a novel real-time PCR assay for detection of high-risk human papillomavirus DNA by targeting the E7 region. J Clin Microbiol. 2014; 52(3): 890-896.
40. Stoler MH, Wright TC, Sharma A, Apple R, Gutekunst K, Wright TL. High-risk human papillomavirus testing in women with ASC-US cytology: Results from the athena HPV study. Am J Clin Pathol. 2011; 135(3): 468-475.
41. Aptima HPV Assay. Accessed August 3, 2022.
42. Borgogna JC, Shardell MD, Santori EK, et al. The vaginal metabolome and microbiota of cervical HPV-positive and HPV-negative women: a cross-sectional analysis. BJOG. 2020; 127(2): 182-192.
43. Alemany L, Saunier M, Alvarado I, Quirós B, J S, Shin H. HPV DNA prevalence and type distribution in anal carcinomas worldwide. Int J Cancer. 2015; 136(1): 1-23.
44. Ratnam S, Coutlee F, Fontaine D, et al. Aptima HPV E6/E7 mRNA test is as sensitive as hybrid capture 2 assay but more specific at detecting cervical precancer and cancer. J Clin Microbiol. 2011; 49(2): 557-564.
45. Castle PE, Eaton B, Reid J, Getman D, Dockter J. Comparison of human papillomavirus detection by Aptima HPV and cobas HPV tests in a population of women referred for colposcopy following detection of atypical squamous cells of undetermined significance by Pap cytology. J Clin Microbiol. 2015; 53(4): 1277-1281.
46. Preisler S, Rebolj M, Ejegod DM, Lynge E, Rygaard C, Bonde J. Cross-reactivity profiles of hybrid capture II, cobas, and

APTIMA human papillomavirus assays: Split-sample study. BMC Cancer. 2016; 16(1): 1-13.
47. BD OnclarityTM HPV Assay. Accessed August 5, 2022. https://www.accessdata.fda.gov/cdrh_docs/pdf16/P160037C.pdf
48. Ejegod DM, Junge J, Franzmann M, et al. Clinical and analytical performance of the BD OnclarityTM HPV assay for detection of CIN2+ lesions on SurePath samples. Papillomavirus Res. 2016; 2: 31-37.
49. Bottari F, Sideri M, Gulmini C, et al. Comparison of onclarity Human Papillomavirus (HPV) assay with hybrid capture II HPV DNA assay for detection of cervical intraepithelial neoplasia grade 2 and 3 lesions. J Clin Microbiol. 2015; 53(7): 2109-2114.
50. Hybrid Capture 2. HC2 High-Risk HPV DNA Test. Accessed August 5, 2022.
51. Cervista HPV 16/18. Accessed August 5, 2022.
52. Cervista HPV HR. Accessed August 5, 2022.