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# CERVICAL CANCER AND HUMAN PAPILLOMA VIRUS: THEIR SCREENING AND DIAGNOSTIC METHODS

**Jigar V Suthar[1], Rajesh K Patel[2], Tushar N Chauhan[1], Sanjay M Dave[3], Kanagee J Suthar[4]**

1. Genetics Group of Gujarat Diagnostic Centre, Mehsana
2. Sandor Animal Biogenics Pvt. Ltd., Hyderabad
3. Department of Biotechnology, Hemchandracharya North Gujarat University, Patan
4. Department of Microbiology, Ratnamani Science College, Becharaji

**ABSTRACT:** Cervical cancer is a cancer arising from the cervix, which is usually also a sexually transmitted disease. The occurrence of the cancer is world- wide leading to deaths. However, the incidence and mortality due to cervical cancer has declined during last few decades in developed countries owing to the widespread use of cervical screening programs. However, approximately 70% of cervical cancers occur in developing countries due to lack of PAP screening and vaccine against causative agent. Almost all cervical cancers are caused by longstanding infection with one of the HPVs. The review article has given more emphasis on prevalence of cervical cancer and its preventive strategies in India especially Gujarat state. India is largest country in population size with less knowledge on sexually transmitted diseases and limited medical resources. Therefore, it needs more focus on cervical screening (PAP) strategy to lower the cervical cancer incidents. The article elucidates details of HPV, genetics of HPV, diagnosis of HPV, molecular diagnosis of HPV, Cervical cancer, strategies for screening methods and available test for cervical cancer diagnosis.

**KEYWORDS:** HPV, Cervical cancer, PAP, Molecular diagnosis.

**Corresponding Author: Mr. Jigar V Suthar***

Genetics Group of Gujarat Diagnostic Centre, Mehsana, India.

Email Address: gajjarjigar90@yahoo.com

## 1.INTRODUCTION

Focus of the article is Human papillomavirus, cervical cancer and the methods for their diagnosis and screening. Our aim is to provide each and every detail related to HPV and cervical cancer which can be known to a health professional or a fresh researcher. This article contains the latest mortality

and incidence based information of India and Gujarat regions. India is the 2nd most populated country in the world and so based on that here we gave a comparison of different screening and diagnosis methods, we also focused on the different screening strategies for lowering the cervical cancer risk. At last the different molecular biology based diagnostic kits detail is given in a table form which are sold in market. It can be helpful to health professional to start a test.

**CERVICAL CANCER**

Cancer is not a single disease. The term cancer includes more than 200 diseases all characterized by the uncontrolled proliferation of cells. Ignoring the body's signal to stop, malignant cells multiply to form tumors in organs and tissues or, in the case of blood cancers, crowd out normal cells in the blood stream and bone marrow. Cancer is always named after body part where it starts, even if it spreads to other body parts later. When cancer starts in the cervix, it is called cervical cancer. The cervix is the lower, narrow end of the uterus. The cervix connects the vagina to the upper part of the uterus (or womb) where offspring are conceived and in which they gestate before birth. Cervical cancer is one of sexually transmitted disease. According to the Globocan-2012 report, the cervical cancer is the 4th most frequent cancer in the world, where it is the 2nd most common cancer in the south East Asia and India (Fig. 1.). The estimated incidence of cervical cancer is 5,27,624 worldwide, estimated prevalence is 4,06,210 and the estimated mortality is the 2,65,672 worldwide in the year 2012 [1,2]. Cervical cancer is persistently increasing worldwide. Annual incident rate of cervical cancer was 9.7 and 2.5 mortality in 100000, where 1450 new cases of this cancer and 430 deaths in year 2000 noted in Canada. In 2014, there were 12,578 women diagnosed for cervical cancer and 4,115 women died out of them in the United States[3,4]. Around 80 percent cases are occurring in developing countries because lack of adequate screening programs [5]. India is the 2nd largest populated developing country, where medical and health care is poor as compare to developed countries hence, the condition is pathetic. In India, cervical cancer is the most common genital cancer [6] and also it is third most frequently diagnosed cancer [7]. Globally, every year 1,22,844 new cases of cervical cancer are detected where, nearly 70,218 women die annually from the disease [1,2]. As shown in the Fig. 2, the incidence rate has higher difference between the breast and cervical cancer in India, whereas the mortality rates are more or less same for both the cancers. However, breast cancer cases are more prevented as compare to cervical cancer in India because of awareness. Screening programs and awareness programs should be organized to reduce the incidence of cervical cancer and subsequently death cases in our country. According to cancer registry program of Ahmedabad urban – annual report - 2011, cervical cancer is second ranked cancer of all other female cancers and it accounts for 9.2% [8]. An another study conducted in Gujarat state of West India revealed that in cervical cancer, 31 (59.6%) patients were infected with HPV 16 and 18. Of these 31 HPV-positive cervical cancer patients, 28 (90.3%) were infected with HPV 16 and 3 (9.7%) were infected with HPV 18 [9].

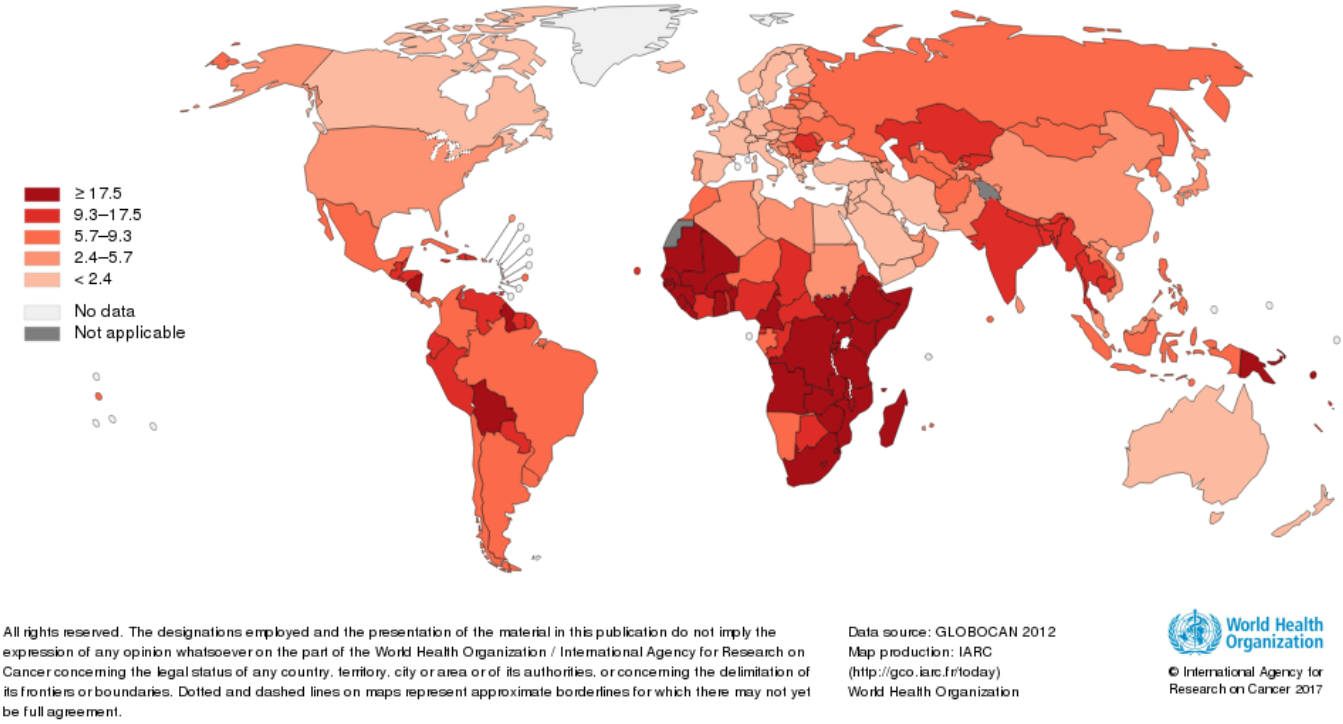


**Fig 1: Estimated age standardized rates of deaths caused by cervical cancer, worldwide in 2012**

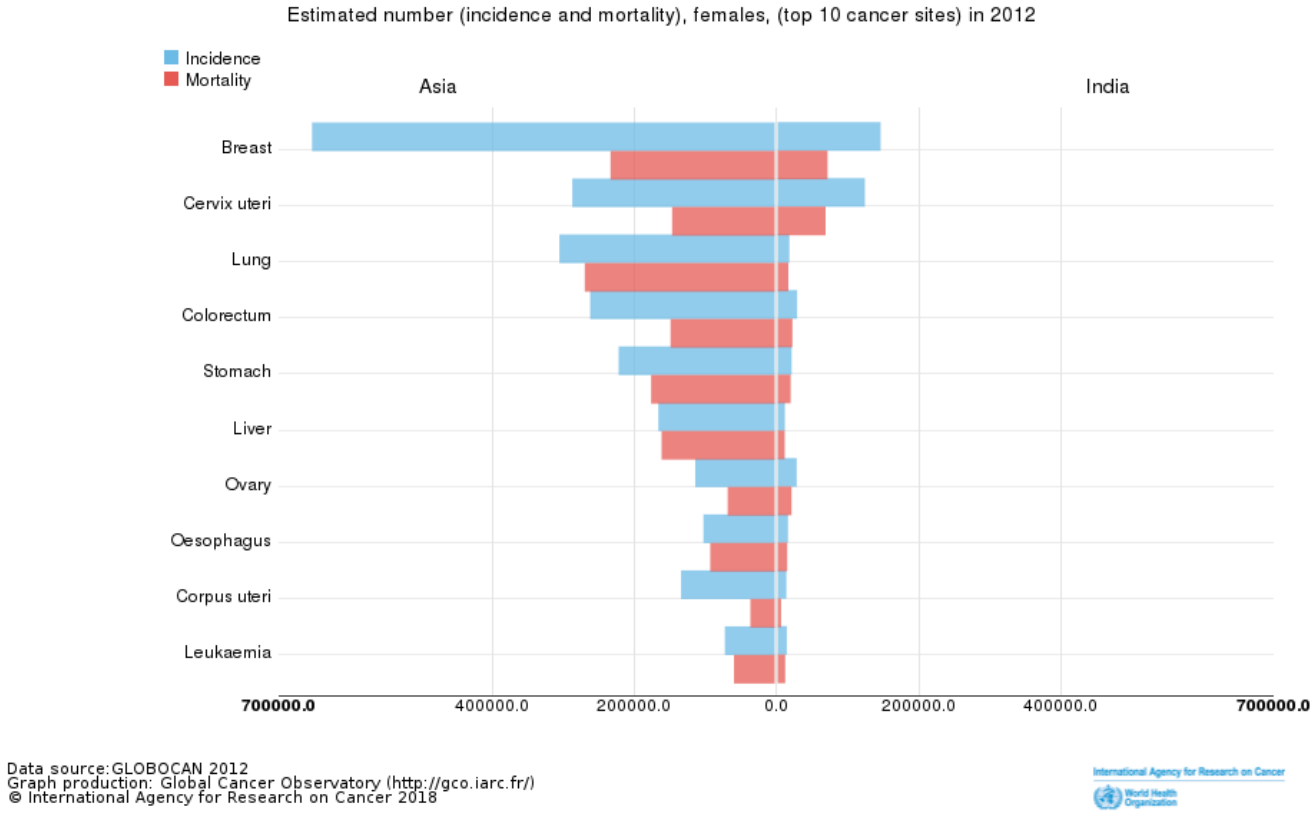


**Fig 2: Comparative estimated numbers (incidence and mortality) in females due to top 10 cancers in Asia and India during 2012**

There are over 100 different kinds of HPV and not all of them cause health problems. Some kinds of HPV may cause problems like genital warts. Some of HPV 6, 11, 16 or 18 cause cancer of the cervix, vagina, vulva, or anus. Cervical cancer is caused by infection of carcinogenic human papillomavirus [10,11]. Cervical cancer is the most important outcome of HPV infection among all the cancers in females [12]. However, anogenital warts and RRP (Recurrent Respiratory Papillomatosis) are non-cancer related outcomes of HPV infection which develop after 2-3 months of infection [13]. It is authenticated that human papilloma virus type 16 and 18 are carcinogenic for cervix that is concluded based on epidemiologic data from case control studies, prospective cohort studies and case series. More data from the same study showed that infection with Human papilloma virus types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 or 66 may also cause cervical cancer [5]. Some data also show HPV type 16 has a causal role in many genital cancers of the vulva, vagina and penis. It has also a causal role in cancer of the anus, oropharynx, and some association with cancer of the larynx and periungual skin [5]. HPV type 18 has also a causal role in many cancers indicated in fig 2. High risk HPV type infection plays a significant role in the initiation and prognosis through various stages of cervical intraepithelial neoplasias (CINs) to invasive cancer [14,15]. Generally the cervical cancer affects women with age group 30 to 50 [16]. Most infections of cervix cured within 2 years, but the persistent infection of cervix with High risk-HPVs can cause cancer. Symptoms of cervical cancer appear in advance stage and it may include irregular vaginal bleeding after sexual intercourse, back pain, leg pain, pelvic pain, fatigue, loss of appetite, loss of weight, discomfort of vagina, odorous discharge, a single swollen leg and many other symptoms may arise at advanced stages [17]. Last 30 years of epidemiologic study indicated that sexual activity influences cervical cancer risk. There are many risk factors for cervical cancer, some important of them are listed below.

1. The type and duration of viral infection, with high risk HPV type and persistent infection predicting a higher risk for progression; low risk HPV types do not cause cervical cancer [18].
2. Host conditions that compromise immunity, poor nutritional status, immunocompromise, and HIV infection [18].
3. Number of sexual partners, age of first sexual intercourse and sexual behavior of the women's partner [19, 20].
4. High risk of cervical cancer later in life can also be transmitted to wives of patient with penile cancer [21].
5. Mortality and incidence rate is high where there is strong association of cervical and penile cancer [22, 23].
6. Well known risk factor for cervical cancer is tobacco smoking [24]. Nicotine metabolite can be found in the cervical mucus of women who smoke and there is high effect of carcinogenic action of cigarette on the cervix [25].
7. Long term use of oral contraceptive was associated with excess risk of cervical cancer [25].

8. Diet can also effect on risk of cervical cancer like carotene and vitamin C. Risk of cervical cancer can be reduced by vitamin A [26, 27].

This cancer can be prevented through regular screening [28], which is necessary for testing pre-cancer and cancer in probable patients who have no symptoms and they are perfectly feeling healthy. HPV infection in the anogenital epithelial induces the histopathologic changes. Viral DNA replications continue at higher rate in infected cells, which turn to be differentiated cells wherein the transcription activates more. Hence, viral protein expression is seen more in differentiated cell layers. The first spectrum of disease is condyloma and cervical intraepithelial neoplasia (CIN) grade 1 also known as mild dysplasia. These initial disease spectrum condyloma, CIN 1 and mild dysplasia are combined in category low grade squamous intraepithelial lesions (LSIL) in the Bethesda system (Fig. 3). The LSIL is characterized with atypia and little basal cell proliferation, and in case of condyloma, presence of cells with an irregular, enlarged nucleus with clear halo (koilocytes). Koilocytes are the proof of cytopathic effect of HPV infection. There is an additional category in Bethesda system known as ASCUS (atypical squamous cells of undetermined significance), used for the cells which are neither clearly normal nor clearly dysplastic. In contrast to LSIL, there is a term HSIL (high grade SIL) in Bethesda system, which includes CIN grade 2 and 3, moderate and sever dysplasia and carcinoma in situ (CIS). Cellular characteristics like sever atypia, abnormal mitotic activity in the more superficial cell layers and replacement of the normal epithelium with immature basaloid cells are seen in HSIL [29, 30]. The precancerous lesions are described as the initial part of the continuous beginning of CIN 1, which can be progress in to CIN 2, CIN 3, CIS and invasive cancer [30]. General thinking of most women patient is that the CIN 1 has poor potential to progress in invasive cancer. In some model, it has been described that the some CIN 1, 2 and 3 can coexist in same woman patient and sometimes CIN 3 develops directly without passing through intermediate CIN 1[31]. Time required for developing CIN 2, 3 is largely vary. In one cohort study, Koutsky et al. (1992) reported the 6 months period was required for the developing CIN 2 – 3 after HPV infection. Capacity of HSIL is greater to progress in to an invasive cancer compare to LSIL because of the involvement of HR HPV infection in HSIL, where LSIL shows the infection of wide range of HPV types from LR to HR [33].

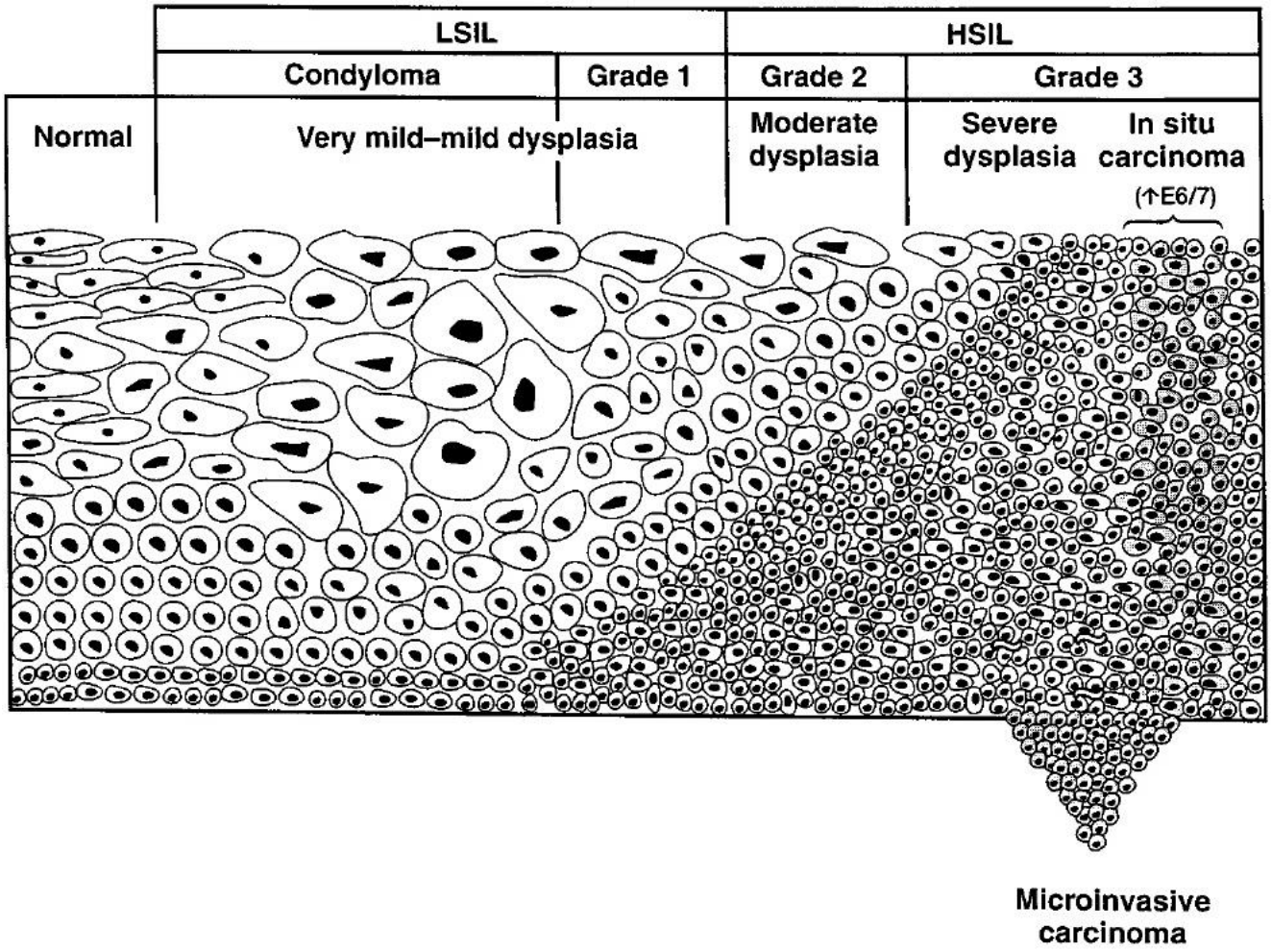


**Fig. 3. Schematic presentation of different grades of intraepithelial neoplasia. Cervical or anal intraepithelial neoplasia grade 1 is characterized by 20–25% replacement of the epithelium with immature cells with high nucleus/cytoplasm ratios. Intraepithelial neoplasia grade 2 is characterized by approx. 50% replacement with immature cells, and grade 3 by complete or nearly complete replacement. Micro-invasion, shown at the bottom, occurs when the cells traverse the basement membrane. Although micro-invasion can rarely occur in conjunction with intraepithelial neoplasia grade 1, it is likelier to occur in conjunction with intraepithelial neoplasia grade 3, as indicated schematically.**

## HPV

### Introduction

Papillomaviridae is an ancient taxonomic family of small, non-enveloped and double-stranded DNA viruses, collectively known as Papillomavirus [33] with more than 240 distinct types classified in 37 genera, therefore, papillomaviruses may be the biggest family of vertebrate viruses [34]. Human Papillomavirus is the most common sexually transmitted virus. Human Papillomavirus is also called HPV, are a group of more than 150 related viruses, and near 100 types of HPVs having different genetic variation and oncogenic potential [36, 37] From the 100 identified HPV types almost 40 can infect genital area [12].

Classification of Papillomavirus

Group: Group-1(dsDNA)

Order: Unassigned

Family: Papillomaviridae [34].

Based on the potential of causing cancer, there are two categories of sexually transmitted HPVs [35].

1. Low risk HPVs (LR-HPVs) include types 6, 11, 40, 42, 43, and 44 [38], which do not cause a cancer, but can cause skin warts on or around the genitals or anus. For example, HPV types 6 and 11 cause 90 percent of all genital warts.

2. High risk HPVs (HR-HPVs) include types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 [38], which can cause a cancer. At least dozen high risk HPV types have been identified. Two of these HPV types 16 and 18 are responsible for the majority of HPV-caused cancers.

Persistent infection of HR-HPV types can cause serious cytological abnormalities or lesion that will progress to cancer, if remain untreated [35]. About 5% of all cancers are caused by high risk HPV infection worldwide [39]. HPV 16 and 18 were the first two cancer-associated HPV types that were isolated and used to design the initial testing systems. These two HPVs were reported largely in early studies on HPV and cervical cancer [38]. These HR-HPVs 16 and 18 can cause approximately 70% of cervical cancer worldwide [37]. HPV is the infectious agent of most common sexually transmitted infections in the United States [35]. In fact, more than half of the sexually active people are infected with one or more HPV type at some point in their lives. A research indicates that 42.5 percent women have genital HPV infection at any point in the time [12, 40]. Human papillomavirus infect human mucosal and cutaneous tissue. The prevalence of Human papillomavirus for genital infection varies between countries (1 to 40 percent in women).

**Virus Genetics**

HPV having double standard DNA as its genome with approximately 8000 bp, containing a capsid and virus size range from 55 to 60 nm [41]. Viral genome containing open reading frame (ORF) with three regions, early replicating (E) later replicating (L) and long control region (LCR) (Fig. 4). These regions bear viral origin of replication and transcription. HPV genomes having 10 open reading frames (ORFs) E1, E2, E3, E4, E5, E6, E7 and E8 are early replicating ORFs whereas L1 and L2 are late replicating (ORFs), which are encoding structural proteins [42]. Taxonomic status of HPV is depends on the variation present in the L1 gene. Different level of variation has been seen in L1 gene in HPV at types (10%), sub types (2 to 10% ) and variants (maximally 2%) [33]. E regions codes for proteins related to replication (E1) and for activation or repression of the viral DNA (E2) [43]. Generally E2 protein of HPV down regulates the production of E6 and E7, so it can hold the host cell in its normal state. Integration of HPV genome into host cell disrupts the E2 gene and so it cannot down regulate the production of E6 and E7. Loss of E2-ORF leads to over expression of protein. E6 and E7 oncogenes which in turn initiates transformations, so in cervical

cancer biopsy, it has been frequently seen the deletion of E2 ORF [44]. E6 and E7 genes of HPV genome can exit the cell cycle blockage and can induce cell to

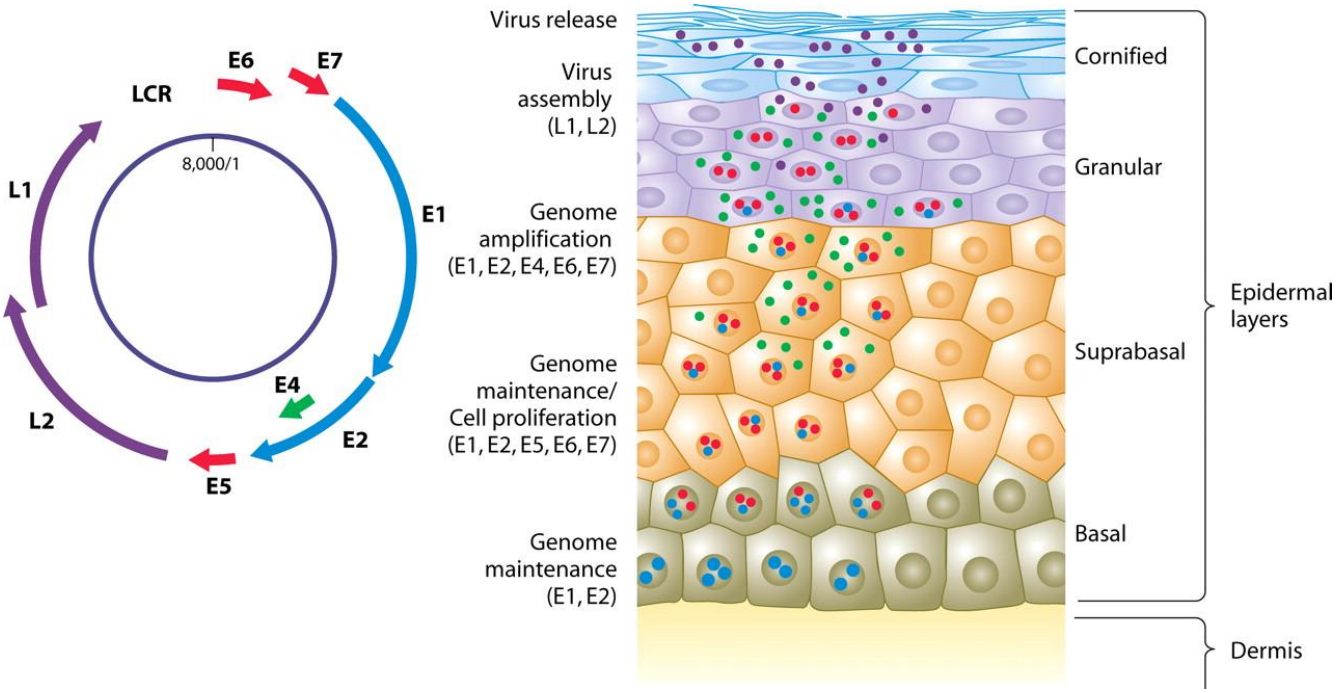


**Fig. 4: Virus genome and life cycle**

fall in S phase in host cells (basal cells). So E6 and E7 will be produced at higher rate and it will drive the cells towards malignancy. Therefore, E6 and E7 m RNA transcripts can be used for diagnose the precursor for lesions of cervical carcinomas [45]. These E6 and E7 with E5 are the viral oncogenes, whose expressions induce the cell transformation and immortalization. Particularly the E6 and E7 proteins inactivates the p53 and pRb tumor suppressor proteins respectively [42]. E6 and E7 proteins of HR HPVs efficiently inactivates the p53 and pRb proteins than of LR HPVs [46].

**Diagnosis**

If cervical cancer diagnosed with pre-invasive state then it can be 100 percent curable [7]. Therefore, the highly specific and sensitive diagnosis has the crucial role to prevent and cure the cervical cancer. Reduction in the incidence of advanced cancer and deaths is directly proportional to have a strong, sensitive and more specific HPV test to detect lesions with high potential for malignant transformation [49]. Carcinoma of the cervix has a slow progression from precancerous lesion to malignancy, therefore it is a great opportunity for early detection and considerably improve prognosis because easy accessibility to examine. There are two main strategies for diagnosis of the risk of getting cervical cancer or had a cervical cancer or its precursor lesions. First one is based on the cell morphology, and it can be checked by PAP smear test, which can give the details about cell morphology but it cannot give any information regarding infection of HPV. The second strategy is based on infection of HPV; whether it is a HR or LR HPV. The HPV DNA testing and genotyping

**Table no.1. Roll of different HPV proteins**

| | Ref –[42] | Ref –[45] | Ref –[43] | Ref –[47] | Ref –[48] |
|---|---|---|---|---|---|
| **E1** | mediates the virus life cycle | ---------- | ---------- | ATP hydrolyse and it is essential for HPV replication | Helicase function; essential for viral replication and control of gene transcription; similar among types Viral |
| **E2** | mediates the virus life cycle | down regulate the production of E6 and E7, so it can hold the host cell in its normal state | two proteins that function as transcription factor and as internal regulators of the viral E6 and E7 oncogenes expression | ---------- | Viral transcription factor; essential for viral replication and control of gene transcription; genome segregation and encapsidation |
| **E3** | not yet identified | ---------- | ---------- | ---------- | Function not known; only present in a few HPVs |
| **E4** | ---------- | ---------- | ---------- | ---------- | Binding to cytoskeletal protein Interaction |
| **E5** | ---------- | ---------- | ---------- | ---------- | Interaction with EGF/PDGF-receptors Interaction |
| **E6** | proteins which regulate the host cell DNA replication and transformation | exit the cell cycle blockage and can induce cell to fall in S phase in host cells | ---------- | ---------- | Interaction with several cellular proteins; degradation of p53 and activation of telomerase |
| **E7** | proteins which regulate the host cell DNA replication and transformation | exit the cell cycle blockage and can induce cell to fall in S phase in host cells | ---------- | ---------- | Interaction with several cellular proteins; interaction with pRB and transactivation of E2F-dependent promoters |
| **E8** | not yet identified | ---------- | ---------- | ---------- | Long distance transcription and replication repressor protein |
| **L1** | structural proteins | ---------- | ---------- | ---------- | Major capsid protein |
| **L2** | structural proteins | ---------- | ---------- | ---------- | Minor capsid protein |

can give detail about the presence or absence of HPV infection and if present then which type of HPV is involved in infection. However, HPV DNA test cannot give any detail about the cell morphology and cancer status. So these both the strategy should be investigated for all patients on regular intervals to lower the cervical cancer incidence. A study concluded that Combination of both cervical cytology and HPV DNA test can diagnose the cervical cancer with greater accuracy [50].

Many techniques are available for screening cancer, like VIA (Visual inspection with 5% Acetic acid), VILI (Visual inspection with lugol's Iodine), and Pap smear test. These are the commonly used methods for screening. According to author, the VILI is better than VIA as color contrast is better in VILI, self-life of lugol's iodine is more than acetic acid. Pap smear test can be used to reduce the 80% incidence of cervical cancer and 70% mortality [7]. Pap smear can screen cytology and related malignancy of endocervical cells, but it cannot screen the infection of HPV and genotype of it. Screening for HPV is as important as to PAP smear test to reduce the cases of invasive cancer generating from a healthy women. HPV cannot propagate by the tissue culture method, so molecular biology techniques can be used for its accurate identification. In most cases, detection of HPV in clinical specimens are based on nucleic probe technology [51]. Molecular biology techniques are the most commonly used for HPV testing and are the "Gold-standard" for diagnosing viral infection. There are various bio-markers which can be used for HR-HPV progression risk investigation [39]. HPV diagnosis based on molecular techniques are more accurate and reliable for detection and typing [52]. At present three assays are available for molecular based diagnosis (Fig. 4.). These three assays are nucleic acid hybridization assay, signal amplification assay and nucleic acid amplification assay. All methods have their own merits and demerits. Nucleic acid amplification is the most common method used for research and diagnosis purpose. HPV presence can be checked by using morphological, serological and clinical findings [51,53]. In development of cervical cancer, HPV generates a humeral immune response against many antigen, but especially against the L1 major capsid protein [54]. Recently, HPV particles have been propagated in vitro in raft culture and xonograft but the amount was not enough to carry out immune based test. So alternative way must be searched to get the enough protein burden for developing immuno based testing [55]. Viral DNA can be a marker for detection of the presence of HPV but it cannot give any information related to productive infection or presence of cervical lesions. Cytological methods are most common and it can detect the cancerous lesions or somewhere clinicians misusing the cytological methods to diagnose viral lesions, so the molecular methods will help here to know the lesions are because of HPV infection or else. Here PAP or colposcopic study with HPV DNA test can help to identify HPV infected women with a risk of developing cervical cancer [23]. Polymerase chain reaction (PCR) is widely used for HPV Screening and it is also a central method for many other molecular biological methods like Sequencing, Restriction fragment length polymorphism (RFLP) and Southern blotting. PCR method is as sensitive and accurate as hybrid capture 2 (HC2) assay which is approved by FDA (Fig. 5.)[56]. With high accuracy and sensitivity, PCR method is also a cheap, quick, and more accessible compare to other methods. PCR with primer sets like MY09/11, GP5+/6+, PGMY 09/11, My09/11 and GP5+/6+ ,

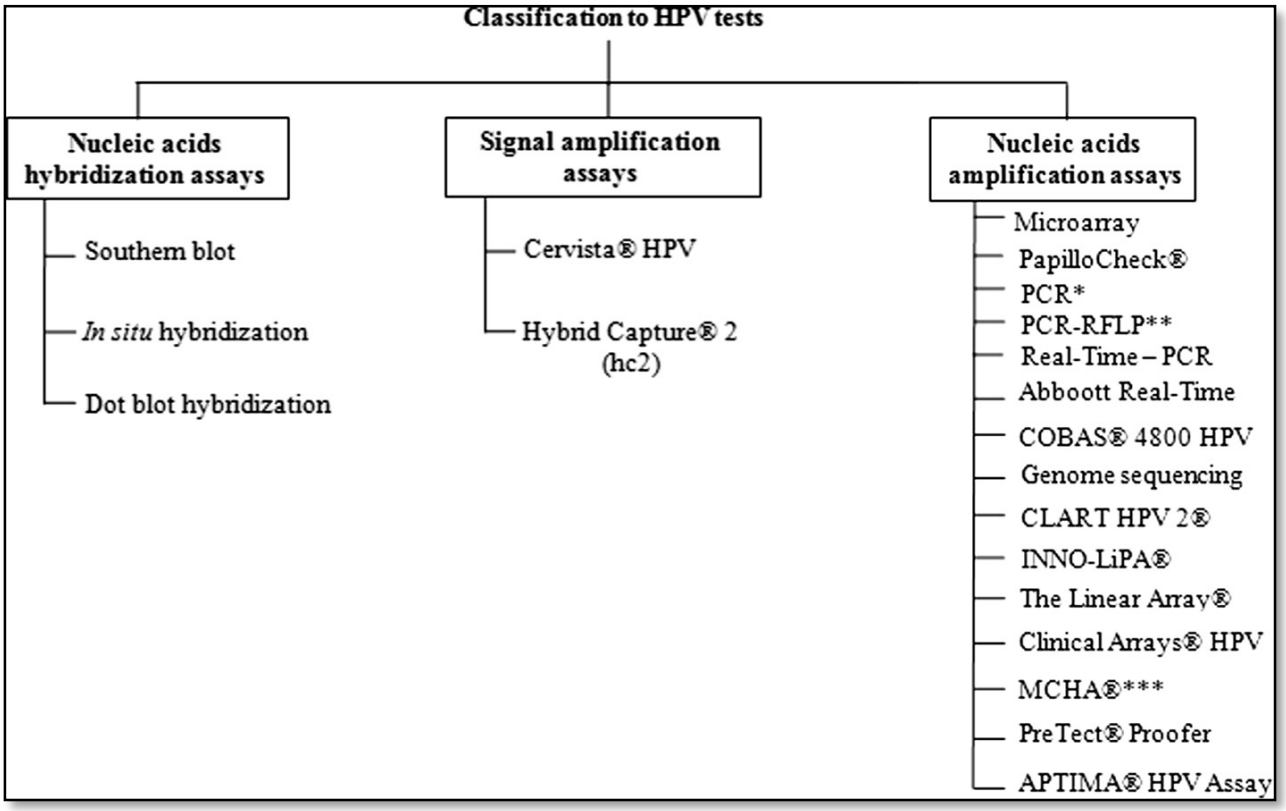


**Fig. 5. Classification of HPV tests [39]**

Nested PCR– with PGMY09/11 and GP5+/6+ and many more are developed and used for screening and research purposes. Now a days, PGMY09/11 with GP5+/6+ nested PCR is more used for screening and diagnosis of HPV. Several primer sets for HPV DNA detections are there but the L1 gene primer set is the most popular. L1 consensus primer system (example- MY09/ 11 or GP5+/ 6+) can detect as 10 to 100 molecules of target HPV DNA from a genital sample [23]. There are some facts and figures about cervical cancer detection methods given in table 1. According to the author, PCR method is as sensitive & specific as Hybrid Capture 2 assay. None other method beside these two has this much sensitivity and specificity [51]. Primers PGMY09 / PGMY11 and GP5+ / GP6+ are consensus primers used for HPV- PCR protocols, which allow amplification of large number of genotypes in a single reaction. These primers target conserved region of the HPV genome, such as L1 gene. Once a amplification process completed then HPV genotypes can be determined separately by using techniques like Restriction fragment length polymorphism (RFLP), linear probe assay, direct sequencing or genotype specific primer [57]. Type specific primers amplify the long control region L1 and E6 / E7 [58]. Molecular assays are the gold standard for HPV identification. PCR method requires very small amount of DNA templet, where Southern blot hybridization method requires large amount of DNA templet, very difficult to do in routine practice, and not always reproducible. In-situ hybridization does not show sensitivity consistently like in PCR & HC2 [56]. Most widely utilized HC2 assay is costly to include it in a routine checkup for many developing countries. So efforts should be made to develop an affordable and sensitive test for HPV

DNA [51]. PCR with MY09/11 primers has clearly low analytical sensitivity compare to nested PCR. Nested PCR RFLP assay can detect precisely low risk HPVs (11, 42, 44, 54, 62, 70, 81, 84) and high risk HPVs (16, 18, 33, 35, 45, 52, 53, 58, 73). This nested PCR RFLP method is suitable for the detection and Characterization of HPV –DNA in public health programs. It is also performed easily in limited resource laboratories to accurately identify the HPV type [57]. Multiplex PCR method for HPV can detect single or multiple HPV infections in sample. Relatively ease and economic accessibility of the multiplex PCR method can potentially have an impact in HPV screening in low income countries. Multiplex PCR method has improved its ability to detect hi-risk HPV types in multiple HPV infected samples makes it an attractive option for HPV testing [59].

The six main possible clinical applications of HPV DNA testing are;

I. Triage of women with equivocal or low grade cytology abnormalities.

II. Follow up of women with abnormal screening results who are negative at colposcopy/biopsy.

III. Prediction of the therapeutic outcome after treatment of cervical intraepithelial neoplasia (CIN).

IV. Primary screening for HPV DNA testing in combination with a pap smear, to detect cervical cancer precursor [60].

V. Gain valuable information on the persistence of certain HPV type [61].

VI. Investigation of regional and country based prevalence of type-specific HPV, to provide baseline values against which the global impact of HPV vaccination can be assessed in the future [62].

After this elaborative description of methods used in the HPV detection, some merits and demerits of these methods are given for HPV DNA test (Table no. 2.). Accuracy of diagnostic method is also depends on the expertise sampling. One study concluded that samples collected by clinicians are showing more prevalence (10.3%) of HPV compare to cervico vaginal samples collected by participant or patient (7%). Author studied 186 samples of different age groups such as 30-35, 36-40, 41-45, 46 & above. In their study they reported 13 (30-35), 10 (36-40), 6 (41-45) & 11 (46 & above) samples with HPV positive from 48, 49, 36 and 53 volunteer respectively. Based on this and many other studies, author concluded that HPV prevalence is not associated with age [63]. Author used multiple consensus primer sets such as L1C1/L1C2 +C2M, MY09/11 & GP5+/6+, type specific primer sets and modified primer sets such as PGMY09/11 & GP5+/6+ (MGP). These all primers are targeting HPV – 16, L1 gene. They concluded from their comparative study of different primer sets that HPV detection in sample with multiple type infection based on PCR by using consensus primers will produce biased results. That will result in misjudgment or produce half results of clinical sample. To overcome this problem they suggested to use modified PGMY09/11 or MGP primers in case of

**Table 1 Characteristics of tests for the detection of cervical cancer and its precursors**

| Test | Test sensitivity/specificity for CIN 2/3 lesions and cervical cancer | |
|---|---|---|
| | Analytical | Clinical |
| **Based on cell morphology** | | |
| Pap smear/tissues [a] | NA | low/high |
| Colposcopy [a] | NA | moderate/low |
| Visual inspection [a] | NA | low/low |
| **Detection of HPV proteins** | | |
| Immunocito/histochemistry [b] | low/high | low/low |
| Electron microscopy [b] | low/high | low/low |
| Western blots [b] | low/high | low/moderate |
| **Detection of HPV genomes** | | |
| Direct methods | | |
| Southern blot [b,c] | moderate/high | moderate/high |
| In situ hybridization [b,c] | moderate/moderate | moderate/moderate |
| Dot blot | low/high | low/high |
| Signal amplification | | |
| Hybrid Capture [d,e,f] | high/high | high/high |
| Target amplification | | |
| PCR [d,e,f] | high/high | very high–high/high–moderate |
| Real-Time PCR [e,f] | very high/high | very high * |
| **Detection of anti-HPV antibodies** | | |
| ELISA peptides | low/low | low/low |
| VLP | moderate/high | low/low |
| Fused E6/E7 | high/moderate | low–moderate/high |

Abbreviations: ELISA, enzyme-linked immunosorbent assay.
* No data were available.
[a] Limited because of their low sensitivity; highly dependent on sampling and tissue preservation; cannot type HPV.
[b] Technically cumbersome and/or time consuming.
[c] Requires DNA and tissue preservation.
[d] Less dependent on sampling; can be done in crude samples.
[e] Suitable for high-throuput testing and automation.
[f] Provides viral load information.

**Fig 6: Test Characteristics for detection of cervical cancer & precursors [51]**

multiple HPV type infection to get better specification [64]. Authors stated that the HPV DNA testing with genotyping is the corner stone which can give better understanding of epidemiological phenomenon and correct prediction of natural history of the infection [65]. One systematic review concluded that, persistence infection of HPV is strongly associated with CIN 2-3/HSIL+. They stated that HPV DNA test can be a valuable marker for diagnosis and research purpose to screen CIN 2-3 in clinical samples, if properly standardized [66]. Molecular biology based techniques are highly sensitive but they cannot differentiate between latent, persistent and active infection. Moreover these techniques need high technical skills [23].

**Table No.2. Advantages and Disadvantages of the molecular methods for HPV detection**

| Method | Advantage | Disadvantage |
|---|---|---|
| **Nucleic acids hybridization assays** | Southern blot is gold standard for HPV genomic analysis.<br>Presence of HPV in association with morphology | Low sensitivity, time consuming, relatively large amount of purified DNA.<br>Southern blot and hybridization cannot use degraded DNA |
| **Signal amplification assays** | Quantitative<br>FDA-approved test<br>Lower false-positive rate<br>High sensitivity to genotyping | Licensed and patented technology<br>Wasn't designed to genotyping individual |
| **Nucleic acids amplification assays** | Flexible technology<br>Very high sensitivity<br>Multiplex analysis | Lower amplification signals of some HPV genotype.<br>Contamination with previously amplified material can lead to false positives |

**Screening Strategy**

Author studied that the high coverage of pre-adolescent vaccination is more worth in the area where screening efficiency and resources are limited or least accurate. But if the area where the screening can be done with pre-adolescent vaccination, it can decrease the most cervical cancer incidence even after the coverage of vaccination is 50%. Authors gave the two strategies for screening programs 1. Most cost effective strategy with high efficiency to lower cervical cancer incident is to use pre-adolescent vaccination with combination of screening three times per life time after age 30. 2. That alone pre-adolescent vaccination by 70% coverage can reduce upto 44% where in combination with screening, it can reduce up to 63% cervical cancer incidence. The strategy of three time screening in whole life of women can prevent the cancer causing by HPV type 16 & 18 and it also prevents the death due to cancer caused by HPV types other than 16 & 18 in the non-vaccinated population. Authors found that HPV-DNA test is more effective than VIA, and also VIA is more effective than cytology visits [67]. Utilizing both cytology and HPV DNA test in combination can increase the screening cost as compare to other health screening tests. HPV-DNA test with genotyping has a very great potential to predict the natural history of the infection and it can also better explain the epidemiological phenomena like cohort effect, clustering etc. In addition, HPV-DNA test is highly reproducible compare to cervical cytology and it is not influenced by age as PAP test because the performance of PAP test is better in women aged >50 [68]. These studies show that the HPV DNA test is better than PAP cytology, and we need the good screening strategy with lower screening cost. So there is an alternative strategy like "Screen and Treat" can overcome this problem of higher

screening cost. This program was set for low resource place. In which only HPV DNA test is utilized for screening and all positive women undergo for treatment. This strategy appeared highly effective and inexpensive for cervical cancer prevention. In Europe, same kind of alternative strategy has been identified for overcome screening cost. They used HPV-DNA test uses a primary screening method and only positive women will go for cervical cytology for knowing further requirements [68]. In India, the HPV prevalence is not associated with the age of females concluded by many studies. This non association is unexplained. It is necessary to know the different high risk HPV type distribution in various geographical regions of the India to maximize the effect of HPV vaccination. Authors observed presence of high risk HPV in 87.8% invasive squamous cell carcinomas, where they did not find any low risk HPV type in carcinoma. Among that HR HPVs, type 16 is most prevalent (66.7%) followed by type 18 (19.4%) in their study for Andhra Pradesh. Based on this data they confirmed that >50% burden of cervical cancer can be lowered by targeting only HPV16 for vaccination program for Andhra Pradesh and South India. For setting up a vaccination strategy for India, more studies of different geographical regions are required. That can conclude the different target for different geographical region to lower the cervical cancer burden in India and also reveal the association of age with HPV. The studies concluded that the HPV- DNA test should be the primary screening method in Andhra Pradesh [63]. The failure of cytological smears in rural India is because of some factors like (1) Poor infrastructure, (2) Lack of trained health professionals and cytotechnician, (3) Absence of organized community based screening programs and (4) Inadequate follow up of abnormal smears [69]. Author reviewed 11 independent studies; 6 from India and 5 from Africa. The total 58,679 women participated in these 11 studies. Author studied the specificity, sensitivity, positive predictive value (PPV), negative predictive value and pooled related accuracy of five different screening tests like VIA, VILI, PAP smear, VIAM and HC2. Based on this study author stated some important points. Sensitivity increases up to >22% and specificity decreases up to ≥3% of these 5 screening tests with the increase in severity from CIN1 to cancer. Cervical cancer or its precursors can be better detected by VIA or VILI than PAP smear and HC2. It has been demonstrated that once in a life time VIA screening reduces the incidence of cervical cancer with 25% and cause specific mortality by 35% [70]. The VILI is the most preferred method of the screening high grade in developing countries among all other methods. Authors reported that the HC2 is 62% sensitive to detect the high grade CIN is very low as compared with other studies where it is expected very high. They also reported that the cytology has the lowest sensitivity (52% for CIN +) among all screening tests studied. Based on their study, they concluded that there is a requirement of developing a more sensitive and highly specific method for confirm the disease [71].

**Diagnostic Kit**

There are many techniques available for the HPV DNA test, and many kits are also available for this test. Many of them are differ in their target strain number and type, where some are differ in the method used for testing. By using internet, we have collected some important information regarding available kits in the markets and their details are described in Table no. 3.

| Sr. No. | Kit name | Manufacturing company | Method used | Type of Test | HPV types targeted |
|---|---|---|---|---|---|
| **1** | HPV High Risk screen | Sacace Biotechnologies | PCR + Gel Ele. | Sc. | ---------- |
| **2** | HPV 16/18 | Sacace Biotechnologies | PCR + Gel Ele. | Sc. + Gen. Kit | 16, 18 |
| **3** | HPV High Risk Typing | Sacace Biotechnologies | PCR + Gel Ele. | Gen. Kit | 16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59, 66 |
| **4** | HPV Low and High Risk Typing | Sacace Biotechnologies | PCR + Gel Ele. | Gen. Kit | HR – (16, 18, 31, 45), Intermediate Risk – (33, 35, 39, 51, 52, 56, 58, 59, 68), LR – (16, 11, 42, 44) |
| **5** | HPV 6/11 | Sacace Biotechnologies | PCR + Gel Ele. | Sc. + Gen. Kit | 6,11 |
| **6** | HPV High Risk screen Real- TM Quant | Sacace Biotechnologies | RT-PCR Qt test | Qt sc. + Gen. Kit | 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 |
| **7** | HPV Genotypes 14 Real-TM Quant | Sacace Biotechnologies | RT-PCR Qt test | Qt sc. + Gen. Kit | 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 |
| **8** | HPV 16/18 Real-TM Quant | Sacace Biotechnologies | RT-PCR Qt test | Qt sc. + Gen. Kit | 16, 18 |
| **9** | HPV 6/11 Real-TM | Sacace Biotechnologies | RT-PCR Qt test | Qt sc. + Gen. Kit | 6, 11 |
| **10** | Many types of kits for HPV | E-COLI | RT-PCR | Sc. + Gen. Kit | ---------- |

| | | | | | |
|---|---|---|---|---|---|
| **11** | IntelliPlex HPV DNA genotyping Kit | PlexBio | PCR + Hyb. | Sc. + Gen. Kit | 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 70, 73, 81 (CP8304), 82, and 83. |
| **12** | Optiplex HPV Genotyping Kit | DiaMex | PCR + Hyb. | Sc. + Gen. Kit | 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82 |
| **13** | Infinifi HPV Genotyping Assay | AutoGenomics | PCR + Micr. | Sc. + Gen. Kit | 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 73, 82, 6, 11, 30, 34, 70, 85 |
| **14** | Linear Array HPV Genotyping Test | Roche | PCR + Line Blot Assay (Hyb.) | Sc. + Gen. Kit | 6, 11, **16**, **18**, 26, **31**,**33**, **35**, **39**, 40, 42, **45**, **51**, **52**, 53, 54, 55,**56**, **58**, **59**, 61, 62, 64, 66, 67, **68**, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39, and CP6108 |
| **15** | F-HPV Typing Kit | NIMAGEN | PCR + Hyb. | Sc. + Gen. | 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, 68 |
| **16** | Cobas HPV Test Kit | Roche | PCR + Hyb. | Sc. + Gen. | 16, 18 |
| **17** | Human Papillomavirus PCR Detection Set | TAKARA | PCR + Dot Hyb. | Sc. + Gen. | 16, 18, 33 |
| **18** | Human Papillomavirus PCR Typing Set | TAKARA | PCR + RFLP | Sc. + Gen. | ---------- |
| **19** | Plasma-Serum HPV (High Risk) PCR Detection Kit | NORGEN Biotech. Corp. | PCR + Gel Ele. | Sc. + gen. | 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 |
| **20** | INNO-LiPA HPV Genotyping Extra Amp | INNOGENETICS / Fujirebio | PCR + reverse hyb. | Sc. + Gen. | ---------- |
| **21** | RealLine HPV high carcinogenic risk, Genotype, qual. | BIORON | RT-PCR | Sc. + Gen. | 16, 18, 35, 45, 31, 33 |
| **22** | RealLine HPV high carcinogenic risk, Genotype, quant. | BIORON | RT-PCR | Sc. + Gen. | 16, 18, 35, 45, 31, 33 |
| **23** | Diagen Hybrid Capture 2 (HC2) HPV DNA Test Kit | QIAGEN | Signal Amplification | Sc. + Gen. | 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 |

## 2. CONCLUSION

In India, good screening strategies should be developed for HPV and should be used in routine checkup to lower the cervical cancer risk. HPV DNA test with PAP smear test is a good option for coast effectiveness and for increasing time interval between two continuous visit. There are many good techniques in molecular biology for HPV DNA detection, but as per the availability of infrastructure and instrumentation, chemical and reagent requirement, expertise and result analyzing skill, the PCR method is most suitable for this purpose. It is also a cost effective and with as good as other highly advanced technique.

## CONFLICT OF INTEREST

The author declares no conflict of interest.

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