Review Article

Early detection of cervical cancer based on high-risk HPV DNA-based genosensors: A systematic review

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Abstract

Human papillomavirus type (HPV) is a common cause of sexually transmitted disease (STD) in humans. HPV types 16 and 18 as the highest risk types are related with gynecologic malignancy and cervical cancer (CC) among women worldwide. Recently, considerable development of genosensors, which allows dynamic monitoring of hybridization events for HPV-16 and 18, has been a topic of focus by many researchers. In this systematic review, we highlight the route of development of DNA-based genosensory detection methods for diagnosis of high risk of HPV precancer. Biosensor detection methods of HPV-16 and 18 was investigated from 1994 to 2018 using several databases including PubMed, Cochrane Library, Scopus, Google Scholar, SID, and Scientific Information Database. Manual search of references of retrieved articles were also performed. A total of 50 studies were reviewed. By analyzing the most recent developed electrochemical biosensors for the identification of HPV, we observed that the sensor platform fabricated by Wang et al. holds the lowest detection limit reported

†These authors contributed equally to the work.

Abbreviations: ADC, adenocarcinomas; AM, Atto Molar; AQ, anthraquinone; AuNPs, gold nanoparticles; Bis-PNA, bis-peptide nucleic acid; CC, cervical cancer; CNOs, carbon nano onions; CV, cyclic voltammetry; DPV, differential pulse voltammetry; EIS, electrochemical impedance spectroscopy; FAM, fluorescein amidite; FDA, fluorescein diacetate; GCE, glassy carbon electrode; GMR, GMI, giant magnetoresistive; G-PANI, graphene-polyaniline; GM, giant magnetoimpedance; HPV, Human papillomavirus type; IDA, interdigitated platinum electrode array; LAMP, loop-mediated isothermal amplification; LBC, liquid-based cytology; LSAW, leaky surface acoustic wave; mAb, monoclonal antibody; MeSH, medical subject headings; MMP, magnetic microparticle; MoS_2, molybdenum sulfide; MPs, magnetizable particles; PANi–MWCNT, polyaniline-multiwalled carbon nanotubes; Pap, papanicolaou; PDMAA, polydimethylacrylamide; PNA, pyrrolidinyl peptide nucleic acid; prGO, porous reduced graphene oxide; QCM, quartz crystal microbalance; QDs, quantum dots; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; Rox, 6-carboxyl-X-rhodamine; SCC, squamous cell carcinomas; SPE, screen printed electrode; SAW, surface acoustic wave; STD, sexually transmitted disease; SWV, square wave voltammetry; TMB, tetramethylbenzidine; VIA, visual inspection with acetic acid; WHO, World Health Organization

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in the literature for the DNA of HPV-16. Up to this date, optical, electrochemical, and piezoelectric systems are the main transducers used in the development of biosensors. Among the most sensitive techniques available to study the biorecognition activity of the sensors, we highlight the biosensors based fluorescent, EIS, and QCM. The current systematic review focuses on the sensory diagnostic methods that are being used to detect HPV-16 and 18 worldwide. Special emphasis is given on the sensory techniques that can diagnosis the individuals with CC. © 2018 BioFactors, 00(00):1–17, 2018

Keywords: *high risk HPV; molecular methods; DNA-based genosensor; systematic review*

1. Introduction

Cervical cancer (CC) caused by the human papillomavirus type (HPV) represents the third most common fatal cancer in women. HPV is one of the most significant sexually transmitted viruses worldwide. Several epidemiologic studies have revealed that there is a strong relationship with HPV and cervical neoplasia, independent of other risk factors [1].

The World Health Organization (WHO) declares that CC is one of the common abnormalities and cause of death in woman. It is estimated in the worldwide about 1.4 million women are living with CC [2].

HPV is subclassified into high-risk and low-risk groups. More than 100 different HPV types are identified and approximately 10 types have been found in CCs. The most common types have found in CC (HPV-16, -18, -31, -33, -35, -45, -52, and -58) and the less-common types are (HPV-39, -51, -56, and -59) [3,4]. HPV-16 and 18 as the high risk types were found in approximately 70% of cases of CC. HPV-18 is the second most carcinogenic after HPV-16 and it accounts for approximately 12% of squamous cell carcinomas (SCC) as well as 37% of adenocarcinomas (ADC) of the cervix worldwide [5,6].

Once HPVs 16 and 18 enter the epithelial cells, viruses begin to make E6 and E7 proteins. Actually, expression of these proteins would interfere with cell function and malignant conversion of keratinocytes. High-risk HPVs, E6 and E7 proteins degrade and inactive the tumor suppressors p53 and pRB, respectively. Therefore, the cells grow in an uncontrolled manner and to evade from cell death [7].

Current methods particularly reverse transcriptase PCR (RT-PCR), although known as an effective tool for detection and typing of viruses, is faced with some disadvantages such as costs and complicated procedures. Therefore, the development of rapid, easy, accurate, and sensitive detection techniques is necessary [8,9].

Up to date, there is no systematic review on sensory methods for diagnosis of HPV-16 and 18. The aim of this systematic review is to deliver a meticulous summary of the developments of all genosensors for the detection of HPV-16 and 18.

2. Methods

2.1. Search strategy

We systemically searched Web of Science; PubMed; Cochrane Library; Scopus; Science Direct and Google Scholar from 1994 to 2018. Our search was restricted to original papers in English and the Persian language. In the search strategy for searching associated HPV detection studies, we used the following words and medical subject headings (MeSH): “high risk human papilloma virus” OR “HPV16” OR “HPV18” AND “electrochemical biosensor” OR “optical method” OR “piezoelectric method” OR “fluorescent method” OR “colorimetric method” AND “detection” OR “diagnosis”. The search was performed by two independent researchers and results were checked by a third researcher (Fig. 1).

2.2. Data extraction

Data were extracted from 50 selected articles. Data included methods, HPVs type, sensor platform, label, detection limit, detection range, response time, reusability, and comments.

2.3. Quality assessment

In order to evaluate the quality of included studies, data extraction and study quality assessment were performed independently by two reviewers.

2.4. Traditional screening detection methods of HPV-16 and 18 in CC

There are three screening detection methods of high-risk HPV types in CC. This includes the conventional papanicolaou (Pap) test and liquid-based cytology (LBC), visual inspection with Acetic Acid (VIA), and HPV molecular techniques.

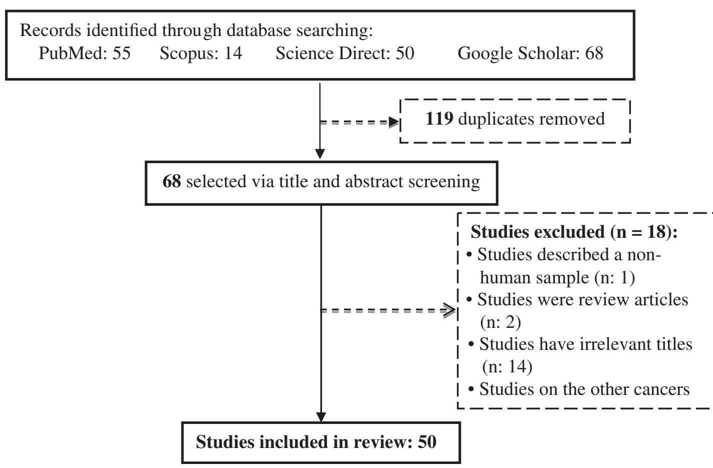

FIG 1 *Flow chart of search strategy.*

TABLE 1 *The sensitivity and specificity of screening laboratory methods for HPV16 and 18*

	Performance	
Screening test	*Sensitivity (%)*	*Specificity (%)*
Pap smear	51	67
LBC	55	78
VIA	63	66
HPV antibody detection	58	97

The Pap smear is the oldest method for screening precancerous changes for squamous CC. This test involves the collection of cells from the cervix and the examination under a microscope to look for abnormalities. Its false-negative rate is 13–70% and its false-positive rates range from 0 to 14% [10].

LBC is the second cytology technique where a brush-like device is used for collecting a cervical sample. The advantages of the LBC method in contrast to the Pap smear include an improving means of slide preparation, producing more homogenous samples, and the increased adoption of sample standardization as well as having a greater sensitivity. On the other hand, the disadvantages of the LBC method is that it is more expensive than the Pap smear and it is also not suitable for a sample with limited resources as well as requiring a trained personnel to carry out the task [11].

The third conventional technique is VIA that is performed in low-resource settings and although it has limited specificity, it is economical. One of the other major limitations of VIA is the lack of reliability in the precursors of CC's area for postmenopausal women due to changes in the endo-cervical junction (Table 1) [12].

Serological markers could be tumor-specific antigens associated with the expression of the oncogenes of high-risk HPV. It should be noted that initially techniques such as Southern blotting, *in situ* hybridization, and dot-blot hybridization used radiolabeled nucleic acid hybridization assays to detect HPV infections in cervical samples. Although these techniques are high-quality they have low sensitivity and require relatively large amounts of purified DNA as well as a lot of time for the performance of procedures [13].

The HPV diagnosis depends on molecular-biology methods that leads to accurate detection and typing of HPVs. These common available techniques include microarray, quantitative reverse transcription-polymerase chain reaction (qRT-PCR), and next-generation sequencing (Table 2) [14].

Microarray is one of the most influential high-throughput methods and it monitors the expression of thousands of miRNAs and DNA in a single testing. Actually in this method by using a probe amplification, the PCR product is hybridized onto a chip and after a washing step, hybridized signals are visualized with a DNA chip scanner [15].

TABLE 2 *Comparison of nucleic acid-based cancer techniques for HPV16 and 18*

Technique	*Specificity*	*Sensitivity*	*Assay time*
Microarray	Moderate	Low	1–2 days
qRT-PCR	High	High	Few hours
Next generation sequencing	High	High	2–5 days

The qRT-PCR is another technique which is used for the sensing of HPVs DNA and can be considered as a gold standard method due to it has high sensitivity and specificity. This technique is based on SYBR Green or TaqMan methods and fluorescence signal measurements of the amplicon, therefore it is moderately expensive. There are several tests commercially available in the market for the detection of HPV DNA or the E6/E7 oncogenes, such as Qiagen's Digene Hybrid Capture® 2 HPV DNA test (Valencia, CA) and Cervista® HPV 16/18, Cobas® HPV test by Roche (Indianapolis, IN) [12].

From the above results, tissue cultures and serological techniques are not suitable for the detection of HPVs. As a result of this, in most laboratories, molecular methods have been used for the diagnosis of HPV-16 and 18.

2.5. Biosensing methods as alternative tools for HPV-16 and 18 detection

A Biosensor is a convenient and transportable analytical device constituted by at least one biological molecule. These devices introduced new opportunities for rapid, simple, economical, sensitive, and specific procedures, particularly for the early diagnosis of infectious diseases [16,17].

Furthermore, the materials with the nanometer scale have been used to reach the nanostructuration of these devices [18–21]. The covalent functionalization of nanoparticles and well-organized immobilization of nucleic acids over the surface of a transducer is necessary for the analyte detection and will perform a measurable response signal proportionate to the analyte concentration. The transducers are classified into electrochemical, optical, piezoelectric and magnetic (Fig. 2). The response obtained from the interaction between the single-strand DNA probe and the HPV target DNA transduced into a measurable signal, which can be processed by computer software. Furthermore, the signal conversion can be recorded via SPR, EIS, DPV, and QCM techniques [22–24].

Figure 3 summarizes the quantity of publications in 26 years of research in DNA biosensors for HPV-16 and 18 detection. When the search included the keyword "electrochemical or optical or piezoelectric or aptameric or

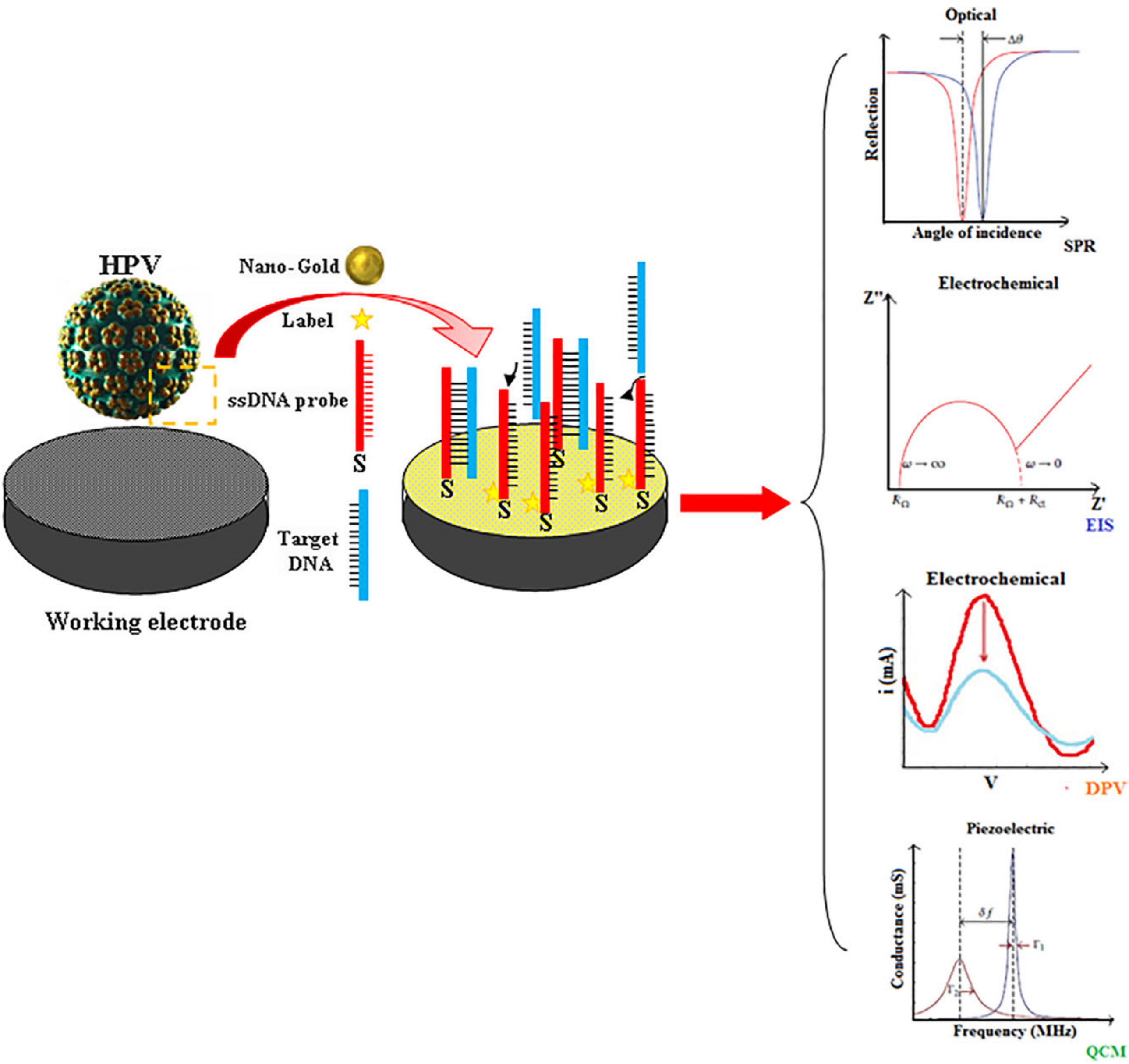

FIG 2 *The design protocol of HPV DNA genosensor with a labeled probe.*

immunosensory", the chart indicates that most devices were based on electrochemical and optical techniques.

In contrast to the conventional identification methods, the biosensors devoted to the molecular diagnosis of HPV types and their significant properties are less complicated and are free of prolonged experimentation processes and purification requirements [25]. The frequency of reported biosensor transducer showed in Fig. 4.

Table 3 summarizes these platforms, which have been applied to HPV-16 and 18 diagnostics, and Table 4 shows the genes and sequence of probes which have been used for HPV-16 and 18 detections.

2.6. Electrochemical biosensors

The plan of electrochemical detection technology is to design a sensitive, selective, and specific detection technique as alternative methods for several of current diagnosis examinations. These platforms measure the output signal by cyclic voltammetry (CV), square wave voltammetry (SWV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) [72–76].

The electrochemical biosensors are favorable diagnostic tools for HPV because of their fast response, simplicity, and low cost instrumentation [25,77,78].

Vernon et al. in 2003 presented a bioelectronics device for the diagnosis of 21 types of HPV. This platform is organized on

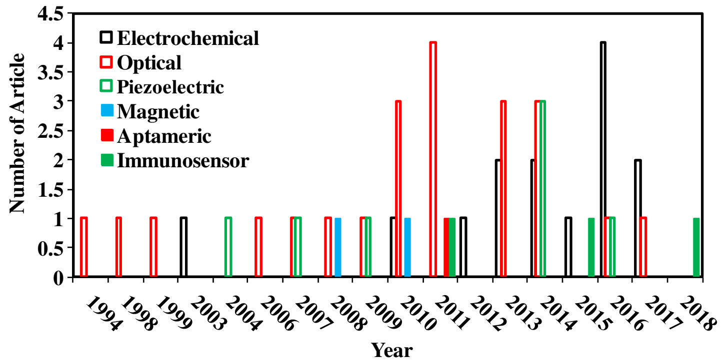

FIG 3 *Number of publications reporting HPV16 and 18 DNA biosensors since 1994 till 2018.*

a gold electrode with a self-assembled monolayer of immobilized oligonucleotides that are specific for each HPV genotypes. In this method, two hybridization steps occurred for the detection of targets, the first between the capture probe and the target, and the second between an adjacent region of the target and ferrocene-labeled signal probe [26].

Civit et al. used an electrochemical genosensor for the detection of two high-risk HPV [16,45] DNA sequences. For the simultaneous detection of several high-risk HPV sequences, a modified and high sensitivity and selectivity chip was reported. This sensor thiolated with HPV16E7p and HPV45E6 probes which exhibited the LOD of 490 and 110 pM, respectively [33].

Wang et al. in 2013 fabricated an electrochemical sensor via depositing Au nanoparticles and immobilization of single-stranded probe DNA on the SWCNT platform. This sensor utilized for the detection of target HPV16 DNA sequences concentration from 1 AM (Atto molar) to 1 μM [37]. The high sensitivity of this biosensor in compare to other reported HPV biosensors was obtained due to the structural platform of optimized genosensor and using the electrochemical impedance spectroscopy.

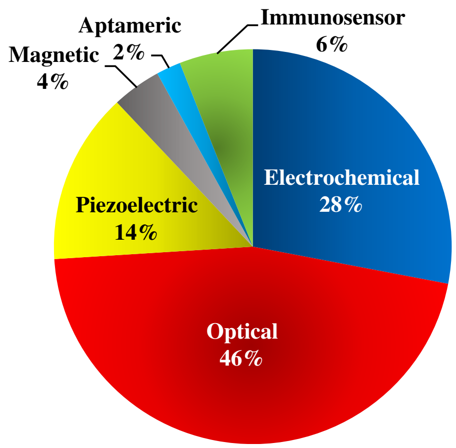

FIG 4 *Frequency of reported DNA biosensor for HPV-16 and 18: electrochemical, optical, piezoelectric, magnetic, aptameric, and immunosensor.*

So far, the most frequent platform used for immobilizing the oligonucleotide probe is a gold electrode which doing the interaction through thiol-gold covalent bonding. The process can be performed in the presence of several labels such as tetramethylbenzidine (TMB), horseradish peroxidase, methylene blue, or hematoxylin which provide an amplified electrochemical response combined with a greater specificity. For example, Campos et al. proposed a gold platform sensor by depositing a cysteine film. In this study, the measurement was based on the reduction signals in the presence of the MB by using a DPV method from 18.75 to 1000 nM and the detection limit was 18.13 nM [31].

In another study by Souza et al., a pencil graphite working electrode was designed for the detection of the E1 HPV gene, similar to a study by Campos et al. who used a MB for recording the signals. The LOD of this assay was reported at 1.49 nM [27].

Jampasa et al. used a screen printed electrode (SPE) immobilized with an anthraquinone (AQ)-labeled pyrrolidinyl peptide nucleic acid (PNA) for identifying HPV L1 gene down to 4 nM [29]. In parallel work, Teengam et al. used the EIS method for the immobilization of the AQ-PNA probe on the graphene-polyaniline (G-PANI) modified electrode. The hybridization between the HPV type16 target and DNA probe was investigated by SWV. The detection limit of HPV type 16 DNA was found at 2.3 nM with a linear range of 10–200 nM [30].

Pursuant to the first report by Ugarte in 1992 on the proposition the Carbon nano onions (CNOs), Bartolome et al. constructed the biosensor modified with small CNOs for the diagnosis of the E7 gene of HPV16 by HRP indicator. The limit of detection of this biosensor was reported as 0.50 nM [32].

Karimizefreh et al. proposed a modified glassy carbon electrode (GCE) with gold nano sheets by an impedimetric method to detect the DNA of HPV type 16 in the presence of hexacyanoferrate as a redox marker. The biosensors respond to target DNA with a concentration range from 1 μM to 1 pM and a detection limit of 0.15 pM. Hence, the authors claimed that the use of gold nanosheets on a GCE distinctly improved the detection and differentiation of HPV compared to using bare gold [38].

Jimanez et al. in 2016 developed a platform sensor based on magnetizable particles (MPs) for the diagnosis of purified HPV16-E6 gene from PUK57 plasmid by SWV technique. Actually, MPs modified by primers coupled with electrochemical and electrophoretic gel detection of the isolated nucleic acid in combination with PCR method acted in a concentration range from 0.15 to 2.5 μM and the LOD of 0.2 nM [28].

Bartosik et al. presented a SPE electrode that assembled by the means of streptavidin-modified magnetic beads and a DNA capture probe to detect HPV16 DNA by using a digoxigenin label. The detection range of this biosensor was 1 pM to around 1 nM [35].

Kowalczyk et al. demonstrated a highly selective biosensing platform based on mercury(II)–mediated thymine base pairs (T–Hg(II)–T) for the detection of a specific DNA sequence characteristic for HPV with the lowest detection limit of 1.2×10^{-5} nmol/L [39].

There have been several studies in the literature on HPV infections, for example Piro et al. reported an immunosensor by

TABLE 3 *Comparison of biosensor technologies for HPV-16 and 18 diagnostics*

Ref.	Method	HPV type	Sensor platform	label	Detection limit	Detection range	Response time	Reusability	Comments
[26]	CV	21 different types of HPV	Gold surface/capture oligonucleotides	Forrecene label	—	—	2–8 h	—	• 86% detection of the HPV types in a clinical sample • Rapid and integrated detection of multiple pathogens
[27]	DPV	HPV-16	PGE	MB	1.49 nM	2–8 nM	15 min	—	• Sensitive, specific and rapid detection method • Low cost and small size device
[28]	SWV	HPV-16	microfluidic chip	Biotin	0.2 μM	0.15–2.5 μM	120 S	—	• Simple, sensitive and cost-effective method • Very effective technique for HPV identification and therapy
[29]	SWV	HPV-16	Carbon surface/ chitosan	AQ	4 nM	0.2–12 nM	15 min	—	• Very high selectivity • inexpensive tool for the early stage detection
[30]	SWV and EIS	HPV-16	Paper base/G-PANI	Label free	2.3 nM	10–200 nM	15 min	—	• Inexpensive and disposable device • Identifies the primary stages of cervical cancer
[31]	DPV	HPV-16	Gold surface/ L-cysteine/HPV16 oligonucleotide	MB	18.13 nM	18.75–10,000 nM	10 min	—	• New portable detection system for HPVs • An effective diagnosis in the early stages of infection
[32]	CV	HPV-16	GCE/CNO	HRP	3.9 nM	—	30 min	Four tests	• Enhanced sensitivities • Higher sensitivities compared to GCE electrode without the CNOs
[33]	CV	HPV-16 HPV-18 HPV-45	Gold surface/ oligoethylene glycol-terminated bipodal thiol	HRP	220 pM for HPV16, 170 pM for HPV18 110 pM for HPV45	0.1–50 nM	20 min	Five tests	• Quantitative detection of DNA in clinical samples • Good specificity for the multiplexed detection
[34]	CV	HPV-16 HPV-45	Gold surface	HRP	490 pM for HPV16 110 pM for HPV45	0.1–50 nM	1 h	—	• High sensitivity and selectivity • High throughput screening assay
[35]	CA	HPV-16 HPV-18	SPE/carbon-based electrode	HRP	—	—	30 min	Five tests	• Good reproducibility, sensitivity and selectivity • Applied into biological samples

[36]	DPV	HPV-16	PGE/ Ag/AgCl	Label-free	16 pg/IL	40–5,000 pg/IL	45 min	—	• Higher sensitivity and specificity compared with the electrophoresis • Alternative tool for cloning analysis in plasmids.
[37]	EIS	HPV-16	SWCNT/gold nanoparticle	—	1aM (Atto molar)	1–1 μM	2 h	Six tests	• Ultrasensitive biosensing • Valuable candidate for achieving early diagnosis
[38]	Impedimetric	HPV-16	GCE/gold nanosheet	Hexacyanoferrate	0.15 pM	1 μM–1 pM	2 h	—	• Good selectivity • Great potential in HPV DNA diagnostics and clinical analysis
[39]	SWV	HPV-18	GCE/carboxyphenyl layer	Hg(II)	1.2×10^{-5} nmol/L	-	1 h	—	• Low cost method • Effective sensing
[40]	Fluorescence PCR	HPV-16, -18, -31, -33, and -35	Aqueous solution	Fluorescence reporter dye	Differential detection of samples mimicking mixed infections up to 1% of the total DNA	10^1–10^6 DNA copies.	75 min	—	Detection of mixed infections
[41]	In situ PCR/FISH/ LSCM	HPV-16, 18 6,11,31,33,35	*In situ* histological sample	Biotin-streptavidin	1–2 DNA copies	1–50 DNA copies per cell	24 h(overnight hybridization)	—	The sensitivity is same as in situ PCR
[42]	FISH	HPV-16 and 18	*In situ* histological sample	Digoxigenin-II-Dutp by random priming	1 DNA copy	1–500 DNA per cell	6 h	—	• Good sensitivity • Removes irrelevant material from the specimen • 100% reliability
[43]	PCR/fluorescence	HPV-16, 18, and 52	Microfluidic chip	HRP/QD	10 copies/μL	1 copies/μL to 10^5 copies/ μL	1 h	—	• Useful platform for sensitive HPV virus genotyping • Transferable for the detection of other disease-related biomolecules
[44]	PCR/ chromatography fluorescence	HPV-16 and 18	Nitrocellulose DNA chip	Biotin	Qualitative	—	30 min	—	• Rapid test • Sensitive test
[45]	PCR/SPR	24 different types of HPV	Gold chip	Label free	Qualitative	—	4 h	—	• Highly sensitive • Effective approach in a clinical setting
[46]	PCR amplification	13 different types of HPV	Nitrocellulose chip	Biotin–avidin	10 to 10^2 Plasmid copies	0–10^5 DNA copies	30 min	—	Good method in the clinical application
[47]	qRT-PCR	HPV-18	Water-soluble QDs	QDs and Cy5	0.2 nM	1.0–50.0 nM	20 min	—	• Simple method • Low cost method • Highly sensitive method
[48]	Fluorescence spectroscopy	HPV-16	Aqueous solution/ Maghemite/ quantum dot	Biotin and QD	Qualitative	—	1 h	—	• Rapid and facile hybridization method • This method is suitable for epidemiological screening

TABLE 3 *(Continued)*

Ref.	Method	HPV type	Sensor platform	label	Detection limit	Detection range	Response time	Reusability	Comments
[49]	Fluorescence microscopy	HPV-16	Silica nanoparticles	Fluorescence dye	Qualitative	—	15 min	—	• The specificity of this method is similar and its sensitivity was better than the current method • Fast and cost-effective procedure
[50]	PCR amplification	HPV-6, 11 16 and 18	microfluidic-based microbeads array platform	Biotin	25 pM	—	30 min	—	• Small consumption of reagents • Simple sample injection • Minimal sample contamination
[51]	Fluorescence spectroscopy	HPV-16 and 18	Silica nanoparticle	Biotin-avidin	13–15 pmol	0–0.78 nM	90 min	—	• A simple and sensitive method for multiplexed DNA detection • The method is quite sensitive
[52]	Optical signal	HPV-6, -11, -16, and -18	Glass	Au/Ag	0.05 pmol/μL	—	—	—	A low-cost method
[53]	Optical signal	HPV-16	In situ histological sample	YOYO-3 dye	Down to 0.07 copies per cell	—	24 h (overnight for hybridization)	—	• A sensitive method • Directly coupled with current sampling methods • Applicable to any DNA detection
[54]	PCR	HPV-16, -18 and -45	Aqueous solution/ particle-based conjugates	FDA/FITC/HRP	10^3 copies/μL	—	10 min	—	• High selectivity • Short incubation times • Directly fluorescent-labeled streptavidin
[55]	PCR	12 different types of HPV	Biochip platform	Streptavidin-Cy5	10^4 copies/μL	—	20 min	—	• Specificity • Cost-efficient • High sensitivity
[56]	PCR	HPV-16	Glass	YOYO-3 dye	0.7 copies per cell	1.44–7000 copies/cell	16 h	—	Without any interference or restrictions with current methods
[57]	Spectrophotometer	HPV-16 and 18	MMP	QD	70 pM for HPV-16 60 pM for HPV-18	—	75 min	—	• Good precision • High accuracy
[58]	Nanoparticle based optical	HPV-16 and 18	PDA Chip	Biotin	30 pM	100 pM to 5 nM	15 min	—	Short time method
[59]	colorimetric	HPV-16 and 18	Aqueous solution/ AuNP	Hydroxynaphthol Blue dye	0.14 nM	—	—	—	• Reliable optical detection method • Not requiring expensive equipments • Read by the naked eye
[60]	LAMP/ colorimetric	HPV-16, 18 45 and 52	Aqueous solution / LAMP	—	—	—	30 min	—	• Simple and rapid test • Sensitive and qualitative method

[61]	LAMP turbidity/ colorimetric	HPV-16 and 18	LAMP/ AuNP	Au	10^2 for HPV16 10° for HPV18	—	25 min	—	• The sensitivity is higher than LAMP-turbidity assay. • The specificity is 100%
[62]	PCR/colorimetric	HPV-16 and 18	MMB	—	10 pM	—	1 h	—	• Fast response • Low-cost • Sensitive assay
[63]	QCM	HPV-6, 11 16 and 18	HPV probes with a disulfide group	Label free	$-\Delta F = 48 \pm 5$ Hz	—	—	—	• Highly sensitive • Speedy and inexpensive measurements • Promising candidate for a diagnostic sensor system
[64]	QCM	HPV-16 and 18	Biotinylated HPV probe via streptavidin anchoring	Label free	$-\triangledown F = \leq 3$ Hz	—	—	—	• Combined multi-sensor method • Good reproducibility • Suitable for detection and genotyping of HPV.
[65]	QCM	11 different types of HPV	Biotinylated HPV probe via avidin anchoring	Label free	$\triangledown F = 4.4$ Hz	—	—	—	• Good accuracy • Good sensitivity
[66]	LAMP-QCM/ Surface-mass detection	HPV-16	SPR	Label free	100copies per cell	—	15 min	—	• 100% specificity • Precision lesser than TaqMan qPCR
[67]	QCM-D	HPV-16	Alkanethiol self-assembling monolayer	—	—	—	—	—	• Distinguishes between HPV-positive and negative cells
[68]	SAW	HPV-18	LSAW/bis-PNA/gold electrode	—	1.21 fg/ml	1–1000 μg/L	38–75 min	—	• Time saving • High selectivity and sensitivity
[69]	SAW	HPV-18	LSAW/bis-PNA	—	1 fg/ml	—	30 min	—	• High sensitivity • Simple • Rapid method
[70]	Magnetic	HPV-18	GMR biochip	—	10 nM	—	<1 h	—	• 90% accuracy • Reliable DNA detection
[71]	Magnetic	HPV-16 and 18	GMI-based micro channel system	—	—	—	less than 2 h	—	• Quick method for genotyping HPV type 16/18 • Time is shortened

AuNP: Au nanoparticle; AQ: anthraquinone; bis-PNA: bis-peptide nucleic acid; Cy5: Cyanine5; CNO: carbon nano-onion; CV: cyclic voltammetry; DPV: differential pulse voltammetry; EIS: electrochemical impedance spectroscopy; FDA: fluorescein diacetate; FISH: fluorescent in situ *hybridization; FITC: fluorescein 5-isothiocyanate; GCE: glassy carbon electrode; G-PANI: graphene polyaniline; GMR: A giant magneto resistive; GMI: giant magneto impedance; HNB: hydroxynaphthol blue dye; HRP: Horseradish peroxidase; LAMP: loop-mediated isothermal amplification; LSAW: leaky surface acoustic wave; MB: methylene blue; MMB: magnetic microbead; MMP: magnetic microparticle; PCR: polymerase chain reaction; PDA: photodiode array; PGE: Pencil graphite electrode; QCM: quartz crystal microbalance; QD: quantum dot; SWV: square wave voltammetry; SAW: surface acoustic wave; SPE: screen-printed electrode; SPR: surface plasmon resonance; SWCNT: single-walled carbon nanotube arrays.*

employing GCE conjugated with copolymer poly (5-hydroxy-1,-4-naphthoquinone-co-5-hydroxy-2-carboxyethyl-1,4-naphthoquinone). This copolymer acted as both an immobilizing and transducing element. This sensor was able to detect the interaction between antigenic peptide L1 and HPV-16 antibody with LOD 50 nM [79]. Furthermore, the similar model was used by Tran et al. in order to produce a film of polyaniline-multiwalled carbon nanotube (PANi–MWCNT) on an interdigitated platinum electrode array (IDA). A peptide aptamer was used as an affinity capture reagent for the detection of the L1 antigen of HPV-16. The most significant advantage of this technique consists of reagent less and multiple detection of antigen–antibody complex formation on well conducting IDA interface of PANi-MWCNT without intermediate steps or any labeling reagents. The range of this study was 10–50 nM and LOD of 490 pM [80].

With the same object, Urrego et al. presented a biomicrosystem consisting of 98 biosensors through the interaction between monoclonal antibody (mAb) 5051 and HPV-16 L1 antigen.

The immobilization of mAb 5051 was performed on a self-assembled monolayer of 4-aminothiophenol, on a poly methyl methacrylate substrate with a gold nanolayer. The authors asserted that the bio microsystem is simple to manufacture, its use does not need specialized personnel and it allows carrying out 98 tests *in situ* simultaneously [81]. In another study, Chekin et al. showed the improvement the biosensing of GCE modified successively with porous reduced graphene oxide (prGO) and molybdenum sulfide (MoS_2) for the sensitive and selective detection of the L1-major capsid protein of HPV16. Using DPV, a detection limit of 1.75 pM could be reached [82].

2.7. Fluorescent biosensors

In the recent years, biochip based on florescent technique has developed into a major research subject, due to being an extremely valuable tool in gene and drug discovery and disease diagnosis. The fluorescence technique for recognizing a certain HPV strain has been used, to provide in situ evidence of the existence of the expected strain including its quantification which can be easily achieved [83].

Chan et al. used organic nanocrystals as labels for quantitative detection of different HPV genotypes (HPV-16, HPV-18, and HPV-45). This project was carried out in a two-step process. Fluorescein diacetate (FDA) was pounded into nanometer-sized crystals and subsequently adsorbed with streptavidin HPV DNA. The fluorescence signal was directly proportional to the amplicon concentration in the range of 10^3–10^5 copies/μL [54].

The fluorescent-labeled probes with different fluorophores are known to be rapid, low cost and disposable DNA biosensors that enable one to provide multiplex detection in a single assay [46].

Wang et al. demonstrated that a sandwich assay could allow multiplex detection of HPV-16 and HPV-18 with a LOD of 0.17 and 0.78 nM, respectively. In this way, avidin-coated silica nanoparticles were interacted with biotinylated capture probe HPV DNA strands. These were then mixed with 64-base HPV-16 and HPV-18 target DNA strands. After hybridization, target DNA strands were selectively captured on the nanoparticles and next, the fluorescein amidite (FAM) and 6-carboxyl-X-rhodamine (Rox) labeled HPV-16 and HPV-18 specific probes were incubated with particles to identify target DNA strands [51].

Xiang suggested that magnetic microparticle (MMP) was also suitable as a capture surface for target HPV DNA strands. The quantum dots (QDs) coated with multiple FAM- or Rox-labeled random DNA were used as labels for targeting HPV-16 and HPV-18, respectively. After hybridization, MMPs were magnetically separated from the sample followed by a heating step to release the labels inside the solutions. Afterwards, HPV-16 and HPV-18 were detected by simply measuring the FAM and Rox fluorescent signal at concentrations down to 70 and 60 pM, respectively [57].

Hong et al. reported a novel detection method for several types of HPV DNA, merging the advantages of QDs and the manipulability of super paramagnetic nanoparticles. Then the Streptavidin-coated magnetic beads were added to the solution which contained the hybridization of target DNA with biotinylated capture probe DNA and QD labeled detection probe DNA. These DNA complexes were immobilized on the magnetic bead surfaces due to biotin–streptavidin binding. Afterwards, the beads were magnetically trapped in the solution and the supernatant was collected to sense remaining QD labeled detection probe DNA. This biosensor was used for the detection of HPV-16 DNA in 160 clinical cervical swab samples, successfully [48].

In addition, Shamsipur et al. w synthesized, a very sensitive and convenient nanobiosensor based on fluorescence resonance energy transfer (FRET). In this project, water-soluble CdTe QDs were developed for the detection of a 22-mer oligonucleotides sequence in HPV-18 gene. The fluorescence intensity found the concentration from 1.0 to 50.0 nM, with a detection limit of 0.2 nM [47].

In another project, Brandstetter et al. presented the detection of low and high risk HPV types by using a polymer-based DNA biochip platform. 36 DNA probes were printed on a substrate in a microarray pattern by polydimethylacrylamide (PDMAA) via a UV-irradiation procedure. This chip indicated the variety of HPV genotypes in samples down to 10^4 copies with an overall LOD of 10 copies [55].

The HPV DNA test was also analyzed in a microfluidic channel by Zhang et al. They used microbeads as capture surfaces for target HPV-16 and 18 DNA. In this research, microbeads were limited to chambers in a microfluidic channel. These beads were then functionalized with the capture probes. After target HPV DNA was located inside the channel and hybridized on the beads, HRP-functionalized gold nanoparticles with secondary HPV DNA probes were incubated with the target. Finally, the fluorescent signal was measured to quantify target HPV DNA down to 1 fM [43].

Yue et al. fabricated a single layer array of microbeads with specific HPV DNA capture probes in a microfluidic platform. In this study, a mixture of different spectrally encoded microbeads was used for multiplex detection of HPV-16 and 18 target DNA. After hybridization of biotinylated target DNA with probe DNA in the platform, a fluorescent label was introduced in a microchannel and the HPV DNA was measured down to 25 pM by the fluorescent signal [50].

Li et al. showed a single molecule imaging system for detecting the HPV-16 target DNA in a capillary channel. After hybridization of target HPV DNA with a particular fluorescent labeled HPV DNA probe, the resultant fluorescent signal was detected down to 0.7 copies per infected cell. It should be noted that in this study, the fluorescence resonance energy transfer (FRET) was used by staining hybridized DNA with YOYO-3 dye as an acceptor to further increase the selectivity of the previous assay [53]. Furthermore, Lee in another work carried out a detection of HPV16 DNA by a dual-probe strategy. A single molecule detection system was used in this study for the detection of HPV-16 DNA with a similar LOD of the previous study [56].

A lateral flow DNA biosensor was presented by Xu et al. to detect 13 HPV genotypes. They introduced a lateral flow biosensor based on fluorescent-labeled probes with different fluorophores which was combined with multiple immobilized probes. The authors declared that this sensor was disposable, and allowed rapid analysis of samples with LOD values of 10 copies/μL for HPV-16, and 10^2 copies/μL for HPV-18 [46]. This result is consistent with findings by Kim et al. utilizing a lateral flow chip with an array of HPV DNA capture probes to detect biotinylated target HPV-16 and 18 DNA by a fluorescence-streptavidin apparatus [44].

Li et al. developed a chip for identification of some HPV genotypes such as HPV-16 and 18 by using gold/silver core-shell nanoparticle labels at concentrations down to 50 nM. First HPV DNA capture probes were immobilized on a glass chip, then the target HPV DNA labeled with nanoparticles was captured on the glass surface through the hybridization method. Afterwards, the existence of HPV nucleic acid sequences was analyzed via measuring optical signal changes on the chip surface using a photodiode sensor [52].

Moreover, Beak et al. suggested a bipolar integrated circuit photodiode array (PDA) chip for HPV-16 and 18 DNA analysis. On the surface of the chip, DNA probes were hybridized with biotinylated target DNA. Anti-biotin antibody-conjugated gold nanoparticles as a label were added and incubated on the hybridized DNA. Silver enhancement solution was then introduced on the surface to precipitate silver metal particles on the gold nanoparticles which blocks the light on the PDA. Thus, target DNA was measured and quantified as a voltage drop on the PDA. The HPV genotypes were analyzed at concentrations down to 30 pM [58].

Palantavida et al. detected HPV-16 inside the infected epithelial cell by using mesoporous silica nanoparticles based on ultra-bright fluorescent method. These particles were functionalized with folic acid to specifically target folate receptors of malignant cells. After 15 min incubation of the particles with the sample, HPV-16 infected cells were easily distinguishable from the normal cells. This method showed better sensitivities (95–97%) than HPV DNA and cell pathology tests (30–80%) [49].

2.8. Colorimetric biosensors

The Colorimetric assay is another technology for the detection and identification of various types of HPV and this spot test would offer speed and simplicity of operation.

Another useful and alternative assay for DNA detection under isothermal conditions is loop mediated isothermal amplification (LAMP). However, it results in a turbid amplified product which is not easily detected by the naked eye.

Lue et al. improved a technique based on LAMP product by using gold nanoparticles (AuNPs) attached to a single-stranded DNA probe under the optimal conditions (incubation time of 20 min at 65 °C) for the detection of HPV-16 and HPV-18. The LAMP-AuNP assay showed higher sensitivity and ease of visualization than LAMP turbidity for the detection of HPV-16 and 18 with stability over 1 year. The proposed LAMP-AuNP colorimetric assay showed a simple, rapid and highly sensitive alternative diagnostic tool for the detection of HPV-16 and HPV-18 in district hospitals or field studies [60].

Chen et al. presented another colorimetric detection method for the diagnosis of HPV-16 and -18 based on a sandwich hybridization which occurred between the target HPV DNA and two HPV DNA probe on gold nanoparticle layers. This colorimetric sensor showed the LOD of 0.14 nM [59].

2.9. Magnetic biosensors

Biosensors with magnetic nanoparticle labels have presented a distinctive alternative in identifying the agent of diseases, such as HPV genotypes. At the surface of the magnetic material, there is a specific biological probe immobilized and ready to interact with its counterpart found on a sample suspected to contain biologic traces of HPV.

Xu et al. reported a multi layered system for the detection of some types [16,18,33,45] of HPVs. In this study, the DNA target of HPV was detected from a concentration as low as10 mP using a giant magnetoresistive (GMR) biochip [70]. Also, Yang et al., presented a similar approach with a giant magnetoimpedance (GMI) based biosensor, wherein impedance changes on the magnetic sensor instead of resistance changes as with the GMR sensor were used to detect the presence of magnetic labels. Based on this sensor, HPV-16 and HPV-18 target DNA were analyzed by using clinical samples [71].

2.10. Piezoelectric biosensors

In several studies, the piezoelectric method has been introduced as an attractive technique due to its simplicity, low instrumentation costs, possibility for real-time and label-free detection and its generally high sensitivity. The recent success in the molecular diagnosis of HPV by electrochemical and optical transducers based on the sequence-specific detection of nucleic acids (DNA or RNA) encouraged the development of the other transducer methods as the piezoelectric biosensors that exploit a secondary but no less important aspect of the electrochemical biosensor.

The quartz crystal microbalance (QCM) sensors consist of cavity resonators constructed over a piezoelectric crystal

TABLE 4 ***Genes and sequences of probes for HPV-16 and 18 detections***

HPV types	*Genes*	*Sequences (5′–3′)*	*Ref.*
HPV16	L1	22mer: GTAGTTTCTGAAGTAGATATGG	[26]
HPV18	L1	22mer: TGGTAGCATCATATTGCCCAGG	[26]
HPV16	E1	21mer: GCAAAGGCAGCAATGTTAGCA	[27]
HPV16	E6	NM[a]	[28]
HPV16	L1	14mer: GCTGGAGGTGTATG	[29]
HPV18	L1	14mer: GGATGCTGCACCGG	[29]
HPV16	NM	14mer: GCTGGAGGTGTATG and AQ-PNA Prob: Ac-(Glu) 3-CATACACCTCCAGC-Lys(AQ)NH2	[30]
HPV16	L1	23mer: ATGCACCAAAAGAGAACTGCAAT	[31]
HPV16	E7	21-mer were purchased from Biomers.net (Ulm, Germany)	[32]
HPV16	E7	24-mer were purchased from Biomers.net (Ulm, Germany)	[33]
HPV18	E6	24-mer were purchased from Biomers.net (Ulm, Germany)	[33]
HPV16	E7	24mer: GAGGAGGAGGATGAAATAGATGGT	[34]
HPV16	NM	(see Supporting Information Table S1) were synthesized by Generi Biotech (Czech Republic)	[35]
HPV18	NM	(see Supporting Information Table S1) were synthesized by Generi Biotech (Czech Republic)	[35]
HPV16	E6	23mer:ATICACCAAAAIAIAACTICAAT (guanine-free)	[36]
HPV16	E7	24mer: ATGGGGTCTGTCCGGTTCTGCTTG	[37]
HPV16	L1	25mer: AAAGCAAAGTCATATACCTCACGTC	[38]
HPV18	NM	20mer: H2N-C6-TGCTGTTCTTCTTGTTCTCG	[39]
HPV16	E1	33mer: FAM-5′-ATAATCTCCTTTTTGCAGCTCTACTTTGTTTTT-3′TAMRA	[40]
HPV18	E1	30mer: TET-5′-CCGCCTTTTTGCCTTTTTCTGCCCACTATT-3′TAMRA	[40]
HPV16	In E6-E7 region	18mer: CCGGACAGAGCCCATTAC	[41]
HPV18	In E6-E7 region	20mer: TAAGGCAACATTGCAAGACA	[41]
HPV16 and 18	NM	NM	[42]
HPV16	NM	See Supporting Information Table S1	[43]
HPV18	NM	See Supporting Information Table S1	[43]
HPV16	NM	Capture probe: 36mer: 5′-ACGCAGTACAAATATGTC --------------------------ACCTACGACATGGGGAGG-3′	[44]
HPV18	NM	Capture Probe: 36mer: 5′-CACTCGTAGTACCAATTT ---TATAGCAGACATGTTGAAG-3′	[44]

TABLE 4 *(Continued)*

HPV types	*Genes*	*Sequences (5′–3′)*	*Ref.*
HPV16 and 18	L1	NM	[45]
13 different types of HPV	NM	NM	[46]
HPV 18	NM	11mer: Cy5-labeled probe: Cy5-5_GCCCATTAACA 10mer: (N)DNA: NH2-(CH2)3-3_AAACCTTCTG	[47]
HPV16	NM	Capture Probe: 74mer: GAGGAGGATGAAATAGATGGTCCAGCTGGACAAG CAGAACCGGACAGAGCCCATTACAATATTGTAACCTTTTG 26mer: TTGCAAGTGTGACTCTACGCTTCGGT Secondary probe: 50mer: GGAGCGACCCAGAAAGTTACCACAGTTATGCACAGAGCTGCAAACAACTA	[48]
HPV16	NM	NM	[49]
HPV16	L1	see Supporting Information Table S2	[50]
HPV18	L1	see Supporting Information Table S2	[50]
HPV16	L1	30mer: FAM-ACAGAAAATGCTAGTGCTTATGCAGCAAAT	[51]
HPV18	L1	30mer: Rox-ACTGAAAGTTCCCATGCCGCCACGTCTAAT	[51]
HPVs16 and 18	NM	NM	[52]
HPV16	E6&E7	NM	[53]
HPVs16 and 18	L1	NM	[54]
HPV16	L1	1)21mer: AAAAATACTAACTTTAAGGAG 2)21mer: CCCCAGGAGGCACACTAGAAG 3)22mer: AAGATGGATCCCCTTAAAAAAT	[55]
HPV18	L1	1)21mer: GATGCTACCAAATTTAAGCAG 2)20mer: CCCCAACTACTAGTTTGGTG 3)21mer: ATAAGGATCCCTATGATAAGT	[55]
HPV16	E7	48mer: GAGGAGGATGAAATAGATGGTCCAGCTGGACAAGCAGAACCGGACAGA	[56]
HPV16	L1	30mer:GTGTGGATAATAGAGAATGTATATCTATGG	[57]
HPV18	L1	30mer: CTGAGGACGTTAGGGACAATGTGTCTGTAG	[57]
HPV16	NM	34mer: TATGTGCTGCCATATCTACTTCAGAAACTACATA	[58]
HPV18	NM	30mer: TGCTTCTACACAGTCTCCTGTACCTGGGCA	[58]
HPV16	L1	30mer: G1: HS-(Ch2)6 ACTGAAAATGCTAGTGCTTATGCAGCAAAT 30mer: G2: GTGTGGATAATAGAGAATGTATATCTATGG-(Ch2)6SH	[59]
HPV18	L1	30mer: G1: HS-(Ch2)6 ACTGAAAGTTCCCATGCCGCCACGTCTAAT 30mer: G2: CTGAGGACGTTAGGGACAATGTGTCTGTAG-(Ch2)6SH	[59]
HPV16 and 18	L1	NM	[60]

TABLE 4 *(Continued)*

HPV types	*Genes*	*Sequences (5′–3′)*	*Ref.*
HPV16	NM	19mer: CCTGCAGGTACCAATGGGG	[61]
HPV18	NM	20mer: TGAACACCCTGTCCTTTGTG	[61]
HPVs16 and 18	NM	NM	[62]
HPV16	NM	20mer: CATACACCTCCAGCACCTAA	[63]
HPV18	NM	20mer: TCTACACAGTGTCCTGTACC	[63]
HPV16	NM	19mer: GCTGCCATATCTATCAGAA	[64]
HPV18	NM	20mer: TTCTACACAGTCTCCTGTAC	[64]
HPV16 and 18	NM	NM	[65]
HPV16	E6	NM	[66]
HPV18	NM	Bis-PNA probe: 5′SH-(CH2)6-(Lys)3-TTTTCTTCCT -(egl)3-TCCTTCTTTT-Lys 20mer: DNA probe labeled with thiol 5′SH-(CH2) 6-TTTTCTTCCTCTGAGTCGCT-3′	[68]
HPV18	NM	Bis-PNA probe: 5′SH–(CH2)6–(Lys)3–TTTTCTTCCT–(egl)3–TCCTTCTTTT–Lys	[69]
HPV16 and 18	NM	NM	[70]
HPV16 and 18	NM	NM	[71]

[a]NM: not mentioned.

substrate, which will accumulate electrical charge in response to the applied mechanical stress. Moreover immobilization biotinylated HPV probes were preferred for the use of QCM biosensors for the detection of HPV viruses [84].

Fu et al. have constructed one of the first HPV genosensors based on QCM piezoelectric for the detection and identification of HPV types from pathologic biopsy samples. The strategy involved the adsorption of HPV oligonucleotides functionalized with disulfide groups atop the surface of a QCM disc. The system presented high sensibility (25 μM), which is comparable to the result obtained by the combination of PCR and dotblot technique [63].

Dell'Atti et al. developed a biosensor based on the long-term stable anchoring between biotin and streptavidin for the identification of HPV types. Their methodology allowed monitoring real-time hybridization by frequency changes, which resulted in HPV-type differentiation. They achieve the identification within a 50 nM detection limit of PCR-amplified short DNA strands [64].

Parsongdee et al. carried out the same research via immobilizing biotinylated probes for 11 high risk types of HPV. This technique showed good sensitivity up to 10^3 copies/μL from PCR products [65].

As an alternative to the previously described DNA biosensors, Mobley et al. used a different approach for the QCM piezoelectric technique; it is by the dissipation frequency monitoring (QCM-D) instead of the resonance frequency. Commonly applied in the fields of biophysics, biomaterials, cell adhesion, and drug discovery, this interfacial acoustic technique is a special type of QCM that serves to analyze the thickness of a film in a liquid environment [67].

2.11. Acoustic detection technologies

Surface acoustic wave (SAW) biosensors have been developed to detect biomarkers by using acoustic waves. A leaky surface acoustic wave (LSAW) biosensor was utilized for quantitative detection of HPV DNA. Bis-peptide nucleic acid (Bis-PNA) probe was grafted on the sensor to capture double stranded target DNA. Acoustic waves were confined on the microfabricated sensor surface to detect HPV target DNA hybridized on the surface and to quantify the presence of the target DNA by measuring phase shifts. This method had a LOD of 1.21 pM [68]. The signal of LSAW bis-PNA biosensor was increased using RecA protein-coated complementary single stranded detection DNA. A LOD of 1 pM for HPV18 was reached using this label [69].

3. Conclusion

The prevalence of HPV infections still remains high in both developed and developing countries despite tremendous efforts for HPV treatment and prevention. Currently, the Pap smear is the main approach for the detection of early lesions of CC. In conjunction with Pap smears, DNA testing of HPV, especially with the capability of detecting high-risk oncogenic subtypes 16 and 18, significantly increases the sensitivity, which facilitates clinical counseling and follow-up examinations. This therefore improves treatment outcomes. However, the proper diagnosis of HPV infections still remains essential for the prevention of CC.

According to the rapid growth in technological improvements for the development of simple, cost-effective, and accurate rapid diagnostic tests, we highlight the fluorescent spectroscopy, EIS and QCM.

The ability to rapidly regenerate the substrate after a diagnosed sample is of great importance and affects directly the cost of the methodology. In this regard, QCM and nitrocellulose substrates have managed to obtain more than 10 diagnoses without losing sensibility. On the other hand evaluating the performance of the biosensors by facing real samples, such as blood and other body fluids, will verify the real potential of these new molecular methods to confirm the clinical diagnosis of HPV.

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