

used in the above-mentioned detection methods is not acceptable to females, especially unmarried females. This greatly reduces the willingness of females to take the HPV testing, thus limits the HPV test participation rate. Meanwhile, males can also carry HPV, so detection of HPV in male population has a positive effect on preventing the occurrence of cervical cancer in the female population. However, in the current clinical practice, there are few HPV tests for men, and the common sampling method for men (i.e., urethral swabs) is very painful. Thus, there is still a great need for new HPV test methods that are less invasive, easy and cheap to perform, without compromising accuracy, sensitivity, and specificity. The present disclosure provides HPV detection methods that use urine samples for HPV DNA detection, so that the sample collection can be achieved in a non-invasive, painless, quick and convenient way. The present disclosure also provides a full set of compositions and methods for urine DNA extraction and purification, and for the detection of fourteen high-risk HPV subtypes and at least two low-risk HPV subtypes. The invention described herein not only increases the participation rate of female population women in HPV testing, but is also very suitable for HPV testing in the male population. Thus, the invention has great social significance for the prevention of HPV related conditions, such as cervical cancer.

SUMMARY

[010] The present disclosure provides primers and probes related to HPVs. In some examples, these primers and probes can be used for detecting and/or identifying a genotype of a human papillomavirus (HPV). In one aspect, provided is a combination of primers and probes comprises one or more groups of primer and probe selected from the groups consisting of:

(1) a forward primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 1, a reverse primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 2, and a probe comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 37;

(2) a forward primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 3, a reverse primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 4, and a probe comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 38;

(3) a forward primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 5, a reverse primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 6, and a probe comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 39;

(12) a forward primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 23, a reverse primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 24, and a probe comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 40;

(13) a forward primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 25, a reverse primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 26, and a probe comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 41;

(14) a forward primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 27, a reverse primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 28, and a probe comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 40;

(15) a forward primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 29, a reverse primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 30, and a probe comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 40

(16) a forward primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 33, a reverse primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 34, and a probe comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 44;

(17) a forward primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 35, a reverse primer comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 36, and a probe comprising the polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to the sequence of SEQ ID NO: 45, and any combination thereof.

[011] In some examples, the combination of primer and probe comprises the primers and probes in (1) and (2); the primers and probes in (3), (4), (5), and (11); the primers and probes in (6), (12), (14), and (15); the primers and probes in (7), (10), and (13); and/or the primers and probes in (8) and (9).

[012] In some examples, the combination of primer and probe comprises: at least one group of primers and probes in (1) and (2) and at least one group of primers and probes in (3), (4), (5), and (11).

[013] In some examples, the combination of primer and probe comprises: at least one group of primers and probes in (1) and (2) and at least one group of primers and probes in (6), (12), (14), and (15).

[026] In some examples, the combination of primer and probe described herein further comprises the group of primers and probes in (16) and/or (17).

[027] In some examples, the combination of primer and probe consists of the group of primers and probes in (1) to (15), or consists of the group of primers and probes in (1) to (17).

[028] In some examples, the combination of primer and probe further comprises a group of primer and probe for a reference control gene. In some examples, the reference control gene is β -Actin (ACTB), wherein the primers for the ACTB gene are SEQ ID NOs: 31 and 32, and the probe for the ACTB gene is SEQ ID NO: 43.

[029] In some examples, each probe as described herein has a fluorescent dye attached to 5' end thereof. In some examples, the fluorescent dye is selected from the group consisting of FAM (fluorescein), TET, JOE, VIC (2'-chloro-7'-phenyl-1,4-dichloro-6-carboxy-fluorescein), HEX (Hexachloro-fluorescein), ROX, TAMRA, Cy3, cy3.5, Cy5, Cy5.5, OregonGreenTM, CALRedTM, Red640, Texas Red, LighterCycler®Cyan500, LighterCycler®, Red610, a biotin binding material, Alexa 647, Alexa 555, 5-(2-aminoethyl)amino-1-naphthalene sulfonic acid (EDANS), tetramethyl rhodamine (TMR), tetramethyl rhodamine isocyanate (TMRITC), fluorescein isocyanate (FITC), and χ -rhodamine.

[030] In some examples, the fluorescent dye on the probes in (3), (4), (5), and/or (11) is the same dye or different dyes having about the same emission wavelength. In some examples, the fluorescent dye on the probes in (6), (12), (14), and/or (15) is the same dye, or different dyes having about the same emission wavelength. In some examples, the fluorescent dye on the probes in (7), (10), and/or (13) is the same dye, or different dyes having about the same emission wavelength. In some examples, the fluorescent dye on the probes in (8) and (9) is the same dye, or different dyes having about the same emission wavelength. In some examples, the fluorescent dye on the probes in (3), (4), (5), and/or (11) are different dyes having about the same or different emission wavelengths. In some examples, the fluorescent dye on the probes in (6), (12), (14), and/or (15) are different dyes having about the same or different emission wavelengths. In some examples, the fluorescent dye on the probes in (7), (10), and/or (13) are different dyes having about the same or different emission wavelengths. In some examples, the fluorescent dye on the probes in (8) and (9) are different dyes having about the same or different emission wavelengths.

[031] In some examples, each probe has a fluorescent dye attached to 5' end thereof, and wherein:

- (i) the probe in (1) has a first dye;
 - (ii) the probe in (2) has a second dye;
 - (iii) the probes in (3), (4), (5), and (11) have a third dye;
 - (iv) the probes in (6), (12), (14) and (15) have a fourth dye;
 - (v) the probes in (7), (10), and (13) have a fifth dye; and
 - (vi) the probes in (8) and (9) have a sixth dye, wherein
- the dye in (iii) to (vi) are the same dye, but different from the dye in (i) and (ii).

[032] In some examples, each probe has a fluorescent dye attached to 5' end thereof, and wherein:

- (i) the probe in (1) has a first dye;
- (ii) the probe in (2) has a second dye;
- (iii) the probes in (3), (4), (5), and (11) have a third dye;
- (iv) the probes in (6), (12), (14) and (15) have a fourth dye;
- (v) the probes in (7), (10), and (13) have a fifth dye;
- (vi) the probes in (8) and (9) have a sixth dye,
- (vii) the probe in (16) has a seventh dye; and
- (viii) the probe in (17) has an eighth dye; wherein

the dye in (iii) to (vi) are the same dye, but different from the dye in (i), (ii), (vii) and (viii).

[033] In some examples, the combination of primers and probes further comprises a group of primers and probe for a reference control gene, wherein the probe for the reference control gene also has a fluorescent dye attached to 5' end thereof, and the fluorescent dye for the control gene is different from other dyes in the combination.

[034] In some examples, each probe described herein has a fluorescent quencher attached to 3' end thereof. In some examples, the fluorescent quencher is selected from the group consisting of DDQ-I, DDQ-II, Dabcyl, Eclipse, Iowa Black FQ, Iowa Black RQ, BHQ-1, BHQ-2, BHQ-3, QSY-7, QSY-9, and QSY-21.

[035] In some examples, the probe in (1) comprises a Cy5 fluorescent dye attached to its 5' end, and a BHQ-2 fluorescent quencher attached to its 3' end; the probe in (2) comprises a FAM fluorescent dye attached to its 5' end, and a BHQ-1 fluorescent quencher attached to its 3' end; the probes in (3) to (15) comprise a VIC fluorescent dye attached to their 5' ends, and a MGBNFQ fluorescent quencher attached to their 3' ends; and the probes in (16) and (17) comprises a FAM fluorescent dye attached to their 5' ends, and a BHQ-1 fluorescent quencher attached to their 3' ends.

[036] In some examples, the probe in (1) comprises a Cy5 fluorescent dye attached to its 5' end, and a BHQ-2 fluorescent quencher attached to its 3' end; the probe in (2) comprises a FAM fluorescent dye attached to its 5' end, and a BHQ-1 fluorescent quencher attached to its 3' end; the probes in (3) to (15) comprise a VIC fluorescent dye attached to their 5' ends, and a MGBNFQ fluorescent quencher attached to their 3' ends; the probes in (16) and (17) comprises a FAM fluorescent dye attached to their 5' ends, and a BHQ-1 fluorescent quencher attached to their 3' ends, and the probe for the reference control gene comprises a ROX fluorescent dye attached to its 5' end, and a BHQ-2 fluorescent quencher attached to its 3' end.

[037] In another aspect, the present disclosure also provides compositions comprising the combination of primer and probe as described herein.

[038] In another aspect, the present disclosure refers to a DNA chip for testing HPV genotypes, comprising one or more polynucleotides selected from SEQ ID NOs: 1 to 45. The present disclosure also provides DNA chips for detecting and/or genotyping HPVs. In some examples, the DNA chips comprise one or more polynucleotide sequence selected from SEQ ID NOs: 1 to 45.

[039] In another aspect, the present disclosure refers to a kit for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample, comprising a combination of primer and probe as described herein. The present disclosure also provides kits for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample, comprising a combination of primer and probe as described herein, PCR buffer, dNTP, MgCl₂, PCR additive, Taq enzyme, and negative control and positive control as quality control.

[040] In some examples, kits used to detect and/or identify the human papillomavirus (HPV) genotype in biological samples include: HPV qPCR mixture, Taq enzyme, negative control, and positive control.

[041] In some examples, the HPV qPCR mixture comprises a primer and probe combination as described herein, a PCR buffer, dNTP, MgCl₂, PCR additives, and deionized water. In some examples, primer and probe combinations include all primer probe combinations in (1) ~ (15) and reference control gene primers and probes (SEQ ID NOs: 31, 32, and 43) with primer concentrations of 0.1μM~1.2μM and probe concentrations of 1/5 ~1 times the corresponding primer concentrations. In some examples, the PCR buffer contains approximately 10~30mM Tris-HCL buffer and approximately 30~70mM KCL, preferably with a Tris-HCL buffer concentration of approximately 20.5mM and a preferred KCL concentration of approximately 51mM. In some examples, dNTP concentration is 0.15mM~0.3mM, preferably approximately 0.25mM. In some examples, the concentration of MgCl₂ is 1.5mM~4mM, preferably approximately 3.0mM. In some examples, PCR additives contains about 0.1~1mg/ml BSA, 0.2%~2% (V/V) formamide, 0.2mM~2mM spermine, 10 mM~30 mM tetramethyl ammonium chloride, 0.01 mM ~ 0.1 mM dithiothreitol (DTT), 0.2% ~ 2% 2-pyrrolidone, preferred PCR additives contains about 0.64 mg/ml of BSA, about 1% (V/V) formamide, about 1 mM spermine, about 21 mM tetramethyl ammonium chloride, about 0.064 mM DTT, and about 1% (V/V) 2 - pyrrolidone.

[042] In some examples, the Taq enzyme contains a Platinum™ Taq DNA Polymerase (Invitrogen™, 10966018) with a concentration of 1~6U/μl, and preferably a Platinum™ Taq DNA Polymerase (Invitrogen™, 10966018) with a concentration of 4U/μl.

[043] In some examples, the negative control is obtained from urine or its DNA diluted 1-1000 times in adults with high risk HPV DNA negative, preferably by approximately 100 times.

[044] In some examples, the positive control is a plasmid containing the high risk HPV L1 gene with a final concentration of 10~10⁵copies/μl prepared using the negative control as diluent. The high risk HPV L1 genotype may be one or more of the 14 high risk HPV genotypes described in the disclosure. Preferably, the positive control contains L1 plasmids of HPV16, HPV18 and HPV45, and their final concentrations are 10³ copies/μl.

[045] The disclosure also provides a kit for detecting and / or identifying the (HPV) genotype of human papillomavirus in biological samples and capable of guiding and evaluating the efficacy of HPV vaccine, including a combination of primers and probes, PCR buffer, dNTP, MgCl₂, PCR additive, Taq enzyme, and negative control and positive control as quality control.

[046] In some examples, the kit used to detect and/or identify human papilloma virus (HPV) genotypes in biological sample for HPV vaccine guidelines and efficacy evaluation includes HPV qPCR mixture I, HPV qPCR mixture II, Taq enzyme, as well as negative control and positive control as quality control.

[047] In some examples, HPV qPCR mixture I includes primer and probe combinations mentioned in the present disclosure, the PCR buffer, dNTP, MgCl₂, PCR additives, and deionized water. In some examples, primer and probe combinations include all primer probe combinations in (1), (2), (5), (6), (8), (10), (12), (13), (14), and (15), as well as reference control gene primers and probes (SEQ ID NOs: 31, 32, and 43), with primer concentrations ranging from 0.2μM to 1.2μM and probe concentrations ranging from 1/5 to 1 times the corresponding primer concentrations. In some examples, the PCR buffer contains approximately 10~30mM Tris-HCl buffer and approximately 30~70mM KCl, preferably with a Tris-HCl buffer concentration of approximately 25.6mM and a preferred KCl concentration of approximately 64.1mM. In some examples, dNTP concentration is 0.15mM~0.3mM, preferably approximately 0.25mM. In some examples, the concentration of MgCl₂ is 1.5mm~4mM, preferably approximately 3.0mM. In some examples, the PCR additives contain approximately 0.1~1mg/ml BSA, 0.2%~2% (V/V) formamide, 0.2~2mM spermidine, 10mM~30mM tetramethylammonium chloride, 0.01mM~0.1mM DTT, and 0.2%~2% 2-pyrrolidone. Preferably, the PCR additives contains approximately 0.64mg/ml BSA, approximately 1% (V/V) formamide, approximately 1mM spermidine, approximately 21mM tetramethylammonium chloride, approximately 0.064mM DTT, approximately 1% (V/V) 2-pyrrolidone.

[048] In some examples, HPV qPCR mixture II including prime and probe combinations mentioned in the present disclosure, PCR buffer, dNTP, MgCl₂, PCR additive, and deionized water. In some examples, primer and probe combinations including all primer probe combinations in (3), (4), (7), (9), (11), (16), (17), and reference control gene primers and probes (SEQ ID NOs: 31, 32, and 43), with primer concentration of 0.2 μM ~ 1.2 μM and probe concentrations ranging from 1/5 to 1 times the corresponding primer concentrations. In some examples, the PCR buffer contains approximately 10~30 mM Tris-HCl buffer and approximately 30~70 mM KCl, preferably with a Tris-HCl buffer concentration of approximately 25.6 mM, preferably with a KCl concentration of approximately 64.1mM. In some examples, the dNTP concentration is 0.15 mM ~3mM, preferably with a dNTP concentration of approximately 0.25 mM. In some examples, the concentration of MgCl₂ is 1.5 mm~4mM, preferably approximately 3.0 mM. In some examples, the PCR additive contains approximately 0.1~1 mg/ml BSA, 0.2%~2% (V/V) formamide, 0.2~2 mM spermidine, 10 mM~30mM tetramethylammonium chloride, 0.01 mM~0.1mM DTT, and 0.2%~2% 2-pyrrolidone. Preferably, PCR additives contain approximately 0.64 mg/ml BSA, approximately 1% (V/V) formamide, approximately 1 mM spermidine, approximately 21 mM

tetramethylammonium chloride, approximately 0.064 mM DTT, and approximately 1% (V/V) 2-pyrrolidone.

[049] In some examples, the biological sample is collected from a human subject. In some embodiments, the biological sample comprises urine of the human subject.

[050] In some examples, the kit further comprises reagents for isolating DNA from the biological sample.

[051] In some examples, the reagents for isolating DNA from the biological sample comprises: a lysis solution, magnetic nanoparticles, a protease, a first washing buffer, a second washing buffer, an elution buffer, or any combination thereof.

[052] In some examples, the lysis solution comprises guanidinium isothiocyanate, Triton X 100, Tris-HCl, EDTA, and isopropanol. In some examples, the guanidinium isothiocyanate has a concentration of about 2 to 6 M. In some examples, the Triton X-100 has a concentration of about 1 to 5%. In some examples, the Tris-HCl has a concentration of about 20 to 50 mM, wherein the lysis solution has a pH of about 6.5. In some examples, the EDTA has a concentration of about 10 to 50 mM. In some examples, isopropanol is added after all other components are mixed together. In some examples, the isopropanol has a dosage of about 50% to 200% (v/v).

[053] In some examples, the lysis solution comprises guanidinium isothiocyanate, Triton X 100, Tris-HCl, EDTA, and isopropanol. In some examples, the guanidinium isothiocyanate has a concentration of about 1 to 2 M. In some examples, the Triton X-100 has a concentration of about 1 to 2%. In some examples, the Tris-HCl has a concentration of about 5 to 10 mM. In some examples, the lysis solution has a pH of about 6-7. In some examples, the EDTA has a concentration of about 3 to 5 mM. In some examples, the isopropanol has a volume of about 50% to 80% (v/v) of the lysis solution.

[054] In some examples, the magnetic nanoparticles have an inner core layer and an outer shell layer, and the inner core layer is composed of core-shell type magnetic nanoparticles, wherein the outer shell layer is composed of SiO₂. In some examples, the magnetic nanoparticles have a diameter of about 100 to 1000 nm, and a concentration of about 50 mg/ml.

[055] In some examples, the first washing buffer comprises guanidinium isothiocyanate, Tris-HCl, NaCl, and ethanol. In some examples, the guanidinium isothiocyanate has a concentration of about 50 mM. In some examples, the Tris-HCl has a concentration of about 20 to 50 mM. In some examples, the first washing buffer has a pH of about 5.0. In some examples, the NaCl has a concentration of about 50 to 200 mM. In some examples, the ethanol has concentration of about 40% to 60% (v/v).

[056] In some examples, the second washing buffer comprises Tris-HCl and ethanol. In some examples, the Tri-HCl in the second washing buffer has a concentration of about 10 to 50 mM, and the second washing buffer has a pH of about 6.0. In some examples, the ethanol has concentration of about 70% to 80% (v/v).

[057] In some examples, the elution buffer is a Tris-EDTA buffer having a pH of about 8.0.

[058] In some examples, the protease is protease K. In some examples, the protease K has a concentration of about 10 to 20 mg/ml.

[059] The present disclosure further provides methods for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample obtained from a subject in need thereof. In some examples, the methods comprise: (a) obtaining DNA from the biological sample; (b) amplifying the DNA by a fluorescent PCR using a combination of primer and probe as described herein; and (c) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR.

[060] The present disclosure further provides methods for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample obtained from a subject in need thereof. In some examples, the methods comprise (a) obtaining DNA from the biological sample; (b) amplifying the DNA by a fluorescent PCR using the kit as described herein; and (c) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR.

[061] In another aspect, the present disclosure refers to a method for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample that was obtained from a subject in need thereof, comprising: (a) obtaining DNA from the biological sample; (b) amplifying the DNA by a fluorescent PCR using a combination of primer and probe as disclosed herein; and (c) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR. The present disclosure further provides methods for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample obtained from a subject in need thereof. In some examples, the methods comprise: (a) extracting DNA from the biological sample and amplifying the DNA by a fluorescent PCR using the kit as described herein; and (b) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR. In another aspect, the present disclosure refers to a method for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample that was obtained from a subject in need thereof, comprising: (a) obtaining DNA from the biological sample; (b) amplifying the DNA by a fluorescent PCR using the kit as disclosed herein; and (c) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR.

[062] In some examples, the methods comprise detecting and/or identifying the presence or absence of DNA of at least one HPV subtypes in the biological sample. In some examples, the methods comprise detecting and/or identifying the presence or absence of DNA of 14 high-risk HPV subtypes in the biological sample through a single test tube, wherein the high-risk HPV subtypes are HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, and HPV68.

[063] In some examples, the methods comprise detecting and/or identifying the presence or absence of DNA of 14 high-risk HPV subtypes in the biological sample through a single test tube, wherein the high-risk HPV subtypes are HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, and HPV68.

[064] In some examples, the methods comprise detecting and/or identifying the presence or absence of DNA of 14 high-risk HPV subtypes and at least one low risk HPV subtype in the biological sample through two test tubes, wherein, high risk HPV subtypes are HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, and HPV68, and low-risk HPV subtypes are HPV6 and HPV11. The presence or absence of 7 high-risk HPV subtypes HPV16, HPV18, HPV35, HPV39, HPV51, HPV56, HPV59, HPV66 and HPV68 in the biological samples are detected and/or identified in one test tube, whereas the presence or absence of 5 high-risk HPV subtypes HPV31, HPV33, HPV45, HPV52 and HPV68, and the presence or absence of 2 low-risk HPV subtypes, HPV6 and/or HPV11, in the biological samples are detected and/or identified in the other test tube.

[065] In some examples, the biological sample is a smear of the cervix, a fresh tissue sample, a fixed tissue sample, a cross-sectional specimen of a tissue sample, a urine sample, a sample comprising exfoliated cells, a peripheral blood sample, a penis swab, or other body fluid. In some examples, the sample is a urine sample.

[066] In another aspect, the present disclosure refers to the use of a combination of primer and probe as disclosed herein for detecting and/or identifying the genotype of a human papillomavirus (HPV). The present disclosure further provides use of a combination of primer and probe or a kit as described herein for detecting and/or identifying the genotype of a human papillomavirus (HPV).

[067] In another aspect, the present disclosure refers to the use of a pharmaceutical composition and/or a medical procedure in the manufacture of a medicament for treating a condition associated with human papillomavirus (HPV) in a subject in need thereof, comprising: (1) detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample obtained from a subject in need thereof, comprising: (a) amplifying DNA extracted from the biological sample by a fluorescent PCR using a combination of primers and probes as disclosed herein; and (b) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR, and (2) that the subject is to be treated with the pharmaceutical composition and/or a medical procedure according to the results in step (1). The present disclosure further provides methods for treating a condition associated with human papillomavirus (HPV) in a subject in need thereof. In some examples, the methods comprise: (1) detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample obtained from a subject in need thereof. In some examples, the step (1) comprises: (a) amplifying DNA extracted from the biological sample by a fluorescent PCR using a combination of primers and probes as described herein; and (b) determining the presence or absence of DNA of one or more HPV subtype in the biological

sample based on the results of the fluorescent PCR. In some examples, the methods further comprise (2) treating the subject with a pharmaceutical composition and/or a medical procedure according to the results in step (1).

[068] In some examples, the condition is a precancerous lesions caused by HPV. In some examples, the pharmaceutical composition comprises an antiviral agent.

[069] In another aspect, the present disclosure refers to the use of a composition targeting selected HPVs in the manufacture of a medicament for vaccinating a human subject in need thereof, comprising: (1) detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample obtained from the human subject in need thereof before and/or after the human subject is vaccinated, comprising: (a) amplifying DNA extracted from the biological sample by a fluorescent PCR using a combination of primers and probes as disclosed herein; and (b) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR, and (2) that the subject is to be vaccinated with the composition targeting selected HPVs based on the results in step (1). The present disclosure further provides methods for vaccinating a human subject in need thereof. In some examples, the methods comprise (1) detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample obtained from the human subject in need thereof before and/or after the human subject is vaccinated. In some examples, the step (1) comprises (a) amplifying DNA extracted from the biological sample by a fluorescent PCR using a combination of primers and probes as described herein; and (b) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR. In some examples, the methods further comprise (2) vaccinating the subject with a composition targeting selected HPVs based on the results in step (1).

[070] In another aspect, present disclosure refers to an *in vitro* method for evaluating vaccination efficacy in a human subject in need thereof, comprising: (1) detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample obtained from the human subject in need thereof after, or before and after the human subject is vaccinated, comprising: (a) amplifying DNA extracted from the biological sample by a fluorescent PCR using a combination of primers and probes of any one of claims 1-31, after, or before and after the human subject is vaccinated; and (b) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR; and; (2) determining vaccination efficacy based on the results in step (1)

of a subject that was vaccinated with a composition targeting selected HPVs. The present disclosure further provides methods for evaluating vaccination efficacy in a human subject in need thereof. In some examples, the methods comprise: (1) detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample obtained from the human subject in need thereof after, or before and after the human subject is vaccinated. In some examples, the step (1) comprises: (a) amplifying DNA extracted from the biological sample by a fluorescent PCR using a combination of primers and probes as described herein after, or before and after the human subject is vaccinated; and (b) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR after, or before and after the human subject is vaccinated. In some examples, the methods further comprise (2) vaccinating the subject with a composition targeting selected HPVs; and (3) determining vaccination efficacy based on the results in step (1).

[071] In another aspect, the present disclosure refers to a kit for the detection and / or recognition of human papillomavirus (HPV) genotypes in biological samples and for the guidance and efficacy evaluation of HPV vaccine, comprising the combination of primer and probe as disclosed herein. In another aspect, the present disclosure refers to a primer, comprising an oligonucleotide sequence having at least 85%, 90%, 95%, or 100% identity to any one of SEQ ID NOs: 1-36, wherein the primer has less than 100, 90, 80, 70, 60, 50, 40, 30, or 20 nucleotides. In yet another aspect, the present disclosure refers to a pair of primers, comprising a forward primer and a reverse primer, each having at least 85%, 90%, 95%, or 100% identity to any one of SEQ ID NOs: 1-36 and each having less than 100, 90, 80, 70, 60, 50, 40, 30, or 20 nucleotides, wherein the forward and reverse primers are selected from the groups consisting of: SEQ ID NOs: 1-2, 3-4, 5-6, 7-8, 9-10, 11-12, 13-14, 15-16, 17-18, 19-20, 21-22, 23-24, 25-26, 27-28, 29-30, 31-32, 33-34, 35-36, and any combination thereof. The present disclosure further provides primers or pairs of primers related to HPVs. In some examples, the primers comprises a oligonucleotide sequence having at least 85%, 90%, 95%, or 100% identity to any one of SEQ ID NOs: 1-36. In some examples, the primers have less than 100, 90, 80, 70, 60, 50, 40, 30, or 20 nucleotides. In some examples, the pairs of primers comprise a forward primer and a reverse primer. In some examples, each primer in the pairs of primers have at least 85%, 90%, 95%, or 100% identity to any one of SEQ ID NOs: 1-36. In some examples, each primer in the pairs of primers have less than 100, 90, 80, 70, 60, 50, 40, 30, or 20 nucleotides. In some examples, the forward and reverse primers in the pairs of primers are selected from the groups consisting of: SEQ ID NOs: 1-2, 3-4, 5-6, 7-8, 9-10, 11-12, 13-

14, 15-16, 17-18, 19-20, 21-22, 23-24, 25-26, 27-28, 29-30, 31-32, 33-34, 35-36, and any combination thereof.

[072] The present disclosure further provides probes related to HPVs. In another aspect, the present disclosure refers to a probe, comprising a fluorescent dye and an oligonucleotide, wherein the oligonucleotide comprises a sequence having at least 85%, 90%, 95%, or 100% identity to any one of SEQ ID NOs: 37-45, wherein the oligonucleotide has less than 100, 90, 80, 70, 60, 50, 40, 30, or 20 nucleotides. In some examples, the probes comprise a fluorescent dye and an oligonucleotide. In some examples, the oligonucleotide comprises a sequence having at least 85%, 90%, 95%, or 100% identity to any one of SEQ ID NOs: 37-45. In some examples, the oligonucleotide has less than 100, 90, 80, 70, 60, 50, 40, 30, or 20 nucleotides.

[073] In some example, the fluorescent dye is attached to 5' end of the probe. In some examples, the fluorescent dye is selected from the group consisting of FAM (fluorescein), TET, JOE, VIC, HEX, ROX, TAMRA, Cy3, cy3.5, Cy5, Cy5.5, OregonGreenTM, CALRedTM, Red640, Texas Red, LighterCycler®Cyan500, LighterCycler®, Red610, a biotin binding material, Alexa 647, Alexa 555, 5-(2-aminoethyl)amino-1-naphthalene sulfonic acid (EDANS), tetramethyl rhodamine (TMR), tetramethyl rhodamine isocyanate (TMRITC), fluorescein isocyanate (FITC), and χ -rhodamine.

[074] In some examples, the probes further comprise comprising a fluorescent quencher. In some examples, the fluorescent quencher is attached to 3' end of the probe. In some examples, the fluorescent quencher is selected from the group consisting of DDQ-I, DDQ-II, Dabcyl, Eclipse, Iowa Black FQ, Iowa Black RQ, BHQ-1, BHQ-2, BHQ-3, QSY-7, QSY-9, and QSY-21.

[075] In yet another aspect, the present disclosure refers to a kit for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample, comprising one or more primers as disclosed herein, and a one or more probes as disclosed herein. The present disclosure further provides kits for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample. In some examples, the kits comprise one or more primers as described herein, and one or more probes as described herein. In some examples, the kits comprise at least 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, or 36 primers as described herein. In some examples, the kits comprise at least 2, 3, 4, 5, 6, 7, 8, or 9 probes as described herein. In some examples, the kits further comprise a lysis solution,

magnetic nanoparticles, a protease, a first washing buffer, a second washing buffer, an elution buffer, or any combination thereof.

[076] In yet another aspect, the present disclosure refers to a method for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a urine sample obtained from a subject in need thereof, comprising: (a) obtaining DNA from the urine sample; (b) amplifying the DNA by a fluorescent PCR using one or more primers as disclosed herein, and a one or more probes as disclosed herein; and (c) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR. The present disclosure further provides methods for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a urine sample obtained from a subject in need thereof. In some examples, the methods comprise: (a) obtaining DNA from the urine sample; (b) amplifying the DNA by a fluorescent PCR using one or more primers as described herein, and one or more probes as described herein; and (c) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR.

BRIEF DESCRIPTION OF THE FIGURES

[077] **Figure 1** depicts a phylogenetic tree showing similarity of L1 genes in 12 high-risk HPV subtypes.

[078] **Figure 2** depicts alignment of L1 genes in 12 high-risk HPV subtypes and positions of probes (P1 to P4) that are designed for recognition of subgroups of these HPVs (HPV31, 33, 35, 58-P1; HPV39, 59, 68-P2; HPV45, 56, 66-P3; and HPV51, 52-P4).

[079] **Figure 3A to Figure 3Q** depict the amplification curves using primers and probes specific to 14 high-risk HPVs and 2 low-risk HPVs, respectively (HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, and HPV68, HPV6 and HPV11) and the amplification curve of control gene β -actin in a multiplex PCR. Figures having more than one curves indicate that the same sample has been tested several times.

[080] **Figure 4A to Figure 4D** depict the amplification curves using primers and probes specific to HPV16, HPV18, HPV33, and HPV6 in a multiplex PCR or in single-plex PCRs.

[081] **Figure 5** depicts HPV16 gene amplification curves in multiplex PCRs using the preferred set of primers and probe (SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 37) or

using a candidate set of primers and probe (SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48).

DETAILED DESCRIPTION OF THE INVENTION

Primers, Probes, and Multiplex PCR for HPV subtype detection and/or genotyping

[082] The present disclosure provides compositions and methods for HPV detection and genotyping.

[083] Among HPV detection methods utilizing real-time PCR, E6/E7, E1 and L1 genes of HPV virus are commonly used as detection targets. Since HPV genotypes are distinguished by its L1 gene, the advantage of using L1 gene as the target to design primer and probe is that

CLAIMS

1. A combination of primer and probe for detecting and/or identifying a genotype of a human papillomavirus (HPV), wherein the combination of primer and probe comprises one or more groups of primer and probe selected from the groups consisting of:

(1) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 1, a reverse primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 2, and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 37;

(2) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 3, a reverse primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 4, and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 38;

(3) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 5, a reverse primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 6, and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 39;

(4) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 7, a reverse primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 8, and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 39;

(5) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 9, a reverse primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 10, and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 39;

(6) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 11, a reverse primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 12,

and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 40;

(7) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 13, a reverse primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 14, and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 41;

(8) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 15, a reverse primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 16, and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 42;

(9) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 17, a reverse primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 18, and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 42;

(10) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 19, a reverse primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 20, and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 41;

(11) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 21, a reverse primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 22, and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 39;

(12) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 23, a reverse primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 24, and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 40;

(13) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 25, a reverse primer comprising a

polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 26, and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 41;

(14) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 27, a reverse primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 28, and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 40;

(15) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 29, a reverse primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 30, and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 40;

(16) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 33, a reverse primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 34, and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 44;

(17) a forward primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 35, a reverse primer comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 36, and a probe comprising a polynucleotide sequence having at least 85%, 90%, 95%, or 100% identity to a sequence of SEQ ID NO: 45; and any combination thereof.

2. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises the primers and probes in (1) and (2).

3. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises the primers and probes in (3), (4), (5), and (11).

4. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises the primers and probes in (6), (12), (14) and (15).

5. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises the primers and probes in (7), (10), and (13).
6. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises the primers and probes in (8) and (9).
7. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises: at least one group of primers and probes in (1) and (2) and at least one group of primers and probes in (3), (4), (5), and (11).
8. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises: at least one group of primers and probes in (1) and (2) and at least one group of primers and probes in (6), (12), (14) and (15).
9. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises: at least one group of primers and probes in (1) and (2) and at least one group of primers and probes in (7), (10), and (13).
10. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises: at least one group of primers and probes in (1) and (2) and at least one group of primers and probes in (8) and (9).
11. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises: at least one group of primers and probes in (1) and (2), at least one group of primers and probes in (3), (4), (5), and (11), and at least one group of primers and probes in (6), (12), (14) and (15).
12. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises: at least one group of primers and probes in (1) and (2), at least one group of primers and probes in (3), (4), (5), and (11), and at least one group of primers and probes in (7), (10), and (13).
13. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises: at least one group of primers and probes in (1) and (2), at least one group of

primers and probes in (3), (4), (5), and (11), and at least one group of primers and probes in (8) and (9).

14. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises: at least one group of primers and probes in (1) and (2), at least one group of primers and probes in (6), (12), (14) and (15), and at least one group of primers and probes in (7), (10), and (13).

15. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises: at least one group of primers and probes in (1) and (2), at least one group of primers and probes in (6), (12), (14) and (15), and at least one group of primers and probes in (8) and (9).

16. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises: at least one group of primers and probes in (1) and (2), at least one group of primers and probes in (7), (10), and (13), and at least one group of primers and probes in (8) and (9).

17. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises: at least one group of primers and probes in (1) and (2), at least one group of primers and probes in (3), (4), (5), and (11), at least one group of primers and probes in (6), (12), (14) and (15), and at least one group of primers and probes in (7), (10), and (13).

18. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises: at least one group of primers and probes in (1) and (2), at least one group of primers and probes in (3), (4), (5), and (11), at least one group of primers and probes in (6), (12), (14) and (15), and at least one group of primers and probes in (8) and (9).

19. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises: at least one group of primers and probes in (1) and (2), at least one group of primers and probes in (3), (4), (5), and (11), and at least one group of primers and probes in (7), (10), and (13), and at least one group of primers and probes in (8) and (9).

20. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises: at least one group of primers and probes in (1) and (2), at least one group of primers and probes in (6), (12), (14) and (15), and at least one group of primers and probes in (7), (10), and (13), and at least one group of primers and probes in (8) and (9).

21. The combination of primer and probe of any one of claim 2 to 20, wherein the combination of primer and probe further comprises the group of primers and probes in (16) and/or (17).

22. The combination of primer and probe of claim 1, wherein the combination of primer and probe consists of the group of primers and probes in (1) to (15).

23. The combination of primer and probe of claim 1, wherein the combination of primer and probe consists of the group of primers and probes in (1) to (17).

24. The combination of primer and probe of any one of claim 1 to 23, wherein the combination of primer and probe further comprises a group of primer and probe for a reference control gene.

25. The combination of primer and probe of claim 24, wherein the reference control gene is β -Actin (ACTB), wherein the primers for the ACTB gene are SEQ ID NOs: 31 and 32, and the probe for the ACTB gene is SEQ ID NO: 43.

26. The combination of primer and probe of any one of claim 1 to 25, wherein each probe has a fluorescent dye attached to 5' end thereof.

27. The combination of primer and probe of claim 26, wherein the fluorescent dye is selected from the group consisting of FAM (fluorescein), TET, JOE, VIC, HEX, ROX, TAMRA, Cy3, cy3.5, Cy5, Cy5.5, OregonGreenTM, CALRedTM, Red640, Texas Red, LighterCycler@Cyan500, LighterCycler®, Red610, a biotin binding material, Alexa 647, Alexa 555, 5-(2-aminoethyl)amino-1-naphthalene sulfonic acid (EDANS), tetramethyl rhodamine (TMR), tetramethyl rhodamine isocyanate (TMRITC), fluorescein isocyanate (FITC), and χ -rhodamine.

28. The combination of primer and probe of any one of claim 1 to 23, wherein each probe has a fluorescent quencher attached to 3' end thereof.

29. The combination of primer and probe of claim 28, wherein the fluorescent quencher is selected from the group consisting of DDQ-I, DDQ-II, Dabcyl, Eclipse, Iowa Black FQ, Iowa Black RQ, BHQ-1, BHQ-2, BHQ-3, QSY-7, QSY-9, and QSY-21.

30. The combination of primer and probe of claim 29, wherein:

the probe in (1) comprises a Cy5 fluorescent dye attached to its 5' end, and a BHQ-2 fluorescent quencher attached to its 3' end;

the probe in (2) comprises a FAM fluorescent dye attached to its 5' end, and a BHQ-1 fluorescent quencher attached to its 3' end;

the probes in (3) to (15) comprise a VIC fluorescent dye attached to their 5' ends, and a MGBNFQ fluorescent quencher attached to their 3' ends; and

the probes in (16) and (17) comprises a FAM fluorescent dye attached to their 5' ends, and a BHQ-1 fluorescent quencher attached to their 3' ends.

31. The combination of primer and probe of claim 29, wherein:

the probe in (1) comprises a Cy5 fluorescent dye attached to its 5' end, and a BHQ-2 fluorescent quencher attached to its 3' end;

the probe in (2) comprises a FAM fluorescent dye attached to its 5' end, and a BHQ-1 fluorescent quencher attached to its 3' end;

the probes in (3) to (15) comprise a VIC fluorescent dye attached to their 5' ends, and a MGBNFQ fluorescent quencher attached to their 3' ends;

the probes in (16) and (17) comprises a FAM fluorescent dye attached to their 5' ends, and a BHQ-1 fluorescent quencher attached to their 3' ends, and

the probe for the reference control gene comprises a ROX fluorescent dye attached to its 5' end, and a BHQ-2 fluorescent quencher attached to its 3' end.

32. A composition comprising the combination of primer and probe of any one of claims 1 to 31.

33. A DNA chip for testing HPV genotypes, comprising one or more polynucleotides selected from SEQ ID NOs: 1 to 45.

34. A kit for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample, comprising a combination of primer and probe in any one of claims 1 to 31.

35. The kit of claim 34, wherein the biological sample is collected from a human subject.

36. The kit of claim 35, wherein the biological sample comprises urine of the human subject.

37. A method for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample that was obtained from a subject in need thereof, comprising:

- (a) obtaining DNA from the biological sample;
- (b) amplifying the DNA by a fluorescent PCR using a combination of primer and probe in any one of claims 1 to 31; and
- (c) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR.

38. A method for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample that was obtained from a subject in need thereof, comprising:

- (a) obtaining DNA from the biological sample;
- (b) amplifying the DNA by a fluorescent PCR using the kit of claim 34; and
- (c) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR.

39. A method for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample that was obtained from a subject in need thereof, comprising:

- (a) extracting DNA from the biological sample and amplifying the DNA by a fluorescent PCR using the kit of claims 35 or 36; and
- (b) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR.

40. The method of any one of claims 37 to 39, wherein the method comprises detecting and/or identifying the presence or absence of DNA of at least seven HPV subtypes in the biological sample.

41. The method of any one of claims 37 to 39, wherein the method comprises detecting and/or identifying the presence or absence of DNA of 14 high-risk HPV subtypes in the biological sample through a single test tube, wherein the high-risk HPV subtypes are HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, and HPV68.

42. The method of any one of claims 37 to 39, wherein the method comprises detecting and/or identifying the presence or absence of DNA of 14 high-risk HPV subtypes and at least one low-risk HPV subtype in the biological sample through a single test tube, wherein the high-risk HPV subtypes are HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, and HPV68, and the at least one low-risk HPV subtype is HPV6 or HPV11.

43. The method of claim 41, wherein the biological sample is a smear of the cervix, a fresh tissue sample, a fixed tissue sample, a cross-sectional specimen of a tissue sample, a urine sample, a sample comprising exfoliated cells, a peripheral blood sample, a penis swab, or other body fluid.

44. The method of claim 43, wherein the sample is a urine sample.

45. Use of a combination of primer and probe of any one of claims 1 to 31 for detecting and/or identifying the genotype of a human papillomavirus (HPV).

46. Use of a pharmaceutical composition and/or a medical procedure in the manufacture of a medicament for treating a condition associated with human papillomavirus (HPV) in a subject in need thereof, comprising:

(1) detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample obtained from a subject in need thereof, comprising:

(a) amplifying DNA extracted from the biological sample by a fluorescent PCR using a combination of primers and probes of any one of claims 1 to 31; and

(b) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR, and

(2) that the subject is to be treated with the pharmaceutical composition and/or a medical procedure according to the results in step (1).

47. The use of claim 46, wherein the condition is a precancerous lesions caused by HPV.

48. The use of claim 46, wherein the pharmaceutical composition comprises an antiviral agent.

49. Use of a composition targeting selected HPVs in the manufacture of a medicament for vaccinating a human subject in need thereof, comprising:

(1) detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample obtained from the human subject in need thereof before and/or after the human subject is vaccinated, comprising:

(a) amplifying DNA extracted from the biological sample by a fluorescent PCR using a combination of primers and probes of any one of claims 1 to 31; and

(b) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR, and

(2) that the subject is to be vaccinated with the composition targeting selected HPVs based on the results in step (1).

50. An *in vitro* method for evaluating vaccination efficacy in a human subject in need thereof, comprising:

(1) detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample obtained from the human subject in need thereof after, or before and after the human subject is vaccinated, comprising:

(a) amplifying DNA extracted from the biological sample by a fluorescent PCR using a combination of primers and probes of any one of claims 1-31, after, or before and after the human subject is vaccinated; and

(b) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR; and;

(2) determining vaccination efficacy based on the results in step (1) of a subject that was vaccinated with a composition targeting selected HPVs.

51. A kit for the detection and / or recognition of human papillomavirus (HPV) genotypes in biological samples and for the guidance and efficacy evaluation of HPV vaccine, comprising the combination of primer and probe in any one of claims 1-31.

52. The kit of claim 51, wherein the primer and probe concentration in the primer and probe composition is 0.1-1.2 μ M, and the primer and probe combination includes all the primer and probe combinations in (1), (2), (5), (6), (8), (10), (12), (13), (14), (15), and the internal gene primers and probes (SEQ ID Nos: 31, 32 and 43).

53. The kit of claim 52, wherein the primer probe concentration in the primer probe composition is 0.1-1.2 μ m, and the primer and probe combination includes all the primer probe combinations in (3), (4), (7), (9), (11), (16), (17) and the reference control gene primers and probes (SEQ ID NOs: 31, 32 and 43).

54. A primer, comprising an oligonucleotide sequence having at least 85%, 90%, 95%, or 100% identity to any one of SEQ ID NOs: 1-36, wherein the primer has less than 100, 90, 80, 70, 60, 50, 40, 30, or 20 nucleotides.

55. A pair of primers, comprising a forward primer and a reverse primer, each having at least 85%, 90%, 95%, or 100% identity to any one of SEQ ID NOs: 1-36 and each having less than 100, 90, 80, 70, 60, 50, 40, 30, or 20 nucleotides, wherein the forward and reverse primers are selected from the groups consisting of: SEQ ID NOs: 1-2, 3-4, 5-6, 7-8, 9-10, 11-12, 13-14, 15-16, 17-18, 19-20, 21-22, 23-24, 25-26, 27-28, 29-30, 31-32, 33-34, 35-36, and any combination thereof.

56. A probe, comprising a fluorescent dye and an oligonucleotide, wherein the oligonucleotide comprises a sequence having at least 85%, 90%, 95%, or 100% identity to any one of SEQ ID NOs: 37-45, wherein the oligonucleotide has less than 100, 90, 80, 70, 60, 50, 40, 30, or 20 nucleotides.

57. The probe of claim 56, wherein the fluorescent dye is attached to 5' end of the probe.

58. The probe of claim 56 or 57, wherein the fluorescent dye is selected from the group consisting of FAM (fluorescein), TET, JOE, VIC, HEX, ROX, TAMRA, Cy3, cy3.5, Cy5, Cy5.5, OregonGreenTM, CALRedTM, Red640, Texas Red, LighterCycler®Cyan500, LighterCycler®, Red610, a biotin binding material, Alexa 647, Alexa 555, 5-(2-aminoethyl)amino-1-naphthalene sulfonic acid (EDANS), tetramethyl rhodamine (TMR),

tetramethyl rhodamine isocyanate (TMRITC), fluorescein isocyanate (FITC), and χ -rhodamine.

59. The probe of any one of claims 56-58, further comprising a fluorescent quencher.

60. The probe of claim 59, wherein the fluorescent quencher is attached to 3' end of the probe.

61. The probe of claim 59 or 60, wherein the fluorescent quencher is selected from the group consisting of DDQ-I, DDQ-II, Dabcyl, Eclipse, Iowa Black FQ, Iowa Black RQ, BHQ-1, BHQ-2, BHQ-3, QSY-7, QSY-9, and QSY-21.

62. A kit for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a biological sample, comprising one or more primers of any one of claim 106 or 107, and a one or more probes of any one of claims 56-61.

63. The kit of claim 62, comprising at least 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, or 36 primers selected from any one of claim 54 or 55.

64. The kit of claim 62 or 63, comprising at least 2, 3, 4, 5, 6, 7, 8, or 9 probes selected from any one of claim 56-61.

65. The kit of any one of claims 62-64, further comprising a lysis solution, magnetic nanoparticles, a protease, a first washing buffer, a second washing buffer, an elution buffer, or any combination thereof.

66. A method for detecting and/or identifying the genotype of a human papillomavirus (HPV) in a urine sample obtained from a subject in need thereof, comprising:

- (a) obtaining DNA from the urine sample;
- (b) amplifying the DNA by a fluorescent PCR using one or more primers of any one of claim 106 or 107, and a one or more probes of any one of claims 108-113; and
- (c) determining the presence or absence of DNA of one or more HPV subtype in the biological sample based on the results of the fluorescent PCR.