

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, the present disclosure provides 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: 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; (2) 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; (3) 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; (4) 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; (5) 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%,

- (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;

[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, and/or a medical procedure, 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), wherein the condition associated with HPV is cervical cancer, a precancerous lesion, a genital wart, a common wart, a plantar wart, a flat wart, a genital lesion, genital inflammation, genital bleeding, a genital lump, a genital bump, or genital itching. 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

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 [aspectexample](#), 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 [aspectexample](#), 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~~39-42, 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, OregonGreen™, CALRed™, 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 [selected from SEQ ID NOs: 1-36 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

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;~~

(31) 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;

(42) 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;

(53) 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;

(64) 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;

(~~75~~) 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;

(~~86~~) 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;

(~~97~~) 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;

(~~849~~) 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;

(~~944~~) 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;

(~~102~~) 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;

(~~113~~) 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;

(1~~24~~) 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;

(1~~35~~) 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, wherein the polynucleotide of the probe has less than 30 nucleotides.~~

~~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).~~

~~32.~~ The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises the primers and probes in (1~~3~~), (4~~2~~), (3~~5~~), and (9~~44~~).

4~~3~~. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises the primers and probes in (6~~4~~), (10~~2~~), (1~~24~~) and (1~~35~~).

~~54~~. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises the primers and probes in (~~57~~), (~~408~~), and (~~113~~).

~~65~~. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises the primers and probes in (~~68~~) and (~~79~~).

~~76~~. 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 (~~13~~), (~~42~~), (~~53~~), and (~~944~~).

~~87~~. 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 (~~46~~), (~~102~~), (~~124~~) and (~~135~~).

~~98~~. 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 (~~57~~), (~~408~~), and (~~113~~).

~~940~~. 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 (~~86~~) and (~~79~~).

~~104~~. 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 (~~13~~), (~~42~~), (~~53~~), and (~~944~~), and at least one group of primers and probes in (~~46~~), (~~102~~), (~~124~~) and (~~135~~).

~~112~~. 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 (~~13~~), (~~24~~), (~~53~~), and (~~944~~), and at least one group of primers and probes in (~~57~~), (~~840~~), and (~~113~~).

~~123~~. 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 (13), (24), (35), and (944), and at least one group of primers and probes in (68) and (97).

134. 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 (64), (102), (124) and (135), and at least one group of primers and probes in (57), (840), and (113).

145. 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 (46), (102), (124) and (135), and at least one group of primers and probes in (68) and (79).

165. 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 (57), (840), and (113), and at least one group of primers and probes in (68) and (79).

176. 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 (13), (24), (53), and (944), at least one group of primers and probes in (46), (102), (124) and (135), and at least one group of primers and probes in (57), (840), and (113).

187. 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 (13), (42), (53), and (944), at least one group of primers and probes in (46), (102), (124) and (135), and at least one group of primers and probes in (68) and (97).

198. 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 (13), (42), (53), and (944), ~~and~~ at least one group of primers and probes in (57), (840), and (113), and at least one group of primers and probes in ~~(86)~~ and (97).

~~1920.~~ 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 (46), (102), (124) and (135), ~~and~~ at least one group of primers and probes in (57), (840), and (113), and at least one group of primers and probes in ~~-(86)~~ and (79).

~~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).~~

202. The combination of primer and probe of claim 1, wherein the combination of primer and probe comprises ~~consists of~~ the group of primers and probes in (1) to (135).

~~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).~~

214. The combination of primer and probe of any one of claim 1 to 203, wherein the combination of primer and probe further comprises a group of primer and probe for a reference control gene.

225. The combination of primer and probe of claim 214, 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.

263. The combination of primer and probe of any one of claim 1 to 225, wherein each probe has a fluorescent dye attached to 5' end thereof.

247. The combination of primer and probe of claim 236, 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.

~~258~~. The combination of primer and probe of any one of claim 1 to ~~203~~, wherein each probe has a fluorescent quencher attached to 3' end thereof.

~~269~~. The combination of primer and probe of claim ~~285~~, 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.

~~2730~~. The combination of primer and probe of claim ~~2529~~, 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 (~~13~~) to (~~135~~) 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.~~

~~2834~~. The combination of primer and probe of claim ~~2529~~, 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 (~~13~~) to (~~135~~) 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.

~~2932~~. A composition comprising the combination of primer and probe of any one of claims 1 to ~~2834~~.

~~3033~~. A DNA chip for testing HPV genotypes, comprising one or more polynucleotides selected from SEQ ID NOs: ~~394~~ to ~~425~~.

314. 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 2831.

325. The kit of claim 314, wherein the biological sample is collected from a human subject.

336. The kit of claim 325, wherein the biological sample comprises urine of the human subject.

347. 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 2831; 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.

358. 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 314; 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.

396. 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 325 or 336; 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.

3740. The method of any one of claims 347 to 369, wherein the method comprises detecting and/or identifying the presence or absence of DNA of at least seven HPV subtypes in the biological sample.

3841. The method of any one of claims 347 to 369, 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.

3942. The method of any one of claims 347 to 369, 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.

4043. The method of claim 3841, 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.

414. The method of claim 403, wherein the sample is a urine sample.

425. Use of a combination of primer and probe of any one of claims 1 to 2834 for detecting and/or identifying the genotype of a human papillomavirus (HPV).

436. Use of a pharmaceutical composition ~~and/or a medical procedure~~ in the manufacture of a medicament, and/or a medical procedure, 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 2834; 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).

wherein the condition associated with HPV is cervical cancer, a precancerous lesion, a genital wart, a common wart, a plantar wart, a flat wart, a genital lesion, genital inflammation, genital bleeding, a genital lump, a genital bump, or genital itching.

~~447~~. The use of claim ~~436~~, wherein the condition is a precancerous lesions caused by HPV.

~~485~~. The use of claim ~~436~~, wherein the pharmaceutical composition comprises an antiviral agent.

~~469~~. 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 ~~2834~~; 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).

~~4750~~. 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-~~2834~~, 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.

~~4851~~. 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-~~2834~~.

~~5249~~. The kit of claim ~~4851~~, 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), (35), (64), (86), (840), (102), (113), (124), (135)~~, and the internal gene primers and probes (SEQ ID Nos: 31, 32 and 43).

~~503~~. The kit of claim ~~4952~~, 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 ~~(13), (24), (57), (97), (944), (146), (157)~~ 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.~~

~~516~~. 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: ~~397-425~~, wherein the oligonucleotide has less than ~~100, 90, 80, 70, 60, 50, 40, 30, or 20~~ nucleotides.

~~527~~. The probe of claim ~~516~~, wherein the fluorescent dye is attached to 5' end of the probe.

~~538~~. The probe of claim ~~516~~ or ~~527~~, 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, OregonGreen™, CALRed™, 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.

~~549~~. The probe of any one of claims ~~516-538~~, further comprising a fluorescent quencher.

~~5560~~. The probe of claim ~~549~~, wherein the fluorescent quencher is attached to 3' end of the probe.

~~564~~. The probe of claim ~~549~~ or ~~5560~~, 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.

~~5762~~. 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 ~~516-564~~.

~~5863~~. The kit of claim ~~5762~~, 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 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. ~~any one of claim 54 or 55.~~

~~6459~~. The kit of claim ~~5762~~ or ~~6358~~, comprising at least 2, 3, or 4, 5, 6, 7, 8, or 9 of the probes ~~selected from any one of claim 56-64.~~

~~605~~. The kit of any one of claims ~~5762-6459~~, further comprising a lysis solution, magnetic nanoparticles, a protease, a first washing buffer, a second washing buffer, an elution buffer, or any combination thereof.

616. 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 selected from SEQ ID NOs: 1-36~~of any one of claim 106 or 107~~, and ~~a~~ one or more probes of any one of claims ~~51-56~~~~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.