Supplementary Materials

A microfluidic-chip-based system with loop-mediated isothermal amplification for rapid and parallel detection of *Trichomonas vaginalis* and human papillomavirus

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1. Detailed information of design and selection of LAMP primers

1.1. Primer design

In order to ensure the performance of LAMP primers, sequences of targeted genes, the actin gene of *Trichomonas vaginalis* (GenBank no. AF237734.1), and the major capsid protein L1 gene of HPV16 (GenBank no. NC_001526.4), HPV18 (GenBank no. NC_001357.1), and HPV52 (GenBank no. X74481.1), were acquired from NCBI GenBank database (https://www.ncbi.nlm.nih.gov/genbank/). These sequences were respectively aligned with those of other species belonging to the same genus, to obtain segments with species specificity and conservation, by the software SnapGene.

With sequences of the segments above-mentioned, primers were designed by PrimerExplorer V5 (http://primerexplorer.jp/lampv5e/index.html), according to the instruction of the software. And plasmids containing these segments were synthesized by Sangon Biotech (Shanghai, China) and used as templates in primer selection. Before selection in practice, the designed primers were examined theoretically with specificity and secondary structure, by NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and another online software UNAFold (http://www.unafold.org/), respectively. Generally, for each target, about 10 sets of primers were synthesized by Sangon Biotech (Shanghai, China) and tested in the following selection.

1.2. Primer selection

A five-step process was carried out in primer selection. The sequences of primers selected and ultimately used in our study are presented in Table S1. The selection of primers detecting *T. vaginalis* was taken, for instance, to introduce the process. Reactions of primer selection were performed in EP tubes by ABI 7500.

Step 1. Selection of secondary structure. LAMP reactions were performed with deionized water as the template for 60 min in 8 independent replications for each set of primers. Primers with all negative results were considered not easy to produce primer dimers, and were selected to Step 2 (Fig. S1).

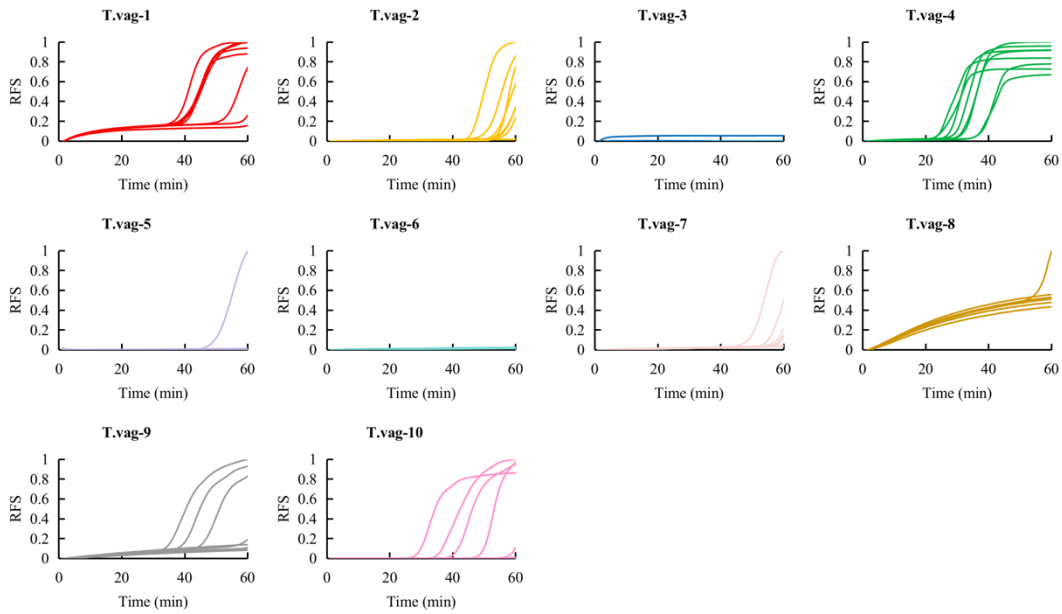

Fig. S1 Selection of the secondary structures of T.vag-1 to T.vag-10. In the 10 sets of primers for *T. vaginalis*, T.vag-3 and T.vag-6 produced all negative results within 60 min in 8 independent replications, respectively, and were selected to Step 2. RFS, relative fluorescence signal.

Step 2. Selection of sensitivity. LAMP reactions were performed with the targeted template of serially diluted concentrations (10^3-10^1 copies/reaction), for 40 min in 3 independent replications, respectively. Primers with all positive results for lower concentrations were considered more sensitive and were selected to Step 3 (Fig. S2).

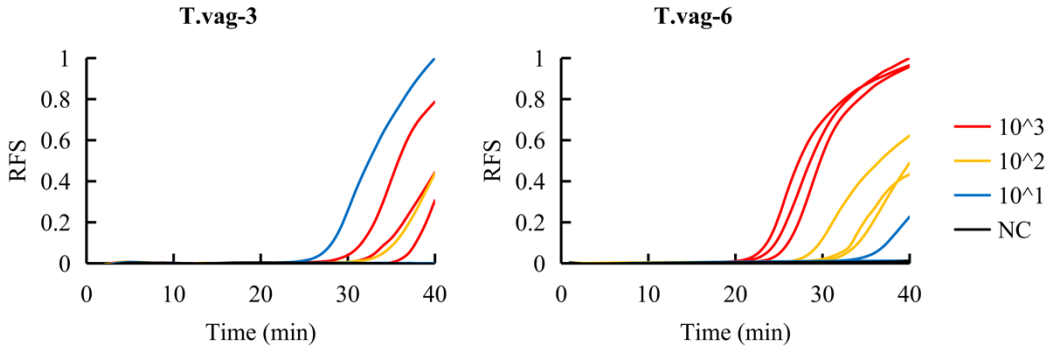

Fig. S2 Selection of the sensitivity of T.vag-3 and T.vag-6. T.vag-6 produced all positive results within 40 min in 3 independent replications, when the concentration of template was low to 10^2 copies/reaction, better than T.vag-3, thus was selected to Step 3. RFS, relative fluorescence signal; NC, negative control, using deionized water as the template; 10^1-10^3 (i.e. 10^1-10^3), the concentration of the template (copies/reaction).

Step 3. Validation of sensitivity. LAMP reactions were performed with the targeted template of the concentration according to the results of Step 2, for 40 min in 20 independent replications. Primers with positive results in at least 15 replications were considered repeatable in sensitivity and were selected to Step 4 (Fig. S3).

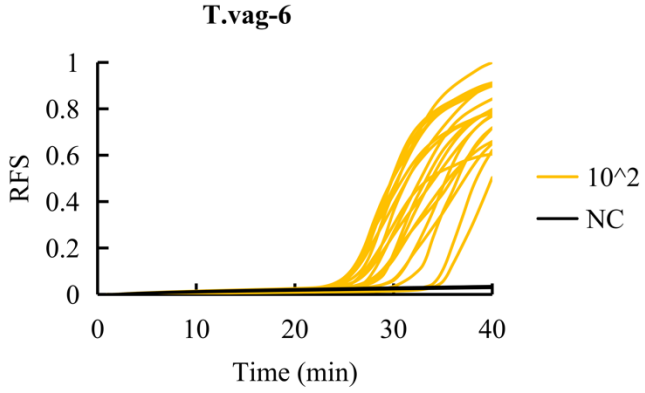

Fig. S3 Validation of the sensitivity of T.vag-6. T.vag-6 produced all positive results within 40 min in 20 independent replications, when the concentration of template was 10^2 copies/reaction, thus was selected to Step 4. RFS, relative fluorescence signal; NC, negative control, using deionized water as the template; 10^2 (i.e. 10^2), the concentration of the template (copies/reaction).

Step 4. Validation of secondary structure. LAMP reactions were performed with deionized water as the template for 50 min in 92 independent replications. Primers with negative results in at least 89 replications were validated of secondary structures not easy to produce primer dimers, and were selected to Step 5 (Fig. S4).

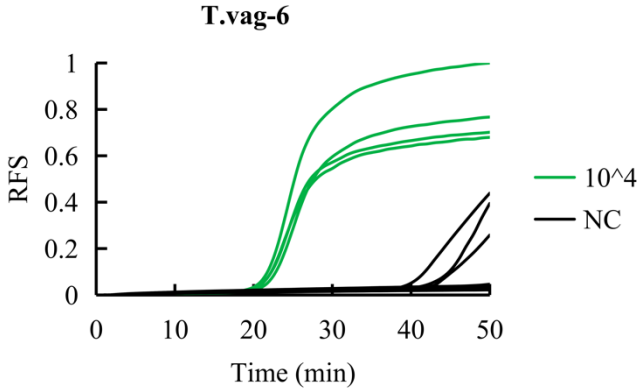

Fig. S4 Validation of the secondary structure of T.vag-6. T.vag-6 produced negative results within 50 min in 89 independent replications, and was selected to Step 5. RFS, relative fluorescence signal; NC, negative control, using deionized water as the template; 10^4 (i.e. 10^4), positive control with the targeted template of 10^4 copies/reaction.

Step 5. Validation of specificity. LAMP reactions were performed with targeted and non-targeted templates of 10^5 copies/reaction, for 40 min in 3 independent replications, respectively. Primers without false positives were validated of good specificity and were ultimately selected (Fig. S5).

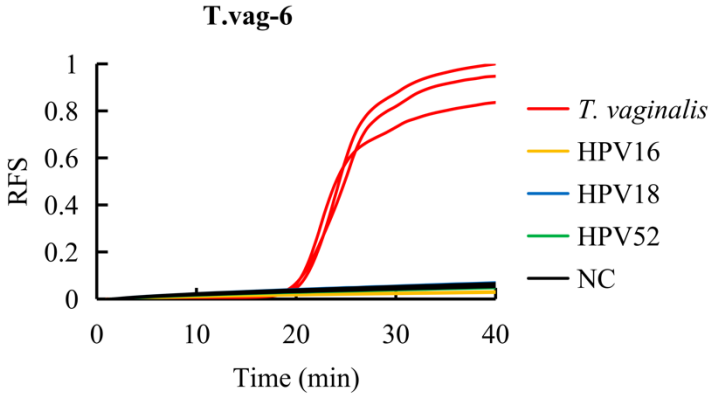

Fig. S5 Validation of the specificity of T.vag-6. T.vag-6 produced positive results only with the targeted template within 40 min in 3 independent replications, when the concentration of all templates was 10^5 copies/reaction, and was selected to detect *T. vaginalis* in our study. RFS, relative fluorescence signal; NC, negative control, using deionized water as the template.

Table S1 Sequences of primers used in this work

Target	Primer name		Sequence (5'-3')
T. vaginalis	T.vag-L	F3	TGGATGGTCAAGCTTCTCAC

Target	Primer name		Sequence (5'-3')
		B3	ATTTCTGGGCAGCGGAAG
		FIP	AGGGCGACATAGCAAAGCTTCTTGTGTG GAGCGTGGCAATGCTTTCA
		BIP	AATGGAGAAGGCCGCTACAGACTGTGT GCATTGCCGATTGTGATGACG
		LF	CGAACGATTTCCTTTTCGGCTGT
		LB	GTCAATTACACACTTCCAGATGGC
HPV16	HPV16-L	F3	GCCATATCTACTTCAGAAACTACA
		B3	GCCTGGGATGTTACAAACC
		FIP	GCAGTTAAGGTTATTTTGCACAGTTTGT GTGTACTAACTTTAAGGAGTACCTACG
		BIP	TTCCACTATTTTGGAGGACTGGATGTGT GAGTATCTTCTAGTGTGCCTC
		LF	CTGTAAATCATATTCCTCCCCATGT
		LB	TTGGTCTACAACCTCCCCC
HPV18	HPV18-L	F3	CGCGTCCTTTATCACAGG
		B3	TGGAATCCCCATAAGGATC
		FIP	GGCACCATATCCAGTATCTACCATAGTG TGTATTGCCCCCCTTTAGAACT
		BIP	TGCAAGATACTAAATGTGAGGTACCGTG TGTGCAGACATTTGTAAATAATCAGGAT
		LF	TCACCATCTTCCAAAACTG
		LB	ATTGGATATTTGTCAGTCT
HPV52	HPV52-L	F3	GGCAATACTGCCACTGTAC
		B3	ATAAAGTCATGTTAGTGCTACG
		FIP	ACGTTGTAACCAGTACGGTTTATTATGT GTGAAGCAGTGCTTTTTTTCCTAC
		BIP	CAGGGCCACAATAATGGCATTGTGTGAG TGGTATCCACAACTGTGA
		LF	GGGATTCTGAGGTTACCATAGAACC
		LB	GTTGGGGCAATCAGTTGTTTG

2. Detailed information of DNA extraction from clinical samples

2.1. Nucleic acids extraction from clinical vaginal swabs

To extract and purify total nucleic acids from vaginal swabs, which may potentially contain various types of cells, we have modified the procedures based on the protocol provided in the DNeasy Blood & Tissue Kit (Qiagen, Germany). The detailed procedures are as follows:

(1) Prepare a buffer system using 1× PBS solution (pH 7.2-7.4), which contains lysozyme and lyticase as specified in Table S2.

(2) Agitate the swab in a 1.5 mL centrifuge tube containing 1 mL of the sample preservation solution. Press the swab against the inner wall of the tube to ensure maximum transfer of the sample before discarding the swab.

(3) Centrifuge the tube for 10 min at 7500 rpm to harvest the cells disattached from the swab. Discard the supernatant.

(4) Add 600 μL of the buffer system prepared in step 1 to respond the pellet and incubate for 60 min at 30°C.

(5) Centrifuge for 10 min at 900 x g. Discard supernatant.

(6) Resuspend pellet in 180 μL Buffer ATL, and add 20 μL Proteinase K. Mix thoroughly by vortexing, and incubate for 60 min at 56°C.

(7) Add 200 μL Buffer AL and mix thoroughly by vortexing.

(8) Add 200 μL ethanol (96–100%) and mix thoroughly by vortexing.

(9) Pipet the mixture obtained in the previous step into the DNeasy Mini spin column placed in a 2 mL collection tube (provided in the kit). Centrifuge at 8000 rpm for 1 min. Discard flow-through and collection tube.

(10) Place the DNeasy Mini spin column in a new 2 mL collection tube (provided in the kit), add 500 μL Buffer AW1, and centrifuge for 1 min at 8000 rpm. Discard flow-through and collection tube.

(11) Place the DNeasy Mini spin column in a new 2 mL collection tube (provided in the kit), add 500 μL Buffer AW2, and centrifuge for 3 min at 14,000 rpm to dry the DNeasy membrane. Discard flow-through and collection tube.

(12) Place the DNeasy Mini spin column in a clean 1.5 ml microcentrifuge tube, and pipet 50-100 μL Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1min, and then centrifuge for 1 min at 8000 rpm to elute.

Table S2 Components and concentration of the buffer system containing lysozyme and lyticase

Components	Concentration
Sorbitol (Solarbio, China)	1 M
EDTA (Solarbio, China)	100 mM
Lysozyme (Aladin, China)	20 mg/mL
Lyticase (Solarbio, China)	200 U/600 μL

2.2. Nucleic acids extraction from cervical mucus samples

To extract and purify HPV DNAs from cervical mucus samples, we implemented specific modifications to the protocol provided in the DNeasy Blood & Tissue Kit (Qiagen, Germany), considering the limited quantity of clinical samples that contain a small viral load. The detailed procedures are as follows:

(1) Centrifuge the 1.5 mL tube containing the cervical sample for 5 min at 13000 rpm. Discard the supernatant.

(2) Add 200 μL 1× PBS solution (pH 7.2-7.4), 20 μL Proteinase K and 200 μL Buffer AL. Mix thoroughly by vortexing, and incubate for 60 min at 56°C.

(3) Add 200 μL ethanol (96–100%) and mix thoroughly by vortexing.

(4) Pipet the mixture obtained in the previous step into the DNeasy Mini spin column placed in a 2 mL collection tube (provided in the kit). Centrifuge at 8000 rpm for 1 min. Discard flow-through and collection tube.

(5) Place the DNeasy Mini spin column in a new 2 mL collection tube (provided in the kit), add 500 μL Buffer AW1, and centrifuge for 1 min at 8000 rpm. Discard flow-through and collection tube.

(6) Place the DNeasy Mini spin column in a new 2 mL collection tube (provided in the kit), add 500 μL Buffer AW2, and centrifuge for 3 min at 14,000 rpm to dry the DNeasy membrane. Discard flow-through and collection tube.

(7) Place the DNeasy Mini spin column in a clean 1.5 ml microcentrifuge tube, and pipet 50-100 μL Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1min, and then centrifuge for 1 min at 8000 rpm to elute.

3. Detailed information of comparison with related works and clinical standards

Table S3 Comparison of microfluidic systems detecting STI pathogens using LAMP

System	Target	Sample	Sensitivity	Specificity	Cost	Efficiency	Ref
Microfluidic chip and detector using digital LAMP	14 HPV genotypes	Plasmid, clinical sample	<60 copies/μL	High when detecting another 13 HPV genotypes	~5 μL LAMP mix per reaction, chip fabrication by 3D printing and soft lithography	>1.5 h for one detection	1
Microfluidic chip and detector using digital LAMP	2 HPV genotypes	Plasmid, clinical sample	700 copies/mL	High when detecting each other HPV genotype	~10 μL LAMP mix per reaction	>1 h for one detection	2
Quartz crystal microbalance sensor using LAMP	1 HPV genotype	Plasmid, clinical sample	100 copies/reaction	High when detecting another 3 HPV genotypes	50 μL LAMP mix per reaction, quartz crystal microbalance fabrication (quartz crystal, gold electrode, etc.)	>1 h for one detection	3
Microfluidic chip and detector using	5 HPV genotypes	Plasmid, cultured sample	1000 copies/μL	High when detecting another 4 HPV	8 μL LAMP mix per reaction	~0.5 h for one detection	4

System	Target	Sample	Sensitivity	Specificity	Cost	Efficiency	Ref
LAMP		(cell line)		genotypes			
Microfluidic chip and detector (iChip-400, Baicare) using LAMP	5 pathogens in lower genital tract	Cultured sample, clinical sample	<300 CFU (copies)/μL	High when detecting another 14 species	~4 μL LAMP mix per reaction	>1 h for one detection	5
Microfluidic-chip-based system with LAMP	*T. vaginalis*, 3 HPV genotypes (up to 22 targets)	Plasmid, cultured sample, clinical sample	<400 copies/reaction	High when detecting another 12 species	~1.41 μL LAMP mix per reaction	<0.75 h for one detection	This work

Table S4 Comparison of the limits of detection to clinically relevant or comparator test standards

Method	Target	Sample	Limits of detection	Specificity	Ref
Wet-mount microscopy	*T. vaginalis*	Vaginal swab sample	100 copies/5 μL	Sensitivity ranges from 38% to 82%, depending on the expertise of the reader, temperature, and the inoculum size.	6
Culture	*T. vaginalis*	Vaginal swab sample	300-500 organisms/mL	Specificity is 100% when testing vaginal swabs	7
Immunochromatographic assay	*T. vaginalis*	Vaginal swab sample	100 copies/5 μL	Specificity is 100% when testing vaginal swabs	6
multiplex PCR	*T. vaginalis, Chlamydia trachomatis* and *Neisseria gonorrhoeae*	Vaginal swab sample	1.5 pg/25 μL reaction	High when detecting common vaginal flora	8
LAMP	*T. vaginalis*	Cultured sample and vaginal swab sample	0.036 ng/μL	High when detecting another 4 species	9
Hybrid Capture II (HC2)	18 HPV genotypes	clinical sample	1.0 pg/mL	High since detected HPV genotypes have no cross-contamination	10
Digital droplet PCR	4 HPV genotypes	Plasmid, clinical sample	~10 copies/20 μL reaction	High when detecting another 8 HPV genotypes	11
LAMP	5 HPV genotypes	Plasmid, clinical sample	10-100 copies/25 μL reaction	High when detecting another 11 HPV genotypes	12
LAMP	*T. vaginalis*, 3 HPV genotypes (up to 22 targets)	Plasmid, cultured sample, clinical sample	< 400 copies/1.41 μL reaction	High when detecting another 12 species	This work

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