Sexually transmitted infection(STI) screening kit

Instruction

PRODUCT NAME

Sexually transmitted infection(STI) screening kit(Fluorescence PCR method)

SPECIFICATION

PR2026-ST01：8 samples/kit, 24 samples/kit

INTENDED USE

This kit is used for in vitro qualitative detection of trichomonas vaginalis, neisseria gonorrhoeae, chlamydia trachomatis, gardnerella vaginalis, haemophilus ducreyi, candida albicans, streptococcus agalactiae, ureaplasma urealyticum, ureaplasma parvum, mycoplasma hominis, mycoplasma genitalium, treponema pallidum, herpes simplex virus type 1&2 nucleic acid in vaginal swabs and urine from Sexually transmitted infection(STI) suspected patients.

PRINCIPLE

This product is a multiplex fluorescent probe-based Taqman® qPCR assay system. Specific primers and probes are designed for the detection of specific genes of 14 common STI/STD pathogens：trichomonas vaginalis, neisseria gonorrhoeae, chlamydia trachomatis, gardnerella vaginalis, haemophilus ducreyi, candida albicans, streptococcus agalactiae, ureaplasma urealyticum, ureaplasma parvum, mycoplasma hominis, mycoplasma genitalium, treponema pallidum, herpes simplex virus type 1&2. Internal control(IC) serves as the monitor to confirm successful extraction and identify possible PCR inhibition for the entire testing system to prevent false negative detection results.In order to avoid aerosol contamination of the amplified products, the UDG enzyme /dUTP system was added to the amplification system to effectively degrade the amplified products and avoid false positive results.

This kit is a fully premix freeze-dried system. Taq enzyme, UDG enzyme, reaction buffer, specific primers and probes required for amplification are all lyophilized in PCR tubes. Detection can be performed directly after adding dissolving solution and extracted nucleic acid.

KIT COMPONENTS

Components	8samples/kit	24samples/kit	Ingredient
ST01 Lyophilized Reagent	8×4 strip tubes	24×4 strip tubes	Specific primer&probes for the detection of target pathogens, dNTP/dUTP Mix, Mg2+, Taq polymerase and UDG enzyme
STI Lysis Buffer	1 mL×1 tube	5 mL×1 tube	Surface active agent, balanced salt solution
One Test Glass Beads	8 tubes×1 Bags	8 tubes×3 Bags	Glass beads
STI Positive Control	400 μL×1 tube	400 μL×1 tube	Plasmid containing every target gene sequence
STI Negative Control	400 μL×1 tube	400 μL×1 tube	Plasmid containing internal control sequence
Dissolving Solution	1 mL×1 tube	1 mL×2 tubes	Stabilizer

Note:

1. Do not mix the components from different batches for detection.
2. When using the same batch of reagents,STI negative and positive control should be tested at least once in one day to ensure that the environment is contamination-free and reagent & instrument are effective.

3. ST01 Lyophilized Reagent should be used immediately after unpacking. If air leakage or deliquescence occurs, do not continue to use.Unpacked but unused ST01 Lyophilized Reagent can be sealed and stored at -20±5℃ no more than 2 months.

4. ST01 Lyophilized Reagent is packed in 0.2mL PCR strip tube. If 0.2mL PCR tube cannot be used by the fluorescent PCR instrument, it is necessary to introduce the liquid in each hole into the applicable consumables before detection.

STORAGE & SHELF LIFE

The kit can be transported at room temperature (no more than one month). It can be stored at -20℃±5℃ for one year. Repeated freezing and thawing should not exceed 7 times.

EQUIPMENTS & MATERIALS REQUIRED BUT NOT SUPPLIED with the kit

1. Disposable powder free gloves and other personal protective equipment；
2. Pipettes (adjustable) and Sterile pipette tips；
3. Vaginal swabs/urine sample collection device；
4. 1.5 mL centrifuge tubes and racks；
5. Bench top centrifuge for centrifuge tubes and PCR tubes；
6. Vortex mixer(Power≥40w)；
7. Centrifuge tube holder for vortex mixer (not necessary,can replace part of manual operation)；
8. Metal bath/water bath (1.5mL centrifuge tube,95℃)；
9. Real-time PCR instrument with FAM/VIC/ROX/CY5 fluorescence channels(ABI7500,Bio-rad CFX96,QuantStudio,SLAN-96S,BTK-96).

SAMPLING & HANDLING

1. Suitable specimen type: Vaginal swab, Urine.
2. Collection:

Vaginal swab sample：After collecting vaginal secretions with a sterile swab, insert them into a sterile container containing normal saline.

Urine sample：The patient should be advised not to urinate for at least two hours prior to sample collection.Collect 10-20 mL of first-catch urine into a sterile container.

3. Storage: The collected specimen should be used for detection within the same day. Otherwise, please store the specimen as follows: Store at 2-8℃ for no more than one week(include transport time); Store at <-20℃ for 6 months, avoiding repeated freeze-thaw cycles.

PROTOCOL

1.Reagent Preparation

Take out the components of the kit, balance them at room temperature, and centrifuge liquid items instantaneously for standby use.

2.Specimen Processing

2.1 Specimen processing

After vaginal swab-normal saline eluent/urine specimens are mixed thoroughly by vortex mixer, take 1mL liquid specimen to 1.5mL centrifuge tubes，and centrifuge at 12000rpm for 10min. After centrifugation, the supernatant should be carefully discarded and ensure the precipitation remains in the tube.

2.2 Nucleic acid releasing

1）Add 100μL STI Lysis Buffer to the above-mentioned centrifuge tube, and then take One Test Glass Beads and pour them into centrifuge tube.

2）Cover the tube tightly and oscillate manually for 2 minutes with vortex mixer (power≥40W), or automatically oscillate for 10 minutes on the centrifuge tube holder of vortex mixer.

3）After oscillation,place centrifuge tube in the metal bath/water bath (preheated in advance) and heat at 95℃ for 2 minutes.

4）Then centrifuge the tube at 12000rpm for 1min, and nucleic acid are in the supernatant. If the nucleic acid cannot be detected immediately, it can be stored at 2-8℃ for no more than 24 hours, and -20℃ for no more than 1 month.

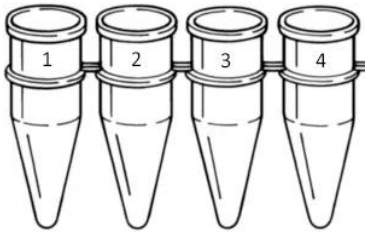
3. Amplification System Preparation

Take out ST01 Lyophilized Reagent according to the number of samples.One 4 strip tubes lyophilized reagent corresponds to one sample.If STI negative control and STI positive control are required,treat them as two sample nucleic acids,no need for nucleic acid extraction.First add 15μL Dissolving Solution in each PCR tube to dissolve the lyophilized powder, then add 5μL nucleic acid from step 2 successively.Each sample nucleic acid should be respectively added to 4 wells of one 4-strip lyophilized reagent.The total volume of each PCR tube is 20μL, cover the tube tightly, mix well by hand flicking(Do not use vortex mixer to mix, strong oscillation will cause enzyme inactivation).Centrifuge PCR tubes at low speed and collect the liquid to the bottom of the tube (avoid bubbles).

4.PCR Amplification

4.1 Put the reaction tubes into PCR instrument and set the names of each reaction well in the corresponding order.

Be sure to carefully observe the serial numbers on the 4-strip tubes, as shown in the figure below. The numbers on the PCR tube correspond to Well 1-4 respectively.Set each well as sample name - well number in the corresponding order, such as sample A-Well 1, sample A-Well 2, etc.



4.2 Settings of detection fluorescence

Select FAM, VIC/HEX, ROX and CY5 four fluorescence channels for detection. The corresponding targets of each fluorescence channel are shown in the table below:

Location	FAM	VIC/HEX	ROX	CY5
Well 1	Trichomonas vaginalis	Neisseria gonorrhoeae	Chlamydia trachomatis	Internal Control
Well 2	Gardnerella vaginalis	Haemophilus ducreyi	Candida albicans	Streptococcus agalactiae
Well 3	Ureaplasma urealyticum	Ureaplasma parvum	Mycoplasma hominis	Mycoplasma genitalium
Well 4	Treponema pallidum	Herpes simplex virus type 1	Herpes simplex virus type 2	

4.3 Amplification program setting

Steps		Temperature	Time	Cycles
1	Pre-denaturation	95 ℃	2 min	1
2	Denaturation	95 ℃	10 s	45
	Annealing, extension, fluorescence acquisition	60 ℃	30 s	

5.Results analysis (please refer to Instrument User Manual）

After the reaction, the results will be saved automatically. According to the analyzed image, please adjust the Start value and End value of the Baseline and Threshold value of common instrument (Start value: 3-15; End value: 5-20). The amplification curves of different detection targets should be analyzed respectively.The threshold line setting principle is recommended that the threshold line just exceeds the highest point of the fluorescence background value of sample.Click “Analysis” to obtain the analysis result automatically, then read the detection result in the “Report” window.

6.Quality Control

The results of STI negative control and positive control in each well and channel shall conform to the following table:

	Channel	Negative Control	Positive Control
Well 1	FAM	No Ct or Ct>40	Ct≤38 with normal amplification curve
	VIC	No Ct or Ct>40	Ct≤38 with normal amplification curve
	ROX	No Ct or Ct>40	Ct≤38 with normal amplification curve
	CY5	Ct≤38 with normal amplification curve	Ct≤38 with normal amplification curve
Well 2/3/4	FAM	No Ct or Ct>40	Ct≤38 with normal amplification curve
	VIC	No Ct or Ct>40	Ct≤38 with normal amplification curve
	ROX	No Ct or Ct>40	Ct≤38 with normal amplification curve
	CY5	No Ct or Ct>40	Ct≤38 with normal amplification curve

TEST RESULTS INTERPRETATION

Since well 1 contains the detection of internal control, the results of well 1 need to be determined before all results are determined. If well 1 gets valid results, the whole test is valid, and then the results of other wells 2, 3 and 4 can be continued to be interpretated. If well 1 produces invalid result, the whole sample test is invalid.

Well 1 Target	Channel	Well 1 valid results				Well 1 invalid result
		Well 1 Negative（-）	Trichomonas vaginalis Positive（+）	Neisseria gonorrhoeae Positive（+）	Chlamydia trachomatis Positive（+）	
Trichomonas vaginalis	FAM	No Ct or Ct>40	Ct≤40	Any results	Any results	No Ct or Ct>40
Neisseria gonorrhoeae	VIC/HEX	No Ct or Ct>40	Any results	Ct≤40	Any results	No Ct or Ct>40
Chlamydia trachomatis	ROX	No Ct or Ct>40	Any results	Any results	Ct≤40	No Ct or Ct>40
Internal Control	CY5	Ct≤40	Any results	Any results	Any results	No Ct or Ct>40

Under the condition that the results of well 1 are valid, the results of other pathogens of wells 2, 3 and 4 shall be interpretated according to the following table:

Target	Well position	Channel	Negative（-）	Positive（+）
Gardnerella vaginalis	Well 2	FAM	No Ct or Ct>40	Ct≤40
Haemophilus ducreyi	Well 2	VIC	No Ct or Ct>40	Ct≤40
Candida albicans	Well 2	ROX	No Ct or Ct>40	Ct≤40
Streptococcus agalactiae	Well 2	CY5	No Ct or Ct>40	Ct≤40
Ureaplasma urealyticum	Well 3	FAM	No Ct or Ct>40	Ct≤40
Ureaplasma parvum	Well 3	VIC	No Ct or Ct>40	Ct≤40
Mycoplasma hominis	Well 3	ROX	No Ct or Ct>40	Ct≤40
Mycoplasma genitalium	Well 3	CY5	No Ct or Ct>40	Ct≤40
Treponema pallidum	Well 4	FAM	No Ct or Ct>40	Ct≤40
Herpes simplex virus type 1	Well 4	VIC	No Ct or Ct>40	Ct≤40
Herpes simplex virus type 2	Well 4	ROX	No Ct or Ct>40	Ct≤40

Note: 1.If multiple pathogen targets are positive, the results indicate mixed infection with multiple pathogens.

2.If invalid result occur, the sample needs to be tested again.

3.If result of Well 1 is well 1 negative, and all the other pathogens results of well 2/3/4 are negative, the sample is interpretated to be negative for all pathogens detected by this kit.

ASSAY LIMITATIONS

1. The detection result of this kit is only for clinical reference, and it should not be directly used as the evidence for clinical diagnosis and treatment. The clinical management of patients should be considered in combination with their symptoms, medical history and exposure history.
2. The detection result can be affected by operations, including specimen collection, storage and transportation. False negative result may occur if there is any mistake in the operation. Cross contamination during specimen treatment may lead to false positive result.
3. The detected target sequences of this kit are the conserved region of genome of pathogens. However, target sequence variations may lead to false negative result.
4. Pathogen not listed in the detection range of this kit cannot be excluded by this method.

PERFORMANCE SPECIFICATIONS

1. Detection limitation: 1000 Copies/mL.
2. Precision: The coefficient of variation (CV, %) of Ct value of within-batch/between-batch precision is ≤ 5%.
3. Accuracy: The conformity rate of negative/positive reference: 100%.
4. Specificity:The target organisms had been analyzed in silico for potential cross-reactivity with the primers or probe sequences.Homology with other species is all below 90%. Specificity analysis showed that there is no cross reaction between the pathogens detected by this kit.

ATTENTIONS

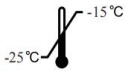
1. This product is only used for in vitro test. Please read this manual carefully before use.
2. The temperature should not exceed 37℃ when transporting the kit. If the outdoor temperature exceeds this temperature, please add ice packs during the transportation of the kit to control the transportation temperature below 37℃.
3. Use sterile, DNase-free and RNase-free tubes and tips during the detection.

4. During the detection, it is necessary to avoid DNase contamination, wear appropriate protective equipments, disposable gloves and masks, and complete the operation in the biosafety cabinet to avoid harmful substances entering the respiratory tract.
5. In case of contact with skin and mucous membrane during use, please rinse immediately with flowing water, which will not cause any harm to the operator.
6. All liquid reagents should be fully melted and mixed at room temperature before use, and centrifuged at 8,000 rpm for a few seconds before use.
7. After use, the packaging and waste liquid must be uniformly treated as medical waste to prevent pollution.

REFERENCES

[1] Fischer L . Screening for Chlamydia and gonorrhea: recommendation statement[J]. American Family Physician, 2015, 91(7).
[2] Hook E 3 . Trichomonas vaginalis--no longer a minor STD (letter; comment)[J]. Sexually Transmitted Diseases, 1999, 26(7):388-389.
[3] Muvunyi C M , Dhont N , Verhelst R , et al. Evaluation of a new multiplex polymerase chain reaction assay STDFinder for the simultaneous detection of 7 sexually transmitted disease pathogens[J]. Diagn Microbiol Infect Dis, 2011, 71(1):29-37.

SYMBOLS

	Date of manufacture		For *In Vitro* Diagnostic Use
	Manufacturer		Refer to the Instructions
	CE mark of conformity		Authorized representative in the European Community
	Batch code		Use-by date
	Do not use if package is damaged		Temperature limit
	Keep away from Sunlight		

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