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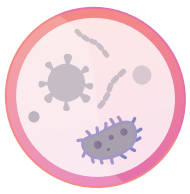

STD Pathogen Panel
Instruction of Use

Direct detection of Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Mycoplasma hominis and Trichomonas vaginalis by Real-Time PCR

For in vitro diagnostic use only
For prescription use only

Name
STD Pathogen Panel

Specification
48 reactions/kit

Intend Use

The STD Pathogen Panel is a qualitative multiplex nucleic acid in vitro amplification testing kit which allowing in two tubes direct detection of Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Mycoplasma hominis and Trichomonas vaginalis in clinical samples of symptomatic and asymptomatic individuals.

The STD Pathogen Panel is intended for detection of CT/NG/MG/MH DNA in male urine specimens, and the detection of CT/NG/MG/MH/TV DNA in female urine specimens, clinician collected female endocervical swab specimens.

Sexually transmitted diseases (STDs) are diseases that are mainly transmitted through sexual contact. It is now the second most common infectious disease. In particular, gonococcal urethritis and non-gonococcal urethritis have biological characteristics such as latent infection and asymptomatic bacterial excretion, which makes it important to prevent and control STD. At the same time, there are many types of pathogenic pathogens of non-gonococcal urethritis, which can be infected alone or in combination with long incubation period and can cause complications. It has brought serious impact to human public health. Studies have confirmed that Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Mycoplasma hominis and Trichomonas vaginalis are the most common pathogens causing urinary and reproductive tract infections. Among them, Neisseria gonorrhoeae causes gonorrhoea, 40 % ~ 50 % of non-gonococcal urethritis (NGU) is caused by Chlamydia trachomatis, and 30 % of NGU is caused by Mycoplasma hominis and Mycoplasma genitalium. Vaginal infection caused by Trichomonas vaginalis are among the most common conditions transmitted sexually. Worldwide, it is estimated that 142.6 million new trichomoniasis cases occur each year.1 Despite being a readily diagnosed and treatable sexually transmitted disease, trichomoniasis is not a reportable disease, and control of the infection has received relatively little emphasis from public health STD control programs

Negative results may be caused by other pathogens not detected in the experiment, which cannot ruled out other infections or be used as the sole basis for diagnosis, treatment, or other management decisions.

Positive results cannot rule out other bacterial or viral infections either.

This kit is for in vitro diagnostic use only. The users should have been professionally trained and obtained relevant qualifications. The health authority should be contacted in time in case of any positive samples.

Principle

This product uses qPCR technology to design specific primers and probes for the Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Mycoplasma hominis and Trichomonas vaginalis. Each test was divided into 2 tubes, one tube for Chlamydia trachomatis, Neisseria gonorrhoeae and Reference gene, and the other tube for Mycoplasma genitalium, Mycoplasma hominis and Trichomonas vaginalis, as shown in Table 1.

During the amplification process, when the probe is complete, because the 3 'quenched group is close to the 5' reporter group, the fluorescence emitted by the reporter group is absorbed by the quenched group, and no fluorescence signal is emitted. When the nucleic acid of the target was contained in the reaction system, the fluorescent probe bound to the template during primer extension was severed by Taq enzyme (5'-3' exonuclase activity), and the reporter group was separated from the quenched group to produce fluorescence signal. The real-time amplification curve can be plotted by fluorescent quantitative PCR instrument automatically according to the detected fluorescence signal, and implement the qualitative analysis of novel coronavirus nucleic acid in the sample.The assay contains an endogenous internal control (CY5 sign in tube A) for monitor specimen collection, nucleic acid extraction and PCR.

Table 1 Arrangement of the targets

	A tube	B tube
FAM	Chlamydia trachomatis	Mycoplasma genitalium
HEX/VIC	Neisseria gonorrhoeae	Mycoplasma hominis
CY5	Reference gene	Trichomonas vaginalis

Materials Provided

Constituent	Component	48 reactions/kit
Dilution Buffer	Nuclease-free water	2mL/vial , 1 vial
STD qPCR Mix A	Primer、Probe、dNTPs、Enzyme	1 vial
STD qPCR Mix B	Primer、Probe、dNTPs、Enzyme	1 vial
STD PCA	Plasmid containing the target gene	1 vial
STD PCB	Plasmid containing the target gene	1 vial
STD NC	Nuclease-free water	2mL/vial , 1 vial

Note: The components in different batch are not interchangeable.

Materials and Equipment Required But Not Provided

• Applied Biosystems™ Real-Time PCR System 7500.
Alternatively, Roche LightCycler® 480 System, or QuantStudio 5 Real-Time PCR System.
• Disposable sampler Inactivated Transport Media (NEST, cat.# 202135)
• Nucleic Acid Extraction Kit (cat. SC903). Optionally, High-throughput Automated Sample Preparation System: SC905/SC906
• Vortex mixer.
• Microcentrifuge.
• Micropipettes (2 or 10 µL, 200 µL and 1000 µL).
• Racks for 1.5 mL microcentrifuge tubes.
• 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach).
• Disposable powder-free gloves and surgical gowns.
• 1.5 mL microcentrifuge tubes (DNase/RNase free).
• 96-well 0.2 mL PCR reaction plates or appropriate optical reaction tube.
• Nuclease-free water

Storage conditions and Expiration period

Store at 2-8℃ away from light for 12 months; No more than 10 days for transportion at room temperature.
Date of manufacture: see label
Expiration Period: See label

Applicable Instrument

ABI7500、ABI QuantStudio 5 and Roche LightCycler480

Specimen Collection and Preparation

1. Types of sample suitable for this product: male urine specimens, female urine specimens and clinician collected female endocervical swab.

1.1 Urine specimens
The STD Pathogen Panel is intended for use with female and male urine specimens from symptomatic and asymptomatic patients.

NOTE: the patient should not have urinated for at least 1 hr prior to specimen collection.

1) Collect 10-20 ml from the first part of urine stream in a clean polypropylene, preservative-free specimen collection cup.
2) Close the cup and label with patient identification and date/time collected.
3) Transport the specimens to the test site at room temperature (18-30℃). Urine samples are stable during 24 hrs at room temperature. If analysis cannot be done within 24 hrs of collection, urine specimens must be stored at 2-8℃ and analyzed within 7 days. It is also posible to freeze urine specimens but in this case do not freeze and thaw urine specimens more than one time.
4) Urine specimens analyzed at external test sites must be transported to the test site within 24 hrs of collection. If room temperature shipment is chosen, specimens must be stored at 2-8℃ prior to shipment and upon arrival in order to ensure that room temperature storage does not exceed 24 hrs of collection.

1.2 Clinician collected female endocervical swab.
The STD Pathogen Panel is intended for use with clinician-collected endocervical swab specimens.
Sample collection: Wash the secretion outside the cervix with sterile normal saline cotton ball, then insert the sample swab into the cervix for 1-2cm, stay for a few seconds and rotate for 2 turns before taking out;

Specimens should be shipped directly in ice packs or with the Disposable sampler Inactivated Transport Media (NEST, cat.# 202135).
For more information on specimen transport, please refer to the Disposable sampler Inactivated Transport Media (NEST, cat.# 202135) package insert. Swab specimens collected with Inactivated Transport Media can be kept up to 20 days at 18-30℃. If the sample is transferred directly, the test should be completed at 2-8℃ for no more than 24 hours or stored at -20℃ for no more than 1 month.

Laboratory Procedures

1. Sample processing

1.1 Urine specimens
Mix upside down the urine specimens, centrifuge 5-10 mL of fresh urine at 10000rpm for 30 min at 4℃ and discard the supernatant. Then, Resuspend the precipitate with 400µL normal saline (0.9% NaCl) and used for the next step of nucleic acid extraction.

1.2 Clinician collected female endocervical swab.
Swab DNA should be collected from fresh a specimen to ensure suitable DNA quality and quantity. DNA should be extracted using the Nucleic Acid Extraction kit manually (cat.# SC903/SC905/SC906, Wuxi Tech-star Technology Co.,Ltd) according to the manufacturer`s Instruction of Use.
800µL Nuclease-free water was added to the STD PCA and STD PCB respectively. Vortex and centrifuge briefly. STD PCA, STD PCB and STD NC after resolution should be processed simultaneously alongside the specimen.

2. Master Mix Setup
Prepare all reagents in preparation area. To begin, take out the kit contents and thaw thoroughly at ambient temperature. Prepare 96-well plates or appropriate optical reaction tube for real-time PCR based on the estimated number of reactions, two tubes are required for each sample.
980µL Dilution Buffer was added to the STD qPCR Mix A and STD qPCR Mix B respectively for re-dissolution, and was completely mixed with tip.
Pipette 20µL of PCR-Mix into each well. Cover and transfer the plate into sample processing area.

The remaining qPCR-Mix, STD PCA, STD PCB must be stored at under -18℃ immediately, and it should be used in 20 days, repeated free zing and thawing times should not exceed 7 times.

3. Sample Addition
Add 5µL extracted sample DNA, Negative control or Positive control, and close the 96-well reaction plate with appropriate lids or optical adhesive film, mix evenly upside down and short spin at 6000rpm.

Make sure that at least one Positive Control and one Negative Control is used per run.

4. Testing
Double-click 7500 software or select Start>>All Programs>>Applied Biosystems>>7500 Software.
Click New Experiment to enter Experiment menu. In the Experiment Properties screen, enter identifying information for the experiment; you can leave other fields empty.

Select 7500 (96 Wells); Quantitation-Standard Curve (for the experiment type); TaqMan Reagents (forreagent); and Standard (for ramp speed).

Click Plate Setup, in the Targets screen, under the tab Define Targets and Samples, set Target 1 with FAM reporter, Target 2 with VIC/HEX reporter and Target 3 with CY5 reporter, set Target 4 with FAM reporter, Target 5 with VIC/HEX reporter and Target 6 with CY5 reporter. Quencher Dye all set: None. Define samples according to the samples in this experiment.

Click Assign Targets and Samples tab, in the target(s) screen, for tube A, select target 1, target 2 and target 3, for tube B, select target 4, target 5 and target 6. In the Samples screen, select samples and controls to include in the reaction plate in corresponding well, and select the sample/target reactions to set up. Select None for passive reference.

Click Run Method. On the Run Method screen, select either the Graphical View tab (default) or the Tabular View to edit the run method. Make sure the thermal profile displays the holding and cycling stages shown below.

Step	Temperature	Time	Fluorescence measured	Cycle
1	95°C	3min	No	1
2	95°C	15sec	No	40
3	58°C	30sec	Yes	

Click Run. In the Run screen, save the experiment. Click START.
After the run completes, unload the instrument and proceed to data analysis.

5. Analysis of result

Click Analysis. In the Amplification Plot screen under Plot Settings tab:

a. In the Plot Type drop-down list, select ΔRn vs Cycle (default).
b. In the Graph Type drop-down list, select Linear.
c. In the Plot Color drop-down list, select Target.

Set the baseline starting point at cycle 3 and ending at cycle 15
Adjust the threshold to be equal to the maximum level of the no-template control curve (i.e., equal to the maximum value of the random noise curve).
Click Analyze. The software analyzes the data with the settings.

Interpretation of result

1. Quality control standards

1.1 STD NC: no typical S-type amplification curve or Ct>38 for FAM, HEX (VIC) and CY5 channels.

1.2 STD PCA&STD PCB: FAM, HEX (VIC) and CY5 channels showed a typical S-type amplification curve and Ct≤33.

STD PCA, STD PCB and STD NC are used to monitor the effectiveness of the instrument and test reagents. The test results of all indexes can only be interpreted when the test results meet the conditions in section 1.1 and 1.2 at the same time.

Reference gene: To ensure the correctness of sample processing, Reference gene designs a set of primers based on the mRNA sequence of human ribonuclease subunit 30. Reference gene is mainly used for quality control of samples for proper processing and to monitor sample-related inhibition during amplification.

2. Result interpretation

Under the premise that STD PCA, STD PCB and STD NC meet the requirements, the following analysis is carried out:

2.1 If the Ct values of any of the 5 indicators detected by this product in their respective channel meet the requirements in the table below, the indicator is positive. As shown in the following table.

	Tube A			Tube B		
	Chlamydia trachomatis	Neisseria gonorrhoeae	IC	Mycoplasma genitalium	Mycoplasma hominis	Trichomonas vaginalis
	FAM	HEX (VIC)	CY5	FAM	HEX (VIC)	CY5
Chlamydia trachomatis positive	Ct＜40	NoCt	No Requirement	NoCt	NoCt	NoCt
Neisseria gonorrhoeae positive	NoCt	Ct＜40	No Requirement	NoCt	NoCt	NoCt
Mycoplasma genitalium positive	NoCt	NoCt	No Requirement	Ct＜40	NoCt	NoCt
Mycoplasma hominis positive	NoCt	NoCt	No Requirement	NoCt	Ct＜40	NoCt
Trichomonas vaginalis positive	NoCt	NoCt	No Requirement	NoCt	NoCt	Ct＜40

If two or more of the above conditions are present in one sample at the same time, it is indicated as mixed infection.

2.2 Negative: All the 5 indicators of the samples to be tested had no Ct value, and the CY5 channel of tube A showed a typical S-type amplification curve, which was judged to be negative. As shown in the following table.

	Tube A			Tube B		
	Chlamydia trachomatis	Neisseria gonorrhoeae	IC	Mycoplasma genitalium	Mycoplasma hominis	Trichomonas vaginalis
	FAM	HEX (VIC)	CY5	FAM	HEX (VIC)	CY5
Negative	NoCt	NoCt	＜40	NoCt	NoCt	NoCt

2.3 Suspected: If all channel of the samples to be tested had no Ct value, the test is invalid. The sample should be extracted and tested again. If the results of the repeated tests are consistent with the previous, re-sampling should be considered.

Limitations

1. The results obtained by this kit should be interpreted in conjunction with other laboratory data and clinical data obtained by the doctor. It should not be used alone as a basis for patient management.
2. Negative results cannot completely exclude pathogen infection, because a concentration of target gene in the sample lower than the test limit or mutation in target sequence can lead to negative results. Improper sample collection, delivery, and handling, as well as improper test operation and experimental environment, can all lead to false negative or false positive results.
3. False positive results may be attributable to non-specific amplification caused by the inhibitors in the sample or cross-reactions of other microorganisms. The cross-reactions observed and detected by this kit and the highest concentrations of some inhibitors are already stated in "Analysis specificity".
4. Diseases caused by other bacterial or viral pathogens cannot be ruled out.
5. The samples collected from the patients who used drug therapy may result in false negative results.
6. The test nucleic acid may remain in the body for a long period of time, regardless of pathogens activity. A positive test result does not necessarily mean that the pathogen is infectious, or that the pathogen is a pathogen that causes clinical symptoms.
7. This test is a qualitative test and does not provide quantitative values nor indicate the quantity of organisms present.

Performance Characteristics

1. Limit of Detection

The pathogens were diluted in the negative swab matrix and tested. LoD is the lowest reproducible sample concentration that can be distinguished from negative samples at 95% confidence level; or the lowest concentration for 19 positive samples out of 20 repeated tests.

Index type	LoD concentration	Positives/tests(percentage)
Chlamydia trachomatis	7.0×10^2 copies/mL	19/20（95%）
Neisseria gonorrhoeae	5.0×10^2 copies/mL	20/20（100%）
Mycoplasma genitalium	5.0×10^2 CCU/ml	20/20（100%）
Mycoplasma hominis	1.0×10^3 copies/mL	19/20（95%）
Trichomonas vaginalis	2.0×10^2 copies/mL	20/20（100%）

2. Analysis specificity

No cross-reactivity were observed with Herpes Simplex Virus 1, Herpes Simplex Virus 2, Neisseria meningitidis, HPV 16, HPV 18, Ureaplasma urealyticum, Escherichia coli, Myocoplasima pneumonia, Candida albicans, Human cytomegalovirus, Treponema pallidum. Meanwhile, endogenous substances and therapeutic drugs have no effect on the test. The details are as follows:

Test substance	Test concentration	Matrix	Result
Endogenous interfering substance			
Human whole blood	5%	Swab	No interference
		Urine	No interference
Bilirubin	1 mg/ml	Urine	No interference
Glucose	1 mg/ml	Urine	No interference
Proteins (Human Serum Albumin)	0.4 mg/mL	Urine	No interference
Drug			
miconazole	1%	Swab	No interference
tioconazole	1%	Swab	No interference
metronidazole	1%	Swab	No interference
Nonoxynol Suppositories	1%	Swab	No interference

3. Precision

The precision reference products were tested with Take three batches of STD Pathogen Panel. Each batch of reagents is tested by 2 people per day, and each person test once in the morning and afternoon. Each concentration is tested 2 times in parallel, and is tested continuously for 5 days. The results met the following criteria.

3.1 Negative sample: The concentration of the test substance is below the minimum detection limit or zero concentration, and the negative detection rate should be 95%.

3.2 Critical positive sample: The concentration of the test substance is slightly higher than the minimum detection limit of the kit, and the positive detection rate should be higher than 95%.

3.3 Medium/strong positive sample: The concentration of the test substance leads to moderate to strong positive result, and the positive detection rate is 100%.

Precautions

1. This product is only suitable for in vitro diagnostic testing, and the experimenter should have received professional training and obtained the relevant work permit.

2. It shall be implemented strictly in accordance with the management norms of gene amplification laboratory promulgated by the relevant competent authorities. The laboratory is divided into three areas for operation (reagent preparation area, sample preparation area and amplification area), items in each area shall not be cross-used, and special instrument and equipment shall be used in each area.

3. The performance characteristics of this test kit for the sample types listed in the section “Intended use” have been identified. The performance of this test kit for other sample types has not been evaluated

4. The tip with filter and centrifuge tube used in the experiment should be autoclaved, and without DNase and RNase. After use, it is directly driven into the disposal bottle containing 1% sodium hypochlorite.

5. All clinical samples should be treated as infectious substances, operation and disposal shall comply with relevant regulations, such as the General guidelines for biosafety in Pathogenic Microbiological laboratory and the Clinical Waste Management Ordinance issued by the Ministry of Health.

Symbols meaning

	Do not re-use		Keep away from sunlight
	Keep dry		Consult instructions for use
	Caution	EC REP	Authorized representative in the European Community
CE	CE Symbol		Do not use if package is damaged
NON STERILE	Non-sterile	IVD	In vitro diagnostic medical device
	Manufacturer	LOT	Batch Code
	Manufacture Date		Symbol for “USE BY”
8°C 2°C	Temperature limit (2-8°C)		

References

1. Kimberly A W, Gail A B. Sexually transmitted diseases treatment guidelines, 2015.[J]. Morbidity and Mortality Weekly Report, 2015, 64 (3):1-137.
2. Organization W H. WHO | Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus[J]. World Health Organization, 2013.
3. Ison C A, Tosswill J, Alexander S. Laboratory diagnosis of sexually transmitted infections[J]. Medicine, 2014, 42(6):310-313.

Basic Information

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Date of approval and amendment of IFU： 2022.06.07
Version number： V1.02