Chlamydia Trachomatis/Ureaplasma Urealyticum/Neisseria Gonorrhoeae DNA Diagnostic Kit (PCR-Fluorescence Probing)

Product identification

Product Name: Chlamydia Trachomatis/Ureaplasma Urealyticum/Neisseria Gonorrhoeae DNA Diagnostic Kit (PCR-Fluorescence Probing)
Reference Number: S3050E
Package Specification: 48 tests/kit, Pre-packaged 24 tests/kit

Intended use

Chlamydia Trachomatis/Ureaplasma Urealyticum/Neisseria Gonorrhoeae DNA Diagnostic Kit (PCR-Fluorescence Probing) is a qualitative in vitro test for simultaneous detection of Chlamydia Trachomatis, Ureaplasma Urealyticum and Neisseria Gonorrhoeae DNA in sterile calcium alginate swab specimens from male urinary tract secretion and female genital tract secretion by applying real-time fluorescence quantitative PCR technique, which can provide molecular diagnosis evidence for the early diagnosis of related sexually transmitted diseases and initial screening of high-risk STD population.

For in vitro diagnostic use only. For professional use only.

Test principle summary and explanation

Summary

Chlamydia Trachomatis (CT) is a prokaryocyte germ of a special cyclogeny and strict intracellular parasitism, which belongs to Bacteriophyta, Rickettsiae, Chlamydiales and Chlamydozoon according to the classification of microbiology, and it is one the most widespread sexually transmitted disease pathogens around the world. After the organism is infected by Chlamydia Trachomatis, it will cause specific cellular immunity and humoral immunity, but the immunity is not strong and stays for a short time, therefore it usually causes persistent infection, silent infection and repeated infection. Chlamydia Trachomatis will infect mucosal epithelial cells of vagina, urethral canal and rectum and cornea, which will cause women to have cervicitis, endometritis, salpingitis and pelvic inflammation, secondary tubal infertility and eccyesis mostly through maternal-neonatal transmission [1, 2]. It will cause men to get nongonococcal urethritis (NGU), epididymitis, prostatitis and proctitis. It may cause infants to get neonatal inclusion conjunctivitis and pneumonia of newborn. Up to now, the main detection methods of Chlamydia Trachomatis include cell culture, enzyme immunoassay (EIA), direct immunofluorescence assay (DFA), real-time PCR technique [3].

Ureaplasma Urealyticum (UU), also known as mycoplasma urealytium, is a kind of minimal prokaryotic micro-organisms between bacterium and virus, which is mainly found from human urogenital tract and transmitted through sexual contact or vertically infecting the fetus by mother. Ureaplasma Urealyticum is one the primary pathogens of nongonococcai urethritis, which can cause various reproductive tract infectious diseases after infection [4]. For males, it can cause nongonococcai urethritis, acute epididymitis and prostatitis. For females, it can cause endometritis, salpingitis and oaritis. The intrauterine infection of ureaplasma urealyticum can cause adverse consequences like abortion, stillborn foetus, premature birth. At present, the laboratory diagnosis methods of ureaplasma urealyticum include isolated culture, immunological methods, molecular biological methods based on nucleic acid detection like real-time PCR technique.

Neisseria Gonorrhoeae (NG), also commonly known as diplococcus of Neisser or gonococcus, is a malgenic pathogen which can cause human gonorrhoea. It belongs to Neisseria species and is one of sexually transmitted diseases of high morbidity in the developing countries. When men get infected by NG, it will usually cause acute urethritis with purulent secretions and serious dysuresia, epididymitis, prostatitis [5]. For women patients, it mainly infects endometria but with slight symptoms in the beginning so it can't be easily detected. If it is not treated in time, then it will proliferate to reproductive system, which can cause urethritis, cervicitis, pelvic inflammation, even infertility. A very few of Neisseria Gonorrhoeae can be transmitted through blood and create propagative gonorrhea, and then cause gonococcal arthritis, eacarditis. So far, the main detection methods of Neisseria Gonorrhoeae include isolated culture, Smear, enzyme immunoassay, real-time PCR technique.

Test principle

By applying real-time fluorescence quantitative PCR technique, this diagnostic kit utilizes a pair of specific primers which are designed to target conserved sequences of CT/UU/NG-DNA, and a specific fluorescence probe, accompanied with other ingredients in CT/UU/NG-PCR Mix, to achieve quick detection of CT/UU/NG-DNA through the change in fluorescent signals.

The PCR detection system uses UNG enzyme + dUTP contamination-proof system which is able to fully degrade possible unwanted side-products to avoid a false positive result.

The PCR detection system uses an internal control to monitor the presence of PCR inhibitors in test samples and assess the quality of nucleic acid extraction by detecting the β-globin of human epidemic cells to determine whether the collected samples can be used for amplification reactions, in order to avoid a false negative result.

Materials provided

This kit is an amplification reaction reagent and contains the following components:

No.	Reagent Name	Specification & Qty.		Main Ingredients
		48 T	Pre-packaged 24T	
1	CT/UU/NG-Enzyme Mix	96 μL/tube × 1 tube	2 μL/tube × 24 tubes	DNA polymerase, Uracil DNA Glycosylase
2	CT/UU/NG-PCR Mix	912 μL/tube × 2 tubes	38 μL/tube × 24 tubes	Primers, probes, dNTPs, 10 x PCR buffer, sterile purified water
3	CT/UU/NG-Positive Reference A (4.0E+07copies/mL)	50 μL/tube × 1 tube	50 μL/tube × 1 tube	Positive clone plasmid containing target gene of chlamydia trachomatis, ureaplasma urealyticum, neisseria gonorrhoeae and β-globin
4	CT/UU/NG-Positive Reference B (4.0E+06copies/mL)	50 μL/tube × 1 tube	50 μL/tube × 1 tube	Positive clone plasmid containing target gene of chlamydia trachomatis, ureaplasma urealyticum, neisseria gonorrhoeae and β-globin
5	CT/UU/NG-Positive Reference C (4.0E+05copies/mL)	50 μL/tube × 1 tube	50 μL/tube × 1 tube	Positive clone plasmid containing target gene of chlamydia trachomatis, ureaplasma urealyticum, neisseria gonorrhoeae and β-globin
6	CT/UU/NG-Positive Reference D (4.0E+04copies/mL)	50 μL/tube × 1 tube	50 μL/tube × 1 tube	Positive clone plasmid containing target gene of chlamydia trachomatis, ureaplasma urealyticum, neisseria gonorrhoeae and β-globin
7	CT/UU/NG-Negative Control	1000 μL/tube × 1 tube	1000 μL/tube × 1 tube	Normal saline
8	CT/UU/NG-Positive Control	50 μL/tube × 1 tube	50 μL/tube × 1 tube	Positive clone plasmid containing target gene of chlamydia trachomatis, ureaplasma urealyticum, neisseria gonorrhoeae and β-globin

Materials required but not provided

Not included in the kit reagent: sterile saline, Multi-type Sample DNA/RNA Extraction-Purification Kit (Magnetic beads method) (Reference Number: S1006E Series) or Sample Release Reagent (Reference Number: S1013E Series) manufactured by Sansure Biotech.

Warnings and precautions

Warnings

Do not mix or exchange components from different kit.

Precautions

1. For *in vitro* diagnostic use only. Please read the manual carefully before any performances.
2. Please learn and be familiar with the operation procedures and precautions for each instrument before test. Please make sure quality control for each test.
3. Laboratory management shall strictly follow management practices of PCR gene amplification laboratory; laboratory personnel must receive professional training; test processes must be performed in separated regions; all consumables should be for single use only after sterilization; required instruments and devices as specified in Test Method should be used for each stage of the experimental operation; all lab devices required in different processes and regions should not be cross-used.
4. All specimens are infectious and they should be handled in a proper way. Wear laboratory coats, protective disposable gloves and change the gloves often to avoid cross-contamination between samples. Handling of specimens and waste must meet relevant requirements outlined in local, state and national regulations.
5. Before use, all reagents must be fully thawed at room temperature and mixed thoroughly.
6. After the addition of the sample in the tube the resulting solution is to be considered potentially biohazardous, handle the reagent with appropriate precautions and good laboratory practice.
7. The safe disposal of the reagents supplied must be carried out according to the instruction contained in the specific Safety Data Sheets and in compliance with the national regulations on disposal of potentially hazardous waste.
8. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established; If you have any questions about the test or the results, please contact Sansure`s customer service hotline +86-731-88883176-6116 or send an email to info@sansure.com.cn/ support@sansure.com.cn

IVD storage, operating conditions and stability

1. The shelf life of the kit is 12 months at -25°C to -15°C and protected from light.
2. The reagents can be stored for a period within the manufacturing date specified on the outside of the package and keep stable when not used. The freeze-thaw cycles of amplification reaction reagent should not exceed five after use.
3. The reagents keep valid and stable before the expiry date on the outer package when transporting for 10 days in a sealed foam box containing ice bags with the temperature lower than 20°C.

Instrumentation

The kit is compatible to Fluorescence Quantitative Analysis System Containing FAM, HEX/VIC, ROX and CY5, such as:

1. Applied Biosystems/7500 Real-Time PCR System
2. Bio-Rad/CFX 96 Deep Well Dx ORM
3. Hongshi/SLAN®-96P Real-Time PCR System
4. Sansure/Portable Molecular Workstation (Model: S-Q36A)

Collecting and preparing specimens

1. Available specimen type: genital tract secretions.
2. Collection of specimens: (Patient is required not to urinate in 2 hours before specimen collection [6]. Patients should not have sex within 24 hours before specimen collection. Refer to Sexually Transmitted Disease Laboratory Operation Manual for collection details.)

For male genital tract secretion: clean urethral orifice with sterile saline, then insert sterile calcium alginate swab into urethra for 2-3 cm, rotate the swab by 1-2 circles to get the specimen.

For female genital tract secretion: clean excessive secretion at cervical mouth, then insert sterile calcium alginate swab 1-2 cm deep in the cervix, rotate the swab for 1-2 circles to get the specimen. The specimen should be collected containing some mucosa. Place the collection swab in a sterile collection tube, seal it and send it for detection.

3. Storage and delivery of specimens:

Specimens collected via the above-mentioned method can be used for immediate detection, or stored at room temperature (20°C) for up to 24 hours, at 4°C for up to 120 hours, or below -20°C for up to 7 months. The freeze-thaw cycles should not exceed 7. Specimens should be transported in a sealed frozen pitcher with ice or in a sealed foam box with ice.

Test procedure

1.Please process according to the following steps for 7500 Real-Time PCR System, CFX 96 Deep Well Dx ORM and SLAN®-96P Real-Time PCR System,:

1.1 Preparation of reagent (performed at "reagent preparation region")

1.1.1 Take out all the components out off the diagnostic kit and equilibrate at room temperature, then vortex each of them respectively for later use.

1.1.2 Preparation of PCR-Master Mix Solution: According to quantity of pretreated specimens, CT/UU/NG-Negative Control, CT/UU/NG-Positive Control and CT/UU/NG-Positive References A~D, pipette appropriate quantity of CT/UU/NG-PCR Mix and CT/UU/NG-Enzyme Mix (CT/UU/NG-PCR Mix 38 μL/test + CT/UU/NG-Enzyme Mix 2 μL/test), fully mix them to make PCR-Master Mix Solution and then centrifuge it at 2000 rpm for 10 seconds. Save it at 4°C and protect from light for later use.

	1 sample	10 samples	24 samples	48 samples
CT/UU/NG-PCR Mix (μL)	38	380	912	1824
CT/UU/NG-Enzyme Mix (μL)	2	20	48	96

Note: The above configuration is just for your reference and to ensure enough volume of the PCR-Master Mix Solution, more volume of the actual pipetting may be required.

1.2. Processing and loading of samples (performed at "specimen processing region") (CT/UU/NG-Negative Control and test specimens are processed together.)

1.2.1 Pretreatment of specimens

Put the sample storage reagent or sample storage solution into a 1.5 mL centrifuge tube (press the cotton swab on the tube wall to dry it and then discard the swab) for later use.

1.2.2 Processing of specimens

Option 1: add 500 μL of test specimens (if the specimens have many secretions or they are very turbid, please centrifuge them at 3000 rpm for 30 seconds and then pipette) to a 1.5 mL centrifuge tube, and use Sansure Biotech's Sample Release Reagent (Reference Number: S1013E Series) to extract the nucleic acid as specified in its manual.

Option 2: add 200 μL of test specimens (if the specimens have many secretions or they are very turbid, please centrifuge them at 3000 rpm for 30 seconds and then pipette) to a 1.5 mL centrifuge tube, and use Sansure Biotech's Multi-type Sample DNA/RNA Extraction-Purification Kit (Magnetic beads method) (Reference Number: S1006E Series) to extract the nucleic acid as specified in its manual.

1.2.3 Loading of specimens

1.2.3.1 According to the quantity of test specimens, CT/UU/NG-Negative Control, CT/UU/NG-Positive Control and CT/UU/NG-Positive References A~D, add 40 μL of PCR-Master Mix Solution into each reaction tube.

1.2.3.2 Add 10 μL of the above pretreated CT/UU/NG-Negative Control and test specimens (fully vortex it and then pipette) as well as CT/UU/NG-Positive Control and CT/UU/NG-Positive References A~D into each reaction tube loaded with PCR-Master Mix Solution. Cover the tube lid (flick the tube and remove the bubbles if exist). Centrifuge it at 2000 rpm for 10 seconds or keep gently swinging the tube until there are no obvious droplets on the tube wall.

1.3. PCR amplification (performed at "amplification and analysis region") (Refer to user manual of each instrument to adjust the settings.)

1.3.1 Place the PCR reaction tube into the specimen well of the amplification device. Set up the CT/UU/NG-Negative Control, CT/UU/NG-Positive Control, CT/UU/NG-Positive References A~D and unknown samples in the corresponding sequence and input sample name.

1.3.2 Select PCR test channel

a. Select FAM channel (Reporter: FAM, Quencher: None) to test UU-DNA.
b. Select HEX or VIC channel (Reporter: HEX/VIC, Quencher: None) to test CT-DNA.
c. Select CY5 channel (Reporter: CY5, Quencher: None) to test NG-DNA.
d. Select ROX channel (Reporter: ROX, Quencher: None) to test the internal control.
e. Set Passive Reference: None.
f. Set Sample Volume: 50.

1.3.3 Set up cycle parameters

	Step	Temperature	Time	Cycle No.
1	UNG enzyme reaction	50°C	2 min.	1
2	Taq enzyme activation	94°C	5 min.	1
3	Denaturation	94°C	15 sec.	45
	Annealing, extension, fluorescence collection	57°C	30 sec.*	
4	Device cooling (optional)	25°C	10 sec.	1

***Note:** Due to the device technical specification of 7500-Real-time PCR System, it can not be set at 30 sec. but 31 sec. or 32 sec.

When the setting is completed, save the settings and carry out the reaction procedure.

2.Please process according to the following steps for Portable Molecular Workstation S-Q36A:

2.1 Preparation of consumables and reagents

2.1.1 Take out the Consumables kits and reagents.

2.1.2 Put Sample Release Reagent (Reference Number: S1013E Series) into the **Well B**; Put CT/UU/NG-PCR Mix into the **Well C**; Put CT/UU/NG-Enzyme Mix into the **Well D**. (The well location information has been marked on the supporting consumables)

2.1.3 Add 20μL sample to be tested or CT/UU/NG-Positive Control or CT/UU/NG-Negative Control into the **Well B** (To avoid bubbles during operation, it is recommended to pipette deeply and release slowly).

2.2 Test procedure (Refer to user manual of each instrument to adjust the settings.)

2.2.1 Click the "  "and "  " button on the instrument display screen to open the door of the instrument and put the prepared consumables into the designated position of the instrument.

2.2.2 Click the "New" on the instrument display screen to enter the new experiment task setting interface.

2.2.3 Select the required experimental project in the drop-down menu of Experimental project, enter the corresponding task name in the Task Name bar, and input and select other items that should be input or selected.

2.2.4 Click "Submit" to submit the experimental task and "OK" to run the instrument.

3. Result analysis (refer to user manual of each instrument to adjust the settings)

When the reactions are completed, results will be saved automatically. After analysis, adjust Start, End and Threshold values of Baseline (Users can adjust them according to the actual situation. Start value can be set at 3-15, and End value at 5-20. Adjust the amplification curve of negative control to be flat or below threshold). Click “Analyze” to implement the analysis, make sure each parameter satisfies the requirement of the below mentioned “4. Quality Control”, then go to “Plate” window to record the Ct value.

4. Quality control

The test result is treated as valid if all the conditions in the table below are met for the same test. Otherwise, the test result is treated as invalid and needs to be re-tested.

	CT/UU/NG-Positive Control	CT/UU/NG-Negative Control	CT/UU/NG-Positive References (A, B, C, D)
Ct value	Typical S-shape amplification curve and Ct value ≤ 38 at channel FAM, HEX/VIC, CY5 and ROX.	No display at channel FAM, HEX/VIC, CY5 and ROX, and monitor whether there is contamination in target gene and endogenous internal control gene (β-globin)	Positive at channel FAM, HEX/VIC, CY5 and ROX.

Reference range

Through the research on reference value, the Ct positive reference value of the diagnostic kit for detecting the target gene is determined to be 38, and the Ct positive reference value for detecting the internal control is to be 40.

Interpretation of test results

Determine the results of test specimens according to the table below:

FAM Channel	HEX Channel	CY5 Channel	ROX Channel	Conclusion
Ct ≤ 38	Ct > 38 or No Ct	Ct > 38 or No Ct	Ct ≤ 40	Positive UU; Negative NG/CT
Ct > 38 or No Ct	Ct ≤ 38	Ct > 38 or No Ct	Ct ≤ 40	Positive CT; Negative NG/UU
Ct > 38 or No Ct	Ct > 38 or No Ct	Ct ≤ 38	Ct ≤ 40	Positive NG; Negative UU/CT
Ct ≤ 38	Ct ≤ 38	Ct > 38 or No Ct	Ct ≤ 40	Positive CT/UU; Negative NG
Ct > 38 or No Ct	Ct ≤ 38	Ct ≤ 38	Ct ≤ 40	Positive CT/NG; Negative UU
Ct ≤ 38	Ct > 38 or No Ct	Ct ≤ 38	Ct ≤ 40	Positive UU/NG; Negative CT
Ct ≤ 38	Ct ≤ 38	Ct ≤ 38	Ct ≤ 40	UU, CT, NG are all positive
Ct > 38 or No Ct	Ct > 38 or No Ct	Ct > 38 or No Ct	Ct ≤ 40	UU, CT, NG are all negative
Ct > 38 or No Ct	Ct > 38 or No Ct	Ct > 38 or No Ct	Ct > 40 or No Ct	1. There are PCR inhibitors existing in test samples if PCR reaction fails. Please dilute the DNA by 10 or 100 times and then retest. If there are fluorescent signals, determine the result as above specified, or recollect the samples and retest. 2. It shows that exfoliative cells are insufficient, and please recollect the samples and retest.

Limitations of the procedure

1. Test results of the diagnostic kit can be used only for clinical reference. The patients’ symptoms, disease histories, other laboratory tests and treatment reactions should all be considered when providing clinical treatment for the patients.
2. Analysis on possibilities of false negative results:
   2.1 Incorrect specimen collection, delivery and processing, and low concentration of pathogens in the specimen all may cause a false negative result.
   2.2 Gene mutation of target gene sequence or sequence change caused by other reasons may cause a false negative result.
   2.3 Inappropriate storage of reagents may cause a false negative result.
   2.4 Other invalidated interference or PCR inhibitors may cause a false negative result.
3. Cross-contamination during sample processing may cause a false positive result.

Performance characteristics

1. Accuracy

Detect enterprise's positive references, national references of human Ureaplasma Urealyticum nucleic acid test and national references in the Neisseria Gonorrhoeae PCR kit, the results are all positive with accurate identification, there are no cross reactions. Detect eight serotypes (D, E, F, G, H, I, J, K) for Chlamydia Trachomatis, fourteen serotypes (type 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14) for Ureaplasma Urealyticum and ten Neisseria Gonorrhoeae liquids (CMCC 29105, 29106, 29400, 29403, 29805, 29806, 29807, 29808, 29816, 29818), the results are all positive with accurate identification.

2. Specificity

While testing enterprise’s negative reference, the results are all negative, and for other pathogens with similar infection location such as Herpes Simplex Virus Type 2, Human Papillomavirus Type 6, Human Papillomavirus Type 16, Mycoplasma genitalium, Mycoplasma hominis chlamydia pneumoniae and Chlamydia psittaci, the detection results are all negative.

3. Precision

For within-lot precision, the coefficient of variation for Ct value (cv, %) is ≤ 5%.

4. Limit of Detection

The lower limit of detection of Chlamydia Trachomatis/Ureaplasma Urealyticum/Neisseria Gonorrhoeae is 400 copies/mL.

5. Interfering substances

The presence of hemoglobin (2 g/L) or phlegm (10%) in the specimens will not cause influence to test results.

List of references

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Symbol key

Symbols	Meanings	Symbols	Meanings
IVD	*In Vitro* Diagnostic Medical Device		Date of Manufacture
	Use-by date		Consult instructions for use
	Temperature Limit		Manufacturer

LOT	Batch Code	REF	Reference Number
Σ	Contains sufficient for <n> tests		Caution
CE	This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.	EC REP	Authorized representative in the European Community /European Union
CONTROL –	Negative Control	CONTROL +	Positive Control
ENZYME MIX	Enzyme Mix	PCR MIX	PCR Mix
REFERENCE + A	Positive Reference A	REFERENCE + C	Positive Reference C
REFERENCE + B	Positive Reference B	REFERENCE + D	Positive Reference D
PRE	Prepackaging	VERSION	Version
	Do not re-use	21 PAP	PAP21: Not corrugated cardboard
	Keep away from light		

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