Mycoplasma genitalium/Mycoplasma hominis/Trichomonas vaginalis Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)

Reference Number

S3250E-48, S3250E-24-P, S3250E-12-S

Product Name

Mycoplasma genitalium/Mycoplasma hominis/Trichomonas vaginalis Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)

Package Specification

48 tests/kit, Pre-packaged 24 tests/kit, Pre-packaged 12 tests/kit

Intended Use

The Mycoplasma genitalium/Mycoplasma hominis/Trichomonas vaginalis Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is intended for the qualitative detection of Mycoplasma genitalium (MG), Mycoplasma hominis (MH), and Trichomonas vaginalis (TV) in human urine samples *in vitro*. The results of detection can be used for auxiliary diagnosis of patients suspected to be infected with MG, MH and TV. The test results of this kit are for clinical reference only and should not be used as the sole standard for clinical diagnosis.

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

Summary

MG, is a sexually transmitted pathogen that causes cervicitis, endometritis, and pelvic inflammatory disease in women[1], and is also responsible for acute and chronic non-gonococcal urethritis (NGU) in 10-35% of men[2].

MH is a pathogen that easily causes pelvic inflammatory disease, bacterial vaginosis, postpartum fever and other diseases in the genitourinary system, often leading to serious diseases, and even some studies have shown that it may be related to female infertility[3].

TV infection is a very common sexually transmitted disease. According to WHO, 170 million people are infected with TV every year in the world. In addition to causing urogenital tract infection, it is also one of the risk factors for HIV infection and cervical cancer. TV infection in women in the second trimester often causes premature rupture of membranes, premature birth, and low birth weight infants. It is also an important cause of non-gonococcal urethritis in men. Like other sexually transmitted diseases, its clinical symptoms are not typical, and clinical diagnosis often relies on laboratory tests[4].

At present, the main method of laboratory detection of the above pathogens, i.e., culture isolation.

Test Principle

This kit has designed three target genes for the specific conserved sequences of MG, MH, and TV, with PCR reaction solution, on the fluorescence quantitative PCR instrument, using real-time fluorescence quantitative PCR detection technology, through the change of fluorescent signal realizes the detection of DNA of MG, MH, and TV.

The PCR detection system uses an internal control to monitor RNase P in human cells for the evaluation of amplification reaction of specimens and monitor the presence of PCR inhibitors, as well as to evaluate the nucleic acid extraction efficiency, in order to avoid a false negative result.

Components of the Diagnostic Kit

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

No.	Reagent Name	Spec. & Qty.			Main Ingredients
		48 T	Pre-packaged 24T	Pre-packaged 12T	
1	MG/MH/TV PCR Mix	936 μL/ tube × 2 tubes	39 μL/tube × 24 tubes	39 μL/tube ×12 tubes	Primers, Probes, dNTPs, $MgCl_2$, PCR buffer
2	MG/MH/TV Enzyme Mix	48 μL/ tube × 1 tube	1 μL/tube × 24 tubes	1 μL/tube × 12 tubes	Taq DNA polymerase
3	MG/MH/TV Positive Control	1000 μL/tube × 1 tube	1000 μL/tube × 1 tube	1000 μL/tube × 1 tube	Plasmid containing MG, MH, TV target sequence and internal standard gene fragment (RNase P)
4	MG/MH/TV Negative Control	1000 μL/ tube × 1 tube	1000 μL/tube × 1 tube	1000 μL/tube × 1 tube	Normal Saline

Note:

1. All contents in this package are prepared and validated for the intended testing purpose. Replacement or modification of any of the package contents will affect the testing performance of the kit. Components contained within a kit are intended to be used together. Do not mix or exchange components from different kits.
2. Not included in the kit reagent: 1.5 mL DNase-free and RNase-free microcentrifuges tubes; 0.2 mL PCR tubes or strips; various models of pipettes and pipette tips (10 μL, 200 μL and 1000 μL tips with filters); microcentrifuges; vortex mixer.
3. Nucleic Acid Extraction-Purification Kit (Magnetic beads method) (S50016E Series) or Sample Release Reagent (S1013E Series) manufactured by Sansure Biotech Inc.

Precautions

1. For *in vitro* diagnostic (IVD) use only.
2. Follow standard precautions. All specimens and Positive Controls should be considered potentially infectious and handled accordingly.
3. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
4. Please read the package insert carefully before operation. The Listeria Monocytogenes Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is only for emergency use as an IVD test. Each step of operation, from specimen collection, storage and transportation, and laboratory testing, should be strictly conducted in line with relevant biosafety regulations and molecular laboratory management.
5. Separate laboratory rooms, dedicated to performing predefined procedures of the assay, are required. a) 1st room: Preparation room—Prepare testing reagent: b) 2nd room: specimen processing—Process the specimen and controls: c) 3rd: Amplification room—PCR conducted.
6. All specimens for detection should be handled as if infectious. 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.
7. 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.
8. 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.
9. 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

Storage and Stability

1. The shelf life of the kit is 12 months at -25°C to -15°C and protected from light.
2. Please refer to the date of manufacture and expiry date printed on the outside of the box.
3. Unopened reagents are valid and stable until the expiry date.
4. Once the reagents are opened, the maximum number of freeze/thaw cycles should not exceed five.
5. The reagents are shipped in sealed foam boxes with coolant below -20°C.

Compatible Instrument

The diagnostic kit is applicable to PCR systems with FAM, HEX/VIC, ROX, CY5 channels such as:

1. Agilent/AriaDx Real-Time PCR System
2. Applied Biosystems/7500 Real-Time PCR System
3. Applied Biosystems/QuantStudio 5 Real-Time PCR System
4. Bio-Rad/CFX 96 Deep Well Dx System
5. Bioer/QuantGene 9600 Fluorescent Quantitative Detection System (Model: S-Q96C)
6. Hongshi/SLAN®-96P Real Time PCR instrument
7. Molarray/Real-time Quantitative Thermal Cycler (Model: MA-6000)
8. Roche/LightCycler® 480 Instrument II
9. Sansure/Portable Molecular Diagnostic System (S-Q37A/S-Q37B)
10. Sansure/Portable Molecular Workstation (Model: S-Q36A)
11. Sansure/Portable Molecule Workstation (Model:S-Q31A/S-Q31B)

Specimen Requirements

1. Applicable specimen type: urine.
2. Collection of specimens:

The patient did not urinate for at least two hours before sampling. After routine disinfection of the urethral orifice, the middle part of the urine was collected in a sterile container.

3. Storage and delivery of specimens:

Specimens to be tested can be immediately processed, or stored at room temperature for up to 24 hours, or at 2°C to 8°C for less than 120 hours, or at-25°C to -15°C for up to 7 months. Freeze/thaw cycles should not exceed seven. Specimens should be transported in a sealed frozen pitcher with ice or in a sealed foam box with ice. Keep the specimen at -70°C or lower for long term storage.

Test Method

1. Please process according to the following steps for AriaDx Real-Time PCR System, 7500 Real-Time PCR System, QuantStudio 5 Real-Time PCR System, CFX 96 Deep Well Dx System, QuantGene 9600 Fluorescent Quantitative Detection System (Model: S-Q96C), SLAN®-96P Real Time PCR instrument, Real-time Quantitative Thermal Cycler (Model: MA-6000), LightCycler® 480 Instrument II.

1. 1. Preparation of reagent (performed at "reagent preparation room")

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

1.1.2 According to the number of samples to be tested, negative controls and positive controls, take the corresponding amount of components in proportion (MG/MH/TV PCR Mix 39 μL/test + MG/MH/TV Enzyme Mix 1 μL/test), fully mixed into PCR Master Mix, centrifuged briefly for later use.

1.2. Sample pre-processing (in the sample processing area, MG/MH/TV-Negative Control needs to be synchronized with the sample to participate in the extraction)

Processing method 1: It is recommended to use the sample release agent (S1013E Series) of Sansure Biotech Inc. for nucleic acid extraction according to its instructions.

Processing method 2: It is recommended to use the nucleic acid extraction or purification reagent (S50016E Series) of Sansure Biotech Inc. to perform nucleic acid extraction according to its instructions.

1.3. Loading of specimens (performed at "specimen processing room")

1.3.1 Add 10 μL of pretreated test specimens, MG/MH/TV negative control, MG/MH/TV Positive Control into a PCR reaction tube.

1.3.2 Add 40 μL of MG/MH/TV PCR Master Mix into each PCR reaction tube. Cover the tube lid and centrifuge the tube at 2000 rpm for 10 seconds

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

1.4.1 Place PCR tubes into the specimen wells of the amplification equipment. Set up the MG/MH/TV Positive Control, MG/MH/TV Negative Control and specimens to be tested in order and input specimen name.

1.4.2 Select PCR test channel:

a) Select FAM channel to detect MG nucleic acid, HEX/VIC channel to detect MH nucleic acid, ROX channel to detect TV nucleic acid.

b) Select CY5 channel to test Internal Control.

1.4.3 Set cycle parameters

	Steps	Temperature	Time	Cycle No.
1	Predenaturation	95°C	5 min.	1
2	Denaturation	95°C	15 sec.	45
	Annealing, extension and fluorescence collection	60°C	30 sec.*	
3	Device cooling	25°C	10 sec.	1

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

***Note:** Due to the technical specifications of 7500 Real-Time PCR System, it cannot be set at 30 sec., but can be set at 31 sec. or 32 sec. When the settings are completed, save the settings and carry out the reaction procedure.

2. Please process according to the following steps for Portable Molecule Workstation (Model: S-Q31A/S-Q31B):

2.1 Preparation of reagent strip

(The well location information has been marked on the supporting consumables)

(1) Put the Tip into **Well H**, and PCR reaction tube into **Well PCR**.

(2) Put Sample Release Reagent (Reference Number: S1013E Series) into the **Well B**; Put MG/MH/TV PCR Mix into the **Well C**; Put MG/MH/TV Enzyme Mix into the **Well D**;

(3) Add 20 μL sample to be tested or MG/MH/TV Positive Control or MG/MH/TV Negative Control into the **Well B** (To avoid bubbles during operation, it is recommended to pipet deeply and release slowly).

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

2.2.1 Place the **Well A** of reagent strip into the instrument towards the outside of the instrument.

2.2.2 Click the “**Lab task**" on the instrument display screen to enter the interface of setting new experimental task.
2.2.3 Select the required experimental project in the drop-down menu of **Lab 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 and start the experimental task successively.
2.2.5 When the Portable Molecule Workstation (Model: S-Q31B) shows "**Please transfer the PCR tube to the 1/2/3/4**" (The S-Q31A shows "Please transfer the PCR tube”) on the interface, take out the PCR tube and cover it well, then centrifuge it instantaneously.
2.2.6 Insert the PCR tube into the PCR amplification module (the “PCR 1/2/3/4” cover has been automatically opened at this time), close the outer cover of the amplification module, then click "**OK**" for amplification detection.

3.Please process according to the following steps for Portable Molecular Workstation (Model: S-Q36A):

3.1 Preparation of reagent strip

(The Well location information has been marked on the supporting consumables)
(1) Put the Tip into **Well E**, and the syringe plunger into **Well G**.
(2) Put Sample Release Reagent (Reference Number: S1013E Series) into the **Well B**; Put MG/MH/TV PCR Mix into the **Well C**; Put MG/MH/TV Enzyme Mix into the **Well D**;
(3) Add 20 μL sample to be tested or MG/MH/TV Positive Control or MG/MH/TV Negative Control into the **Well B** (To avoid bubbles during operation, it is recommended to pipet deeply and release slowly).

3.2 Test Procedure (Refer to user manual of each instrument to adjust the settings)

3.2.1 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.
3.2.2 Click the **“New”** on the instrument display screen to enter the new experiment task setting interface.
3.2.3 Select the required experimental project in the drop-down menu of **Lab project**, enter the corresponding task name in the **Task Name** bar, and input and select other items that should be input or selected.
3.2.4 Click **"Submit"** to submit the experimental task and **"OK"** to run the instrument and start the experimental task successively.

4. Please process according to the following steps for Portable Molecular Diagnostic System (S-Q37A/S-Q37B):

4.1 Pre-run preparation

4.1.1 Load the amplification reagent component assembly into the extraction reagent component (Nucleic Acid Extraction-Purification Kit, Reference Number : S50016E-12A) to compose the test reagent cartridge;
4.1.2 Open the seal plug of the sample loading hole, add 350μL sample or MG/MH/TV Positive Control or MG/MH/TV Negative Control into the sample loading hole (To ensure Diagnostic System have 300μL samples for nucleic acid extraction); or use transfer pipet from the extraction reagent kit to pipette sample into the sample loading hole (When sample enter the lower bubble of transfer pipet indicates enough sample has been taken). Then close the seal plug.

4.2 Test Procedure

4.2.1 Click the "**Specimen**" button on the instrument display screen to open the door of the instrument and enter the new experiment task setting interface.
4.2.2 Put the prepared consumables into the designated position of the instrument.
4.2.3 Enter specimen information, 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.
4.2.4 Click "**Submit**" to submit the experimental task and "**OK**" to run the instrument.

Reading test results

1. Result analysis (Refer to user manual of each instrument for the settings)
Results will be saved automatically when reactions are completed. Adjust Start, End and Threshold values of Baseline of the graph according to analysis results (Users can adjust the values according to the actual situation. Start value can be set between 3-15, and End value between 5-20. Adjust the amplification curve of negative control to be flat or below threshold). Click “**Analyze**” to implement the analysis and make sure each parameter satisfies the requirements given in “5. Quality Control”. Go to “**Plate**” window to record Ct value.

2. 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 repeated.

	MH-MG-TV-Positive Control	MH-MG-TV-Negative Control
Ct Value	Ct≤35 at FAM, HEX/VIC, ROX and CY5 channel	Ct>39 or no display at FAM, HEX/VIC, ROX and CY5 channel

Reference Range

Through the research on cut off value, the Ct cut off value of target gene is determined to be 40, the Ct cut off value of Internal Control is determined to be 40.

Explanation of Detection Result

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. The table below describes the result interpretation concerning the use of the controls mentioned above. The end user is required to review fluorescent curves before final interpretation. All the positive curve should be typical S-shape amplification curve or without plateau for weakly positive samples (38 ≤ Ct ≤ 40).

FAM	HEX/VIC	ROX	IC (CY5)	Results
+	Not considered	Not considered	Not considered	MG Positive
Not considered	+	Not considered	Not considered	MH Positive
Not considered	Not considered	+	Not considered	TV Positive
-	-	-	+	MG, MH, TV Negative
-	-	-	-	Invalid

Result of (-): Ct value >40 or No Ct; Result of (+): Ct value ≤ 40.
Invalid Result: There is no typical S-shape amplification curve or Ct > 40 or No Ct detected for Target (FAM, HEX/VIC, ROX) and Internal Control (CY5), indicating that the specimen concentration is too low, or there are interfering substances that inhibit the reaction. If upon retest, the result is invalid again, another fresh sample should be collected and tested.
For technical assistance of test results, please contact local distributor or email: info@sansure.com.cn.
Note: For samples with positive results, the results of internal control are not required.

Limitations of Detection Method

1. Test results of the diagnostic kit can only be used as an aid in clinical diagnosis. Symptoms and physical signs, disease history, other laboratory examinations and therapeutic reactions of the patients should be comprehensively considered for the clinical diagnosis and treatment.
2. False positive and false negative results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology.
3. Mutation in the target sequence or changes caused by other reasons in the sequence may lead to false negative results.
4. Improper reagent storage may lead to false negative results.
5. Unverified interfering substances or PCR inhibitors may lead to false negative or invalid results.
6. Use of this assay is limited to personnel who are trained in the procedure.

Product Performance Index

1. Accuracy: This kit detects the positive reference products of the enterprise, and the results are all positive, and the negative reference products of the enterprise are tested, and the results are all negative.
2. Specificity: This kit is compatible with Escherichia coli, Enterococcus faecium, Enterococcus faecalis, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii, Candida albicans, Ureaplasma urealyticum, Neisseria gonorrhoeae, human genomic DNA and other positive samples have no cross-reaction. The negative reference products of the testing companies were all negative.
3. Limit of detection: The limit of detection of this kit is 500 copies/mL.
4. Precision: The coefficient of variation (CV%) of Ct value of the within-run precision is ≤ 5%.
5. Possible interfering substances in specimens: Common drugs for the treatment of genital tract infections (such as doxycycline, azithromycin, moxifloxacin, levofloxacin, etc.) do not significantly interfere with the test results of the kit at normal dose concentrations.

Bibliography

[1] Xinying L, Huachun Z. Interpretation of 2021 CDC guidelines on the diagnosis and treatment of Mycoplasma genitalium. Journal of Diagnosis and Therapy on Dermato-vener. 2021,28(06).
[2] Baumann L, Cina M, Egli-Gany D, et al. Prevalence of Mycoplasma genitalium in different population groups: systematic review andmeta-analysis. Sex Transm Infect. 2018;94(4):255–62.
[3] Huifang Z, Jianzhong Z. Establishment of real-time PCR assay to detect Mycoplasma hominis. Disease Surveillance. 2012,27(03).
[4] Brooke Webb, Andrea Crampton. Increased diagnostic yield of routine multiplex PCR compared to clinician requested testing for detection of Trichomonas vaginalis. Pathology 2021 Feb;53(2):257-263.

Symbols

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
CONTROL -	Negative Control	CONTROL +	Positive Control
ENZYME MIX	Enzyme Mix	VERSION	Version
PRE	Prepackaging	PCR MIX	PCR Mix
	Do not re-use	21 PAP	PAP21: Not corrugated cardboard
	Keep away from light	UDI	Unique device identifier
EC REP	Authorized representative in the European Community	CE	This product fulfills the requirements of the European Directive 98/79/EC for *in vitro* diagnostic medical devices.

Contact information

Sansure Biotech Inc.
Add.: No. 680, Lusong Road, Yuelu District, 410205 Changsha, Hunan Province, PEOPLE’S REPUBLIC OF CHINA
Tel.: +86-731-88883176
Fax: +86-731-88884876
Web: www.sansureglobal.com

Obelis s.a.
Bd. Général Wahis 53, 1030 Brussels, BELGIUM
Tel.: + (32) 2.732.59.54
Fax: + (32) 2.732.60.03
E-Mail: mail@obelis.net