RepoDx®

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***Mycoplasma genitalium*（MG）DNA qPCR Detetion Kit**

**Package insert**

**For In Vitro Diagnostic Use Only**

REF

**For use with *ABI 7500 Real-Time PCR System***

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## 1. Intended Use

The kit is suitable for qualitative detection of *Mycoplasma genitalium* DNA in male urethral secretions and female urethral /vagina secretion samples. It is only used for clinical auxiliary diagnosis. In clinical application, the clinician should also consider the actual situation of each case, and the test conclusion of this kit cannot be used as the only basis for clinical diagnosis.

**Indications:** Urogenital tract inflammation caused by infection with *Mycoplasma genitalium*.

## 2. Principle

The kit is based on the principle of fluorescence PCR method and Taqman fluorescence probe technique, PCR amplification of target DNA and detection of cleaved dual-labeled oligonucleotide detection probe specific to the target DNA. The Master Mix reagent contains primers specific to MG DNA and human genomic DNA. The detection of amplfied DNA is performed using target-specifc dual-labeled oligonucleotide probes that permit independent identification of MG amplicon.

## 3. Kit Contents

| NO. | Label | Main Compositions | Number | Volume /tube |
|---|---|---|---|---|
| 1 | MG PCR Mix | Probes, dNTP, Mgcl2, DNA Polymerase, UDG enzyme | 1 | 1.44ml |
| 2 | MG Positive control | 2000copies/μL MG target gene plasmid, 2000copies/μL human genomic target gene plasmid | 1 | 0.20ml |
| 3 | MG Negative control | 2000 copies/μL human genomic target gene plasmid | 1 | 0.20ml |
| 4 | Package insert | // | 1 | 1 sheet |

**Notes：** Different batches of components cannot be mixed
Self-made normal saline
This kit does not contain sampling and extraction components

## 4. Reagent Storage and Handling

- This kit must be stored below -18 °C and protected from light.
- Repeated freeze-thaw cycles should be less than 6 times.
- The kit can be stored for 12 months before opening. Once opened, any unused portion can be stored up to 1months.

## 5. Specimens Applied to Test

5.1. Male urethral secretions and female genital tract secretions.

5.2 Swab sample collection

5.2.1 The sampling method is referred to the "Venereal Disease Laboratory Operation Manual".

5.2.2 Suitable swab types: cotton swabs, calcium alginate swabs, polyester fiber swabs.

5.3 The sampled swab should be placed in a sample collection tube containing 1mL of normal saline, fully oscillated to wash the swab and dried by squeezing against the tube wall.

5.4 Known interfering substances: hemoglobin, feminine cleansing, nonoxynol ether suppository, lubricating fluid, mucin.

5.5 Storage and transportation of samples:

Samples can be used immediately

or stored at 2-8 ° C (no more than 7days)

or stored below -18 ° C (no more than 12months)

or stored below -70° C (long-time storage)

The sample should be transported by curing with ice or sealing with foam boxes and ice packs. The frozen samples should be thawed at room temperature. Please avoid repeated freezing and thawing.

## 6. Instructions for Use

### 6.1 Preparation of the Reaction Mix（Reagent preparation zone）

6.1.1 Take MG PCR Reaction Mix out from the kit, thawed at room temperature, vortex lightly and briefly centrifuge for use

6.1.2 Prepare PCR Reaction buffer based on number of Samples (n) and add 45μL MG PCR Mix into each reaction tubes (sum to n+2 tubes), of which '2' refers to MG positive and negative controls.

### 6.2 Sample Preparation（Sample preparation zone）

It is recommended to use a validated nucleic acid extraction reagent (Record No.: Sutong Machinery No. 20180035), and perform nucleic acid extraction according to the instructions.

6.2.1 Transfer 200μL of the solution to a 1.5 mL tube containing 1000μL of normal saline, centrifuge at 12000 rpm for 2 minutes, and discard the supernatant using a pipette.

6.2.2 Add 1 mL of normal saline to the 1.5 mL tube, shake until there is no visible precipitate, centrifuge at 12000 rpm for 2 minutes, and discard the supernatant using a pipette.

6.2.3 Add 50μL MG positive and negative sample to two new 1.5ml tubes separately.

6.2.4 Add 50μL DNA extraction buffer to tubes with sample (6.2.2), and tubes with positive and negative controls (6.2.3).

**Notes:** DNA extraction solution should be mixed upside down timely before and during use.

6.2.5 Scatter the sediment by the vortex shaker. Put it into 85±5°C dry bath or water bath for 10±2 minutes. Centrifuge at 12000rmp for 2 minutes, and the supernatant is ready for PCR assay.

**Notes:** The DNA sample is deserved to PCR immediately, or stored below -18 °C (no more than 18 months, no more than 3 times of freezing and thawing). When used again, the extracted DNA should be fully melted, mixed and centrifuged transiently. The supernatant can be taken for PCR assay.

### 6.3 Sample Adding (Sample preparation zone）

Add MG negative control, MG positive control and the tested sample (5μL each) into the prepared PCR tube. After capping the tube tightly, centrifuge in a low speed transiently.

**Notes:** Do not touch the sediment at the bottom of the tube when the sample is pipetted.

### 6.4 PCR Amplification (PCR amplification zone）

6.4.1 Sample setting: Set the serial number of samples, positive and negative control according to the type of the sample.

6.4.2 Fluorescence detector selection: Select two detectors for each sample which are FAM and JOE. Set both Quencher and Passive Reference as None.

6.4.3 Reaction conditions setting:

| steps | | Temperature (℃) | Time (seconds) | cycles |
|---|---|---|---|---|
| 1 | UDG enzyme reaction | 37 | 300 | 1 |
| 2 | Pre-denaturation | 95 | 300 | 1 |
| 3 | denaturation | 95 | 10 | 45 |
| | Anneal, extend and detect fluorescence | 60 | 60 | |
| MG gene: FAM; Internal control: JOE | | | | |

Reaction volume: 50μL

6.4.4 Save files, run program

**6.5 Data Analysis**

After the reaction, set the baseline within 3-15 (Baseline Cycle can vary within a certain range according to the actual situation). The principle of the fluorescence threshold setting is that the threshold line just exceeds the highest point of the negative control amplification curve (irregular noise line) and the Ct value is shown as 'Undetermined'. The general MG fluorescence threshold is 2.0E+04~2.0E+05, and the IC fluorescence threshold is 1.0E+04~1.0E+05. The recommended MG fluorescence threshold is 5.0. E+04 and IC fluorescence threshold is 2.0E+04. The results can be automatically analyzed using the instrument software.

**6.6 Quality Control**

6.6.1 MG Negative control: There was no obvious S amplification curve in FAM detector and Ct result of FAM is shown as 'Undetermined'; There is a standard S amplification curve in JOE detector, Ct value of JOE is no more than 34.00.

6.6.2 MG positive control: There exists a standard S amplification curve in both FAM and JOE detectors, the Ct value of FAM is no more than 32.00 and the Ct value of JOE is no more than 34.00.

6.6.3 The result for positive and negative control must conform to explanation in 6.6.1 and 6.6.2, otherwise PCR failure and you need repeat this experiment.

**7. Reference Range**

7.1 There is no obvious S amplification curve in FAM detector, and the Ct value is shown as 'Undetermined'. The result is negative for MG.

7.2 There is obvious S amplification curve in FAM detector, and the Ct value is more than 38.00, but no more than 45.00. The result is in the gray zone of detection for MG

7.3 There is obvious S amplification curve in FAM detector, and the Ct value is no more than 38.00. The result is positive for MG

**8. Interpretation of Data**

Under the premise of effective experiment, the results of the detection of *genital mycoplasma* in the sample are judged as follows：

| JOE | FAM | Result |
|---|---|---|
| Ct<37.00 | Ct≤38.00 | Positive for MG |
| | 38.00<Ct≤45.00 | Detection gray zone |
| | Undetermined | Negative for MG |
| 37.00≤Ct | Ct≤38.00 | Positive for MG |
| | 38.00<Ct≤45.00 | Detection gray zone |
| | Undetermined | repeat sample collection |
| Undetermined | Ct≤38.00 | Positive for MG |
| | 38.00<Ct≤45.00 | Detection gray zone |
| | Undetermined | PCR failure |

It is recommended to repeat the experiment, when the result shows detection grey zone. If the repeated experiment of MG detector shows that Ct value is no more than 45.00, the result is positive, otherwise, the result is negative.

It is recommended to dilute the DNA extracted from the sample by 10 times and repeat the experiment, if the result is PCR failure. If there is a signal, the result is determined by the signal above, otherwise, re-sampling.

**9. Procedural Limitations**

9.1 The kit is used for clinical auxiliary diagnosis. The clinical treatment of the patients should be combined with other medical examinations.

9.2 False negative or false positive results may occur when samples are collected, handled, transported, or stored improperly.

9.3 Possibility analysis of false negative results

Unreasonable sample collection, transfer, handling, or excess-low sample concentrations may lead to false negative results.

Variations in the target sequence to be tested or sequence changes resulting from other causes may lead to false negative results.

It will lower the possibility of false negative results when sampling of the same patient in different times and in multi-sites.

**10. Performance Indexes**

10.1 The lowest detection limit of this kit is 50 Copies/Reaction.

10.2 The coincidence rate was 100% when tested by negative and positive corporate reference products.

10.3 The coefficient of variation is less than 5% within the same lots.

10.4 Specific tests showed that there is no cross reaction with the other pathogens of the genital and urinary tract secretions (including CT, UU, HSV2, HPV, MH, NG).

**11. ⚠ Warnings and Precautions**

11.1 The kit is for in vitro diagnostic use only.

11.2 Read this instruction carefully before using. The operator should have received professional training in gene amplification or molecular biology testing, and have relevant qualifications. The laboratory should have reasonable biosafety preparedness facilities and protective procedures.

11.3 The entire experimental process should be carried out in three areas (reagent preparation area, sample processing area, PCR amplification area). These three areas are independent. Equipment, supplies and work clothes are dedicated for use in designated zones without cross-use; Please clean the workstations immediately after the experiment.

11.4 The product should be fully melted at room temperature, mixed and centrifuged at low speed transiently.

11.5 The positive and negative controls should be set at each test. Do not mix reagents of different batches. Use the kit before the expiry day.

11.6 The samples should be as fresh as possible, and the extraction process should be strictly protected against DNA enzyme contamination and DNA degradation caused by improper operation.

11.7 The DNA sample should be kept below -18° C, fully melted at room temperature, mixed and centrifuged before use.

11.8 Try to avoid air bubbles when dispensing the reaction solution. Before placing it into the instrument, check whether the PCR tubes are tightly closed to avoid contamination of the instrument and the environment.

11.9 The tested samples should be seen as contagious substances. The operation and handling of it should meet the requirements of related regulations.

**12. References**

12.1 Svenstrup HF,et al.Development of a quantitative real-time PCR assay for detection of *Mycoplasma genitalium*.J Clin Microbiol. 2005 Jul; 43(7):3121-8.

12.2 Chloé Le Roy, Sabine Pereyre, Cécile Bébéar. Evaluation of Two Commercial Real-Time PCR Assays for Detection of *Mycoplasma genitalium* in Urogenital Specimens. J Clin Microbiol.2014 March;52(3):971-3.