Neisseria gonorrhoeae, Ureaplasma urealyticum Nucleic Acid Detection Kit (Fluorescent PCR)

Instructions for Use

Effective Date: Jan 10, 2022

For professional use only.

For in vitro diagnostic use only.

IVD -25℃ -15℃ CE

REF BSJ22S1

INTENDED USE

Neisseria gonorrhoeae, Ureaplasma urealyticum Nucleic Acid Detection Kit (Fluorescent PCR) is used for the qualitative detection of Neisseria gonorrhoeae and Ureaplasma urealyticum nucleic acid in male urethral swabs and female cervical swabs from suspected cases. The kit is used for the auxiliary diagnosis and epidemiological surveillance of Neisseria gonorrhoeae and Ureaplasma urealyticum infection, cannot be used as the basis for the diagnosis or exclusion of cases alone.

For professional use only.

For in vitro diagnostic use only.

PRINCIPLE

The kit uses real-time fluorescent PCR technology, primers and Taqman probes are designed in the conserved regions of Neisseria gonorrhoeae (NG) and Ureaplasma urealyticum (UU). The probe specifically binds to a DNA template in the middle of the amplified region of the primers, during the PCR extension reaction, the exonuclease activity of the Taq enzyme cleaves the 5' terminal fluorescent group from the probe, leaving it free from the reaction system, because of detaching from the shielding of the 3' end fluorescence quenching group, thereby emitting fluorescence which can be detected by the instrument, realizing automatic detection of the pathogen nucleic acids in the fully enclosed reaction system. At the same time, internal gene was act as a non-competitive internal control during the extraction and detection process. In addition, UDG enzyme and DUTP anti-contamination measures were added in this kit to avoid false positive results.

The kit detection system uses three fluorescent groups labeled with different colors, and is independently detected in different wavelength regions. The FAM, HEX and CY5 channels are selected for detection of Neisseria gonorrhoeae, Ureaplasma urealyticum and internal reference. At the same time, the kit was set with positive

and negative control, and the negative control was internal reference gene plasmid, positive control was Neisseria gonorrhoeae and Ureaplasma urealyticum DNA diluted with internal reference gene plasmid.

COMPONENTS

Components		Main Ingredients	BSJ22S1 32 tests/kit
Amplification reagent	PCR Buffer	dNTP, Mg^{2+}, Tris Taq enzyme, UDG enzyme	416μL×1
	Primer Probe mix	Primers and Probes	64μL×1
Control	Positive Control	Plasmid DNA fragment of NG, UU diluted with internal reference gene	500μL×1
	Negative Control	Internal reference gene plasmid	500μL×1

a. *The positive control and negative control need to be set to monitor the test body and the operating environment; the negative control and positive control have been packaged in the kit.*

b. *The components of different lots cannot be mixed for use.*

c. *Equipment or materials required but not provided: Specimen collection kits, Nucleic acid extraction kits; PCR tubes and caps, etc.*

APPLIED INSTRUMENT

The kit can be applied to Hangzhou Bioer Technology Co., Ltd. Fluorescence Quantitative Detection System, LineGene 9600 Plus (FQD-96A) or QuantGene 9600 (FQD-96C). The instrument should contain at least three channels of FAM, HEX and CY5.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use (IVD). For professional use only.
- Read the Instructions for Use carefully before operation. The appropriate operations from specimen collection, storage and transportation, and laboratory test should be strictly manipulated in line with relevant regulations of biosafety and molecular laboratory management.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC). Handling samples in the biosafety cabinet, to ensure operator safety and avoid environmental pollution. Place harmful samples

and reagents properly. Discard the waste in special containers. Wipe the table, centrifuge, and equipment frequently with 1.0% sodium hypochlorite or 70 % ethanol. The laboratory and the ultra-clean workbench need UV-treated periodically and after each experiment.

- All the articles in each district are for special use which cannot allow to be exchanged for avoiding pollution. The workbench should be cleaned immediately after the completion of each experiment.
- Use disposable gloves without fluorescent substances, disposable special centrifuge tubes, etc.
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents, while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- The false positive or negative testing result can be led by poor quality of specimen, incorrect operations in sample collection, transportation or laboratory processing, or limitation of the technology. Operator should understand well the principles of the procedures and its limitation in performance in advance and avoid any potential mistakes intentionally.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product.
- Separate laboratory areas are recommended to performing predefined procedures of the assay. Area I: Reagent preparation area-reagent required for preparing amplification. Area II: Sample processing area-processing of tested samples and controls. Area III: PCR detection region-PCR amplification detection.
- The separation of the reaction solution should avoid the generation of air bubbles as far as possible. Before the amplification, pay attention to check whether the caps of each reaction tube are tightened to avoid contaminating instrument.
- Samples should be completely put into the reaction solution when adding samples. No samples should adhere to the tube wall and the cap should be tightened as soon as possible after adding samples.
- Both the kit and nucleic acid products are all stored at -20 °C. Before using, they should be fully thaw out at room temperature, mixed and then instantaneous briefly centrifugation. RNA should be maintained on cold-block or on ice during preparation and use to ensure stability.
- After amplification, please take out the reaction tube immediately, seal it in the special plastic bag, put it in the designated place, and wait for unified treatment.
- Dispose of used / unused kit reagents and human specimens according to local, state, and federal regulations.

STORAGE AND PERIOD OF VALIDITY

1. The kit should be stored at -25°C ~ -15°C away from light, and avoid repeated freeze-thaw. The kit can be stored for 3 days at 2-8 °C after opening.
2. The kit can be stored for up to 12 months if all components are kept in the manner above. Do not use after the stated expiry date.
3. The kit can be transported in foam box sealed with ice bags or dry ice at 2-8°C or lower.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORTATION

1. Specimens: Male urethral swab and female cervical swab.
2. Collection: Specimens of all types are collected by conventional methods.
3. Storage: It is recommended that specimens be processed as soon as possible after collection. If specimens are not processed immediately, they should be stored at 2-8 °C for up to 3 days. If a delayed processing is expected, the specimens should be stored at -25°C ~ -15°C for no more than 3 months. Specimens should not be frozen and thawed frequently.
4. Transportation: Specimen should be packaged and transported in accordance with the requirements of infectious agents. Specimen should be transported with 0°C curling bottle or foam box sealed with ice.

SPECIMEN PRETREATMENT (SPECIMEN DISPOSAL AREA)

Follow the instructions of the nucleic acid extraction and purification kit.

For Automatic extraction: It is recommended to use MagaBio plus Virus DNA/RNA Purification Kit III (Cat: BSC86) to purify the nucleic acid with Gene Pure Series Nucleic acid extractor.

⚠ **Note: The negative control, positive control and unknown specimen need to be tested in the same experiment.**
It's recommended to prepare the reagent ahead of specimen pretreatment to ensure that the reagents are not contaminated.

USING OF THE KIT PCR REACTION (PCR TEST AREA)

1) Reagent prepares

Thaw out the reagents at room temperature. Mix gently and centrifuge all reagents for a few seconds.

Make RT-PCR reagents according to the quantity of specimens and controls as below (N means the number of **specimens and controls**):

Reagents	PCR Buffer	Primer Probe Mix
Dosage/ test	13μL	2μL
Dosage	(N+1) ×13μL	(N+1) ×2μL

Distribute 15 μL mixed PCR reagents into each PCR tubes, and then transfer the

reaction plate to sample processing area.

2) Adding sample

Add 10μL negative control, 10μL extracted product, 10μL positive control into different PCR tubes. Cap the PCR tubes immediately to prevent cross contamination.

Note: Do not label on the scanned area of the reaction tubes!

3) PCR reaction

Place the reaction tubes on a PCR instrument.

It is recommended to choose FAM, HEX and CY5 channels to collect fluorescent signals.

Set fluorescent signals detecting at 60°C, liquid volume is 25μL.

Set reaction procedure as below:

Step	Temperature	Duration	Number of cycles
1	50°C	2 min	1
2	95°C	1 min	1
3	95°C	10 sec	40
	60°C	20 sec	

QUALITY CONTROL STANDARDS

Expected performances of controls are as below:

Control	FAM (NG)	HEX (UU)	CY5 (IC)	Interpretation of Test Results
Positive Control	All the three channels yield Ct Value≤30 with "S" amplification curve			All requirements are met in the same experiment, indicating that the experiment is valid, otherwise it is invalid.
Negative Control	No Ct Value		Ct Value≤30 with "S" amplification curve	

RESULT ANALYSIS AND JUDGMENTS

Expected performances of specimens are as below:

FAM (NG)	HEX (UU)	CY5 (IC)	Result Judgment
Ct Value ≤36.1, with "S" curve	Ct Value ≤37.6, with "S" curve	No specific requirement	NG and UU virus nucleic acid Positive.
Ct Value ≤36.1, with "S" curve	Ct Value >37.6, or no Ct Value	No specific requirement	NG nucleic acid Positive.
Ct Value >36.1,	Ct Value ≤37.6,	No specific	UU nucleic acid Positive.

or no Ct Value	with “S” curve	requirement	
Ct Value >36.1, or no Ct Value	Ct Value >37.6, or no Ct Value	Ct Value ≤38, with “S” curve	NG and UU nucleic acid Negative.
Ct Value >36.1, or no Ct Value	Ct Value >37.6, or no Ct Value	Ct Value > 38; with “S” curve; or no Ct Value	Invalid, repeat test.

NOTE:

1. When the specimen test result is suspicious, it needs to be re-extracted and tested again, and the re-test results are still within this range, and it is positive. Otherwise, it is negative.
2. Both NG and UU test results are positive, which indicates the multiple pathogens infection at the same time.

LIMITATIONS

1. The kit is only used for the qualitative detection the presence of NG and UU in specimens. Neither the quantitative value nor the rate of increase can be determined by the qualitative test.
2. The results of the test are just for clinical reference. The test should not be used as sole criteria for diagnosis. Results should be considered in conjunction with the clinical information and other data available to the physician. Negative result does not preclude *NG* or *UU* infection and should not be used as the sole basis for the diagnosis, treatment or other patient management decisions. The result should not use for monitoring treatment of NG or UU.
3. An incorrect result may occur by incorrect operation in sample collection, transportation or processing.
4. A false negative result may occur: (a) Unreasonable sample collection, processing, transportation and storage conditions, and low concentration of target genes in samples; (b) Variation of Neisseria gonorrhoeae and Ureaplasma urealyticum gene sequences or other causes; (c) Unverified other interfering substances, such as endogenous or exogenous substances introduced into the sample.
5. False-negative results may occur if inadequate numbers of organisms are present in the specimen due to improper collection, transport or handling.
6. A false positive result may occur by aerosol pollution or operating errors.
7. For any suspected cases, it’s recommended to re-extract and/or retest with a new lot of kit or confirmed with another available method.

PERFORMANCE INDICATORS

Performance validation was conducted with Bioer’s Fluorescence Quantitative

Detection System, LineGene 9600 Plus (FQD-96A) or QuantGene 9600 (FQD-96C). Since clinical positive specimen was unavailable, positive control was prepared for the validation. The positive control was Plasmid DNA fragment, which contains the target fragments of Neisseria gonorrhoeae, Ureaplasma urealyticum.

★ Limit of Detection (LoD): The positive reference standard was diluted into 1000 copies/mL, 500 copies/mL, 250 copies/mL and 100 copies/mL, then were tested by 3 lots of kits. Each concentration was tested with 20 replicates. The testing data demonstrated that the kit can detect NG and UU with detection rate equal or higher than 95% at the concentration equal or higher than 500 copies/mL.

★ Analytical sensitivity: 8 positive reference standards and 8 negative reference standards were tested by 3 lots of kits. The positive coincidence rate was 100%, and the negative coincidence rate was 100 %.

★ Analytical specificity: No cross reactivity has been observed by testing the clinical positive specimens such as HPV16, HPV18, HSV II, Treponema pallidum, Mycoplasma hominis, Staphylococcus aureus, Escherichia coli, Gardnerella vaginalis, Candida albicans, Trichomonas vaginalis, Lactobacillus frioris, Adenovirus, Human cytomegalovirus, Streptococcus b, Lactobacillus casei, Human genomic DNA.

★ Analytical specificity: The potentially interfering substances were spiked into positive control, then tests were performed by 1 lots of kits. The tested substances blood, mucin, cervical mucus, vaginal lubricant, anti-inflammatory lotion, azithromycin and levofloxacin showed no influence on the detection.

★ Precision: Positive controls and low positive controls reference were tested by 3 lots of kits with 10 replicates by 2 operators for 20 days. The results showed that the variation coefficient (CV) of within-day, between-day, within-batch and between-batch were less than 5%.

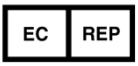
REFERENCES

[1] CW Geraats-Peters et al. Specific and Sensitive Detection of Neisseria gonorrhoeae in Clinical Specimens by Real-Time PCR. J Clin Microbiol,2005.43(11):5653-5659.

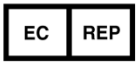
[2] J Yi et al. Detection and biovar discrimination of Ureaplasma urealyticum by real-time PCR.Mol. Cell Probes,2005,19:255-260.

[3] CH Boel et al. Evaluation of Conventional and Real-Time PCR Assays Using Two Targets for Confirmation of Results of the COBAS AMPLICOR Chlamydia trachomatis/Neisseria gonorrhoeae Test for Detection of Neisseria gonorrhoeae in Clinical Samples. J Clin Microbiol,2005,43(5):2231-2235.

SYMBOL DESCRIPTION

	Manufacturer		Catalogue number
	CE mark		Authorized representative in the European community
	Batch code		Consult instructions for use
	In vitro diagnostic medical device		Temperature limitation
	Caution		Use by date
	Positive Control		Negative Control

HANGZHOU BIOER TECHNOLOGY CO., LTD.
1192 BinAn Rd., Binjiang District, 310053 Hangzhou, China
Website: www.bioer.com.cn
TEL: +86-571-87774575 FAX: +86-571-87774565



MedNet EC-REP GmbH
Borkstrasse 10, 48163 Muenster, Germany

TECHNICAL SUPPORT

Please dial phone number +86-571-87774567-5211 or 87774575, by fax to +86-571-87774553, or by email to reagent@bioer.com.cn.