Group B Streptococcus Nucleic Acid Detection Kit (Fluorescent PCR)

Instructions for Use

Effective Date: Jan 10, 2022

For professional use only.

For in vitro diagnostic use only.

REF BSJ07M1

INTENDED USE

Group B Streptococcus Nucleic Acid Detection Kit (Fluorescent PCR) is an in vitro diagnostic test for qualitative detection of Group B Streptococcus in anal or vaginal swab.

The kit is used for the auxiliary identification diagnosis and epidemiological surveillance of Group B Streptococcus infection, cannot be used as the basis for the diagnosis or exclusion of cases alone.

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PRINCIPLE

Group B Streptococcus Nucleic Acid Detection Kit (Fluorescent PCR) is based on in vitro Real time PCR combining fluorescent probing. Primers and a sequence-specific fluorescence probe were designed tailored to CAMP gene of Group B Streptococcus. The probe of Group B Streptococcus is oligonucleotide attached fluorophores at the 5' end with FAM as reporter and 3’ end with quencher. In a meantime, specific primers and probe on basis of human β-Globin gene were developed as internal reference with fluorophores CY5 attached at 5’ end as reporter. During the PCR procedures, the DNA polymerase cleaves the probe at the 5’ end and separates the reporter dye from the quencher dye when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. Monitoring the fluorescence intensities during Real Time allows the qualitative detection of Group B Streptococcus in specimens.

COMPONENTS

Components	BSJ07M1	Main Ingredients

Kit size		48 tests/kit	
Amplification reagent	PCR reaction buffer	624μL×1	dNTP, Mg^{2+}, Tris, DNA polymerase
	Detection solution	336μL×1	specific primers, and probe
Control	Positive control	500μL×1	Solution containing fragment of GBS gene
	Negative control	500μL×1	Solution containing internal reference gene plasmid

a. *The positive control and negative control need to be set to monitor the test body and the operating environment; the negative 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. pipette and pipette tips, vortex, etc.*

APPLIED INSTRUMENT

The kit can be applied to Bioer's Fluorescent Quantitative Detection System, QuantGene 9600 (FQD-96C) and LineGene 9600 Plus (FQD-96A).The instrument should contain channels of FAM and CY5.

WARNINGS AND PRECAUTIONS

- For professional in vitro diagnostic use (IVD). Do not use after expiration date.
- Read the package insert carefully before performing the test. 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 pipette by mouth. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled. Wash hands thoroughly after handling specimens and kit reagents.
- 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. Avoid DNA contamination of reagent.
- 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.
- The extracted nucleic acid sample should be used immediately after extraction.
- 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°C ~ 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 uses after the stated expiration date.
3. The kit can be transported in foam box sealed with ice bags or dry ice at not higher than 8°C.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORTATION

1. Specimen: Anal swab specimens or vaginal swab specimens
2. Collection: Specimens can be 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 24hours. If a delayed processing is expected, the specimens should be stored at -70°C or lower. Specimens should not be frozen and thawed frequently.

SPECIMEN PRETREATMENT (SPECIMEN DISPOSAL AREA)

Follow the instructions of the nucleic acid extraction and purification kit.
It is recommended to use **MagaBio plus Virus DNA /RNA Purification Kit III** (BSC86) to purify the nucleic acid. The Gene Pure Series Nucleic acid extractor is recommended to use to extraction nucleic acid automatically.

⚠ **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. Gently mix and centrifuge all reagents for a few seconds.
Make PCR reagents according to the quantity of specimens and controls as below (N means the number of **specimens and controls**. An extra blank control is highly recommended to prevent the loss of reaction mix.):

Reagents	PCR reaction Buffer	Detection solution
Dosage/ test	13μL	7μL
Dosage	(N+1) ×13μL	(N+1) ×7μL

Distribute 20 μL mixed PCR reagents into each PCR tubes, and then transfer the reaction plate to sample processing area.

2) Adding sample

Add 5μL negative control, 5μL extracted product, 5μL positive control into different PCR tube. 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 and Cy5 channels to collect fluorescent signals.

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

Set reaction procedure as below:

Step	Temperature	Duration	Number of cycles
1	37°C	2 min	1
2	95°C	1 min	1
3	95°C	5 sec	45
	60°C	10 sec	

QUALITY CONTROL STANDARDS

Expected performances of controls are as below:

	FAM	CY5	Interpretation of Test Results
Positive Control	Ct Value ≤35	Ct Value ≤35	All the requirements should meet in the same experiment, otherwise it is invalid.
Negative Control	No Ct Value	Ct Value ≤35	

RESULT ANALYSIS AND JUDGMENTS

Expected performances of specimens are as below:

Ct Value for FAM	Ct Value for CY5	Result Judgment
Ct Value ≤40, with "S" curve	Ct Value ≤35	Group B Streptococcus nucleic acid Positive
No Ct		Group B Streptococcus nucleic acid Negative

If the CT ＞ 40, repeat the experiment. If the CT appears, it will be judged as positive; otherwise, it will be judged as negative.

NOTE:

1. For the fluorescence signals of CY5, if the Ct value is more than 38, the test is invalid.

2. For suspicious or invalid test result, retest the same processed sample first. If the test is invalid / suspicious upon retesting with the processed sample, re-process another aliquot of the same specimen.

LIMITATIONS

1. The kit is an in vitro nucleic acid amplification test for the qualitative detection the presence of Group B Streptococcus. 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.
3. The quality of specimen obtained is of extreme importance. Good laboratory practices and changing gloves between handling patient specimens are recommended to avoid contamination of specimens or reagents. An incorrect result may occur by incorrect operation in sample collection, transportation or processing.
4. A false negative result may occur by very low concentration of target virus in the specimens, mutations within the viral genome covered by the kit's primers and/or probe, and unproved external interference factors.
5. Due to the limitation of detection threshold and detection range, negative results do not preclude infection with Group B Streptococcus. The test result should not be the sole basis of a patient management. Follow up testing/ analysis should be performed.
6. False negative or false positive result may occur by incorrect operation in sample collection, transportation, processing, aerosol pollution or operating errors.

PERFORMANCE INDICATORS

Performance validation was conducted with the Quantgene 9600 series fluorescent quantitative PCR detection system from Bioer.

The kit can be applied to Bioer's Quantgene 9600 fluorescent quantitative PCR detection system, Linegene 9600 fluorescent quantitative PCR detection system and fluorescent quantitative PCR detection systems from other company with at least channel of FAM and CY5.

Since positive specimen of Group B Streptococcus was unavailable, positive control was prepared for the validation. The positive control was trace back to ATCC,which contains GBS-III, GBS-V, GBS-Ia, GBS-Ib.

★ Limit of Detection (LoD): The positive reference standard was diluted into 1000 copies/mL, 500 copies/mL, 200 copies/mL and 100 copies/mL, then were tested by 3 lots of kits. Each control was tested with 20 replicates. The testing data demonstrated that the kit can detect the Group B Streptococcus with detection rate equal or higher than 95% at the concentration equal or higher than 200 copies/mL.

★ Analytical sensitivity: 4 positive reference standards and 10 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 for specimens list below: Herpes simplex virus type II, Legionella haemophilus, Ureaplasma urealyticum, gonococcus, Candida albicans, Klebsiella pneumoniae pneumonia, Chlamydia trachomatis, Human papillomavirus, Bacillus pertussis, Staphylococcus aureus

★ Analytical specificity: The potentially interfering substances: Blood, cervical mucus, human lubricant, vaginal wash, miconazole nitrate, phenylmercury acetate were spiked into weak positive controls; the tests were performed by 3 lots of kits. The tested substances showed no influence on the detection.

★ Precision: Positive controls and low positive controls 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-lot, between-lots, between-operators and between-days were less than 5%.

REFERENCES

[1] State Food and Drug Administration Decree No. 6 "Guiding Principles for the Preparation of Instructions for in vitro Diagnostic Reagents"

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[3] Baker C J , Edwards M S , Kasper D L . Role of antibody to native type III polysaccharide of group B Streptococcus in infant infection.[J]. Pediatrics, 1981, 68(4):544-549.

[4] Place K , Rahkonen L , Nupponen I , et al. Vaginal Streptococcus B colonization is not associated with increased infectious morbidity in labor induction[J]. Acta Obstetricia Et Gynecologica Scandinavica, 2021.

MBOL 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

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