GBS Test

Instructions for Use

For Professional Use

REF **GBS-P1-10**

For Use with FlashDx-1000-E System

CE IVD **For *In vitro* Diagnostic Use**

Revision A. May 2022

Proprietary Name

GBS Test

Common or Usual Name

GBS Test

Packing Specification

10 tests/box

Intended Use

GBS test is a rapid nucleic acid microarray-qPCR test intended for *in vitro* qualitative detection of Group B *Streptococcus* (GBS) in vaginal swab collected from individuals with or without symptoms, or other epidemiological reasons to suspect of GBS infection. The test is run using FlashDx-1000-E or other compatible FlashDx systems.

Positive results are indicative of the presence of GBS bacterial DNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out viral infection or co-infection with other bacteria. The agent detected may not be the definite cause of disease. Negative results do not preclude GBS infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Principle of the Procedure

GBS test is an in vitro diagnostic test for qualitative detection of nucleic acid from group B streptococcus bacteria. The test is performed on FlashDx-1000-E Automatic Nucleic Acid Detection System. The test is a single-use disposable cartridge containing lyophilized and liquid reagents for sample processing, DNA amplification and detection. Once user closes the lid after sample is added, cartridge becomes self-contained and this can minimize cross-contamination between samples.

A microarray of specific probes is prepositioned on inner surface of amplification chamber to detect specific amplification products. When target DNA is amplified, corresponding microarray spots can light up in an exponential manner similar to those during real-time qPCR such as TaqMan assay. This test uses conserved sequence of *cfb* and *sip* gene of *Streptococcus agalactiae* (GBS) as a targeted region. Within the cartridge, human RNase P (RP) primers and probes are also used as an internal control to monitor the full process starting from sample processing to amplification, microarray hybridization and signal detection.

User first transfers sample from recommended universal transport medium (UTM), 0.9% saline or validated transfer medium where sampling swab has been stored, into sample chamber of cartridge and close lid of chamber. The cartridge is loaded into the instrument loading bay according to on-screen instruction. Once user clicks to start the process, system automatically handles sample processing, amplification and detection process. The instrument collects fluorescence signals of each microarray spot in real-time during amplification and automatically generates test result through analysis of amplification curves (fluorescence signal change).

Main Components

Each box contains following components listed in Table 1:

Table 1：Main Components

Serial No.	Components	10 tests/Box		Main Ingredients
		Specification	Quantity	
1	Cartridge	1 test/bag	10 bags	Primers, probes, dNTPs, $MgCl_2$, DNA polymerase and buffer.
2	Disposable Transfer Pipettes	/	10 - 12	/

Storage Conditions and Handling

1. Store GBS cartridge at 2-8°C.

2. Do not open cartridge pouch until you are ready to perform test. Do not use the cartridge if the pouch is broken. Once the pouch is open, use the cartridge within 15 minutes.

3. See production date and expiration date on the label.

Compatible Instrument

FlashDx-1000-E and other compatible Automatic Nucleic Acid Detection System

Requirements for Samples

1. Specimen type: vaginal swab

2. Specimen Collection, Transport, and Storage

2.1 Vaginal swab collection procedure

Carefully insert the swab into vagina about 2 inches (5 cm) inside the opening of the vagina and gently rotate the swab clockwise for 10 to 30 seconds. Make sure the swab touches the walls of the vagina so that moisture is absorbed by the swab and then withdraw the swab without touching the skin. Place swab in the tube containing 3mL or 5mL of recommended UTM or saline. Rotate the swab 5 times rubbing it against the wall of the tube. Break swab at the indicated break line if necessary and cap the specimen collection tube tightly.

2.2 Requirements for sampling containers

Sampling swabs should be rayon swabs (polyester fiber, polyester or rayon head), flocking swabs (nylon fiber) or other non-cotton, non-calcium alginate swabs, and the handle should be made of non-wood materials. If customers choose to use any particular type of transport medium, please verify before using our products. It has been verified that preservation solutions such as saline solution and TE buffer can also be used.

Note: Inactivating UTM containing guanidine salt is NOT compatible with this test.

2.3 Sample transport and storage

Specimens should be tested within 30 minutes at room temperature and within 4 hours at 2-8°C. Specimens should not be stored for more than 48 hours at 2~8°C. Certain UTM will allow samples to be stored for longer period but they should be validated to avoid sample degradation. If it is anticipated that specimens may be tested after 24 hours, specimens should be stored at -70°C (not more than 30 days) and shipped with dry ice. Avoid repeated freezing and thawing. If proper care is not taken with sample, it can lead to potential false negative result. Necessary information such as sample number, date of onset and sample collection date should be collected and attached to sample during sample collection, shipping and storage.

Detection Method

Test cartridge contains all reaction reagents needed, and no additional reagent preparation is required.

1. Sample testing

1.1 Preparation of test cartridge

Open the aluminum foil pouch and take out test cartridge.

Note: Please verify that the test panel printed on pouch is for GBS test before opening. Once the aluminum foil pouch is open, it is necessary to load sample and run test cartridge within 15 minutes. Extended storage can affect test performance.

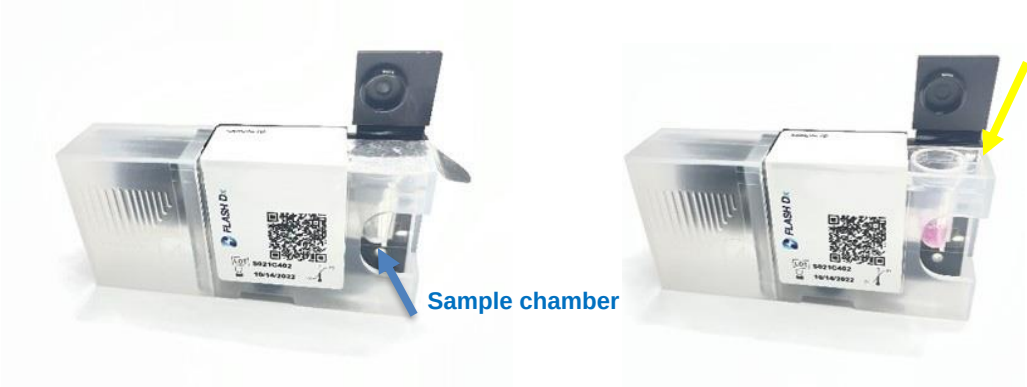

Figure 1. Left: Open cartridge showing the lyo pellet at the bottom of sample chamber; Right: Make sure that there is no foil left on the top of sample chamber.

1.2 Pipetting

1.2.1 Place test cartridge with label upright, barcode facing forward. Make sure that the white lyophilized pellet in sample chamber is located at the bottom. If not, please gently tap the cartridge on tabletop until the lyophilized pellet falls to the bottom.

1.2.2 Remove sealing aluminum foil on the top of sample chamber completely to fully expose opening. Then use a disposable transfer pipette (supplied) or a laboratory pipette to transfer 120µL of sample solution into the sample chamber and dissolve lyophilized reagent completely. Be careful not to introduce air bubbles during pipetting.

Note: When using a disposable transfer pipette, squeeze its top bulb completely and then place the pipette tip well below the liquid surface in the specimen transport tube. Slowly release the top bulb to completely fill the pipette stem with sample before removing it from the specimen collection tube. Some liquid may also be in the overflow reservoir. Insert the tip of the pipette into the sample chamber without touching the lyophilized reagent, squeeze the top bulb of the transfer pipette completely again to empty the liquid in the pipette stem.

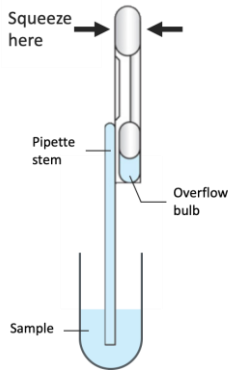

Figure 2. Transfer Pipette

1.2.3 Firmly close sample chamber lid until it is flush with the rest of test cartridge top surface. Make sure there is a tight seal and there should be no gap between chamber lid and cartridge body.

Note: It is important to remove foil completely to ensure a tight seal between lid and sample chamber.

1.3 Run test

Important note: This section only lists basic steps of running the test. Please refer to FlashDx-1000-E user manual for comprehensive instructions.

1.3.1 Input information: Sample information is entered by scanning sample barcode or manually through on-screen keyboard.

1.3.2 Load cartridge: Remove transparent protective cover of cartridge. Hold the test cartridge with chip side pointing leftwards (2-D barcode facing forward). Press button on the instrument's touchscreen and waiting for the loading bay to move out. Put test cartridge into the loading bay, press it down to feel a soft click. The instrument should now detect a cartridge in place. Click button on the touchscreen again to retract the loading bay.

Figure 3. Left: Fully closed sample port lid is flush with the rest of the cartridge top.

Right: Load the cartridge into the instrument's loading bay.

1.3.3 Start test: Instrument automatically recognizes QR code on the test cartridge and select the appropriate test. Select the corresponding sample type as needed. After confirming the program is correct, click button on the touchscreen to start the test. The instrument should start to run test automatically.

1.3.4 Test result: test process takes about 50 minutes. On-screen display will show progress and test results will be saved after test is completed.

Result Report

Once test is completed, the instrument will automatically report results as negative, positive, undetermined or invalid.

Result Interpretation

Test result is interpreted automatically by the instrument according to internal reference controls and detected targets. Presence of a positive readout of internal control or at least one positive target is a prerequisite for the validity of test result. When the test result is valid, a target is either labelled as ⊕, ⊖, or "UD", which represents positive, negative or undetermined result, respectively.

Table 2. GBS Possible Results

Result Text	GBS *cfb*	GBS *sip*	RP Control
GBS Positive	Any *cfb* or *sip* assay(s) tested positive (+)		+ / –
GBS Negative	–	–	+
GBS Undetermined	Any *cfb* or *sip* assay(s) UD while the rest tested negative (–)		+
Invalid	–	–	–

Re-test

To retest a UD or invalid result, use a new cartridge. The invalid result can result from sample not containing enough sample, failed cartridge or sample interference. If feasible, collect a new sample, otherwise use the leftover sample from the original specimen. Follow testing procedure as previously described. Put on a clean pair of gloves and use a new transfer pipette.

If test result is still invalid, no further testing on this sample is recommended. Certain samples may contain too high level of inhibitors and interfere with test.

Limitations of Test Method

1. Test result of this kit should be combined with the patient's clinical symptoms and other relevant medical examination results for comprehensive analysis, and should not be used as a sole basis for patient management.

2. There is a risk of false negatives if bacterial nucleic acid has sequence variations.

3. Unreasonable sample collection, transportation and handling, as well as improper experimental operation and environment may lead to false negative or false positive results.

4. Positive and negative predicted values largely depend on prevalence rate. Test performance may vary with prevalence rate and sampled population.

5. Nucleic acid fragments may appear in body for a long time, and has nothing to do with bacterial activity. Positive result does not necessarily mean active infection by corresponding bacteria or clinical symptoms was caused by corresponding bacteria.

6. Invalid results may result from insufficient sample collected.

7. Other interferences or PCR inhibitors that have not been verified may cause false negative results.

Performance Characteristics

1. Clinical Evaluation

GBS test was evaluated with 96 valid vaginal swab specimens.

A total of 97 samples were collected from patients suspected of GBS infection by the healthcare provider, inactivated and sent back to lab. These samples were eluted in saline, frozen and stored in -70C freezer. At time of test, these samples were thawed and tested in lab in parallel, either by FlashDx GBS kit or by a leading NMPA approved extraction-based qPCR assay, following manufacture suggested protocol. One of these samples were ruled invalid due to non-detection of RP control, suggesting failures during specimen collection process. The other 96 results were then analyzed, compared and summarized below (Table 3).

Table 3. GBS Performance Results

		qPCR Test	
		Positive	**Negative**
FlashDx GBS Test	**Positive**	77	0
	Negative	0	19
	Total	77	19
PPA*		100% (95% CI: 95.3% - 100%)	
NPA*		100% (95% CI: 86.3% - 100%)	

* These values depend on disease prevalence, which is estimated to be 25% in pregnant woman based on CDC estimate (https://www.cdc.gov/groupbstrep/about/fast-facts.html)

Analytical Performance

1. Analytical Sensitivity (Limit of Detection)

Studies were performed to determine the analytical LoD of GBS test. The LoD was established using one lot of reagent and a serial dilution of GBS reference material (ATCC13813) prepared in UTM and presumed negative swab clinical matrix. The lowest concentration level with observed hit rates greater than or equal to 95% in the LoD determination study were 1000 copies/mL for all targets combined. Verification of the estimated LoD claim was performed on different reagent lots in replicates of 20 cartridges with greater or equal to 95% detection rate.

2. Reproducibility

Different lots of cartridges were tested with enterprise precision reference samples (weak positive reference sample with ~3 x LoD and negative reference sample). Each reference samples were tested with 10 cartridges. All results were concordant.

3. Analytical specificity

3.1 Cross-reaction

The target primers and probes are designed based on conserved region of *cfb* and *sip* genes. Test does not cross-react with samples positive for various pathogens including legionella, *bacillus pertussis*, *haemophilus influenzae*, *staphylococcus aureus*, *streptococcus pneumoniae*, *streptococcus pyogenes*, *klebsiella pneumoniae* and *candida albicans*. Additional *in silico* analysis did not reveal significant cross-reaction sequence overlap with *candida glabrata, chlamydia trachomatis, clostridium difficile, neisseria gonorrhoeae*, rotavirus and norovirus and *yersinia enterocolitica*.

3.2 Interfering substances

Potentially interfering substances, including purified mucin, blood and other drugs listed in table below, have been tested in the samples at listed concentration. No significant interference is detected at the level tested, based on triplicate detection of reference material at 3X LoD.

Table 4. Interfering Substance

Substance	Concentration
purified mucin	50 µg/mL
whole blood	0.1%(v/v)
tobramycin	100 µg/mL
Azithromycin	100 µg/mL

Beta Estradiol	1.2 ng/mL
Doxycycline	100 ug/mL

Limitations

1. This test can be used for *in vitro* diagnosis only.

2. Test does not contain any infectious substances and will not infect humans or other animals. Testing sample should be handled as a potential source of infection, and its operation should be carried out in a microbiological and biomedical laboratory with biosafety protection facilities and protocols to protect operators from being affected during work.

3. Clinical laboratory shall strictly follow the *Administrative Measures for Clinical Gene Amplification Laboratories of Medical Institutions* (WBYZF [2010] No. 194 or effective version) and other regulatory standards related to molecular biology laboratories and clinical gene amplification laboratories.

4. Sample types, sample collection and handling methods specified in the instructions for use should be strictly followed, otherwise test performance cannot be guaranteed.

Interpretation of Symbol

Symbol of figure	Name of mark	Symbol of figure	Name of mark
	Batch code		Expiration date
	Limit of temperature		Do not re-use
	Manufacturer		Date of production
	Catalog number		Consult the instruction manual
	*in vitro* diagnostic medical device		CE marking - European Conformity
	Authorized representative in the European Community		Biological risks

Quality Control (QC)

External QC controls (run controls) are not required to use this test kit. Positive and negative control samples are not supplied with the kit.

If certain labs procedures require controls to show that GBS Test is working properly, they can be separately ordered and used in independent cartridges for quality control (FlashDx Cat #QCSL20101). Other heat-inactivated GBS bacteria or purified DNA may also serve the purpose but verification should be performed in advance.

To run control samples, dissolve FlashDx control sample as specified in instruction, or dilute positive control to appropriate concentration first when using other control samples. Then load 120μL of diluted positive control or negative control to a cartridge and run test as a normal sample. The system should generate report of positive detection of GBS, and negative report, respectively. Follow instruction of control samples for storage, expiration and freeze-thaw cycles as specified in vendor instruction.

References

1. Centers for Disease Control and Prevention (https://www.cdc.gov/coronavirus/2019-ncov/index.html).
2. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical laboratories (http://www.cdc.gov/biosafety/publications/).
3. Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline.

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