GBS Assay (Panther Fusion® System)

For *in vitro* diagnostic use.

Rx only.

CONTENTS

General Information

Intended Use

The Panther Fusion® GBS assay is an automated qualitative *in vitro* diagnostic test utilizing real-time PCR for detection of Group B *Streptococcus* DNA from either Lim or Carrot enrichment broth cultures of vaginal/rectal swabs from antepartum women following 18-24 hours incubation.

This test is performed on the Hologic Panther Fusion system and is intended to aid in determining the GBS colonization status of antepartum women. This assay does not diagnose or monitor treatment for GBS infections. The Panther Fusion GBS assay does not provide susceptibility results. Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.

Summary and Explanation of the Test

Group B *Streptococcus* (GBS), *Streptococcus agalactiae*, is a Gram-positive bacterium associated with transient colonization throughout the body, including, but not limited to, the vagina, gastrointestinal tract, and urethra.[1] It is rare for GBS to cause disease in healthy individuals, but it can cause serious illness in patients that are immunocompromised, the elderly, and newborns.[2] The primary health concern is early-onset neonatal infection (early-onset disease, EOD), which occurs within 7 days after birth and is caused by vertical transmission from mother to infant during labor and delivery. Vertical transmission occurs when GBS from a colonized woman ascends the vagina to the amniotic fluid after onset of labor and/or rupture of membranes.[3, 4] Infants that acquire early-onset infection typically present with respiratory symptoms or signs of sepsis within the first 24 to 48 hours following delivery[5]; meningitis is also observed, but with less frequency.

The primary strategy for addressing and preventing EOD is intravenous intrapartum (during labor) antibiotics. This strategy has been extensively studied and has been shown to be extremely effective at reducing the incidence of vertical transmission of GBS. In order to effectively apply an intrapartum antibiotic protocol it is important to accurately identify mothers that would benefit. In 2002 and again in 2010 the CDC updated its GBS prevention guidelines and recommended a universal culture-based screening approach to determine which women are candidates and should receive intrapartum antibiotics.[6, 7]

Principles of the Procedure

The Panther Fusion system fully automates specimen processing, including cell lysis, nucleic acid capture, amplification and detection for the Panther Fusion GBS assay. An internal control (IC-X) is added automatically to each specimen via the working Fusion Capture Reagent-X (wFCR-X) to monitor for interference during specimen processing, amplification and detection caused by reagent failure or inhibitory substances.

> ***Note:*** *The Panther Fusion system adds the IC-X to the FCR-X. After the IC-X is added to the FCR-X, it is referred to as wFCR-X.*

Sample processing and nucleic acid capture: Specimens are first incubated in an alkaline reagent (Panther Fusion Enhancer Reagent-X; FER-X) to enable cell lysis. Nucleic acid released during the lysis step hybridizes to magnetic particles in the FCR-X. The capture particles are then separated from residual specimen matrix in a magnetic field by a series of wash steps with

a mild detergent. The captured nucleic acid is then eluted from the magnetic particles with a reagent of low ionic strength (Panther Fusion Elution Buffer).

PCR amplification and fluorescence detection: Single unit dose PCR master mix is reconstituted with the Panther Fusion Reconstitution Buffer and combined with the eluted nucleic acid into a reaction vial. PCR-based target amplification subsequently occurs with target-specific forward and reverse primers, generating a fluorescence signal. The Panther Fusion GBS software computes a cycle threshold (Ct) result to qualitatively determine the presence of the analyte. The analyte targets and corresponding fluorescent channels used in the Panther Fusion GBS assay are summarized in the table below.

Analyte	Gene Targeted	Channel
GBS	SIP and Cfb	FAM
Internal Control	Not applicable	RED677

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. Carefully read this entire package insert and the *Panther Fusion System Operator's Manual*.

C. The Panther Fusion Enhancer Reagent-X (FER-X) is corrosive, harmful if swallowed and causes severe skin burns and eye damage.

D. Only personnel adequately trained on the use of this assay and in handling potentially infectious materials should perform these procedures. If a spill occurs, immediately disinfect using appropriate site procedures.

E. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.[8]

F. Use only supplied or specified disposable laboratory ware.

G. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents.

H. Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

I. Expiration dates listed on the Aptima Specimen Transfer tubes pertain to the transfer of sample into the tube and not to testing of the sample. Specimens collected/transferred any time prior to these expiration dates are valid for testing provided they are transported and stored in accordance with the appropriate package insert, even if these expiration dates have passed.

J. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

K. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of bacteria or other organisms. Ensure that specimen containers do not come in contact with one another, and discard used materials without passing them over any open containers. Change gloves if they come in contact with specimens.

L. Do not use the reagents and controls after the expiration date.

M. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements* and *Panther Fusion System Test Procedure* for more information.

N. Do not combine any assay reagents or fluids. Do not top off reagents or fluids; the Panther Fusion system verifies reagent levels.

O. Avoid microbial and ribonuclease contamination of reagents.

P. Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.

Q. Do not use the assay cartridge if the storage pouch has lost its seal or if the assay cartridge foil is not intact. Contact Hologic Technical Support if either occurs.

R. Do not use fluid packs that are damaged or leaking. Contact Hologic Technical Support if this occurs.

S. Handle the assay cartridges with care. Do not drop or invert assay cartridges. Avoid prolonged exposure to ambient light.

T. Some reagents of this kit are labeled with risk and safety symbols.

Note: *Hazard communication information for labeling of globally marketed products reflects the US and EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com.*

US Hazard Information

Panther Fusion Oil

Polydimethylsiloxane 95-100%

WARNING

H315 - Causes skin irritation
H319 - Causes serious eye irritation
P264 - Wash face, hands and any exposed skin thoroughly after handling
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P337 + P313 - If eye irritation persists: Get medical advice/attention
P302 + P352 - IF ON SKIN: Wash with plenty of soap and water
P332 + P313 - If skin irritation occurs: Get medical advice/attention
P362 - Take off contaminated clothing and wash before reuse

Panther Fusion Enhancer Reagent-X (FER-X)

Lithium Hydroxide Monohydrate 5-10%

DANGER

H302 - Harmful if swallowed
H314 - Causes severe skin burns and eye damage
P264 - Wash face, hands and any exposed skin thoroughly after handling
P270 - Do not eat, drink or smoke when using this product
P260 - Do not breathe dust/fume/gas/mist/vapors/spray
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P310 - Immediately call a POISON CENTER or doctor/physician
P303 + P361 + P353 - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower
P363 - Wash contaminated clothing before reuse
P304 + P340 - IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
P310 - Immediately call a POISON CENTER or doctor/physician
P301 + P312 - IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell
P330 - Rinse mouth
P301 + P330 + P331 - IF SWALLOWED: rinse mouth. Do NOT induce vomiting
P405 - Store locked up
Dispose of contents/container to an approved waste disposal plant

EU Hazard Information

Panther Fusion Oil

Polydimethylsiloxane 100%

WARNING

H315 - Causes skin irritation
H319 - Causes serious eye irritation

Panther Fusion Enhancer Reagent-X (FER-X)

LITHIUM HYDROXIDE MONOHYDRATE 5-10%

DANGER

H302 - Harmful if swallowed
H314 - Causes severe skin burns and eye damage
P260 - Do not breathe dust/fume/gas/mist/vapors/spray
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P303 + P361 + P353 - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P310 - Immediately call a POISON CENTER or doctor/physician
P280 - Wear protective gloves/protective clothing/eye protection/face protection

Reagent Storage and Handling Requirements

A. The following table provides storage and handling requirements for this assay.

Reagent	Unopened Storage	On Board/ Open Stability[1]	Opened Storage
Panther Fusion GBS Assay Cartridge	2°C to 8°C	60 days	2°C to 8°C[2]
Panther Fusion Capture Reagent-X (FCR-X)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Enhancer Reagent-X (FER-X)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Internal Control-X (IC-X)	2°C to 8°C	(In wFCR-X)	Not applicable
Panther Fusion Elution Buffer	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Oil	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Reconstitution Buffer I	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion GBS Positive Control	2°C to 8°C	Single use vial	Not applicable-single use
Panther Fusion Negative Control	2°C to 8°C	Single use vial	Not applicable-single use

When reagents are removed from the Panther Fusion system, return them immediately to their appropriate storage temperatures.

[1] On board stability starts at the time the reagent is placed on the Panther Fusion system for the Panther Fusion GBS assay cartridge, FCR-X, FER-X and IC-X. On board stability for the Panther Fusion Reconstitution Buffer I, Panther Fusion Elution Buffer, and Panther Fusion Oil Reagent starts when the reagent pack is first used.

[2] If removed from the Panther Fusion system, store the assay cartridge in an air-tight container with desiccant at the recommended storage temperature.

B. wFCR-X and FER-X are stable for 60 days when capped and stored at 15ºC to 30ºC. Do not refrigerate.

C. Discard any unused reagents that have surpassed their on board stability.

D. Avoid cross-contamination during reagent handling and storage.

E. **Do not freeze reagents.**

Specimen Collection and Storage

Specimens - Clinical material collected from patient placed in an appropriate transport system.

Samples - Represents a more generic term to describe any material for testing on the Panther Fusion system including specimens and controls.

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during specimen handling steps. For example, discard used material without passing over open tubes.*

A. Specimen Collection and Enrichment

1. Collect vaginal/rectal swab(s) according to standard technique using a flocked swab. Immediately place the swab specimen into a non-nutritive media (Liquid Stuart's or Amies).
2. After collection, swabs can be stored at 15°C to 30°C for up to 48 hours.
3. Inoculate swab(s) directly into the desired enrichment broth (Lim or Carrot).
4. Incubate aerobically for 18 to 24 hours at 35°C to 37°C according to standard technique.
5. After enrichment, specimens can be stored under one of the following conditions:
   - 15°C to 30°C for up to 24 hours or
   - 2°C to 8°C for up to 5 days.

B. Specimen processing

1. Prior to testing on the Panther Fusion system, resuspend the enriched specimen and transfer 1 mL of the specimen to the Aptima Specimen Transfer Tube containing 2.9 mL of Specimen Transport Medium (STM).
2. After transfer, specimens can be stored under one of the following conditions:
   - 15°C to 30°C for up to 72 hours or
   - 2°C to 8°C for up to 5 days.

Note: *It is recommended that specimens transferred to the Aptima Specimen Transfer Tube are stored capped and upright in a rack.*

C. Storing samples after testing

1. Samples may be left on the Panther Fusion system or removed and tested later as long as the total time on board does not exceed the storage condition described in Step B.
2. Removed samples should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be shipped, remove the penetrable cap and replace with a non-penetrable cap. If samples need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping previously tested and recapped samples, specimen tubes must be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube. Avoid splashing and cross-contamination.

Specimen Transport

Maintain specimen storage conditions as described under *Specimen Collection and Storage*.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Reagents and Materials Provided

Assay Packaging

Components[1]	Cat. No.	Storage
Panther Fusion GBS Assay Cartridges 96 Tests Panther Fusion GBS assay cartridge, 12 tests, 8 per box	PRD-04484	2°C to 8°C
Panther Fusion GBS Assay Controls Panther Fusion GBS Positive Control tube, 5 per box Panther Fusion Negative Control tube, 5 per box	PRD-04485	2°C to 8°C
Panther Fusion Internal Control-X 960 Tests Panther Fusion Internal Control-X tube, 4 per box	PRD-04476	2°C to 8°C
Panther Fusion Extraction Reagent-X 960 Tests Panther Fusion Capture Reagent-X bottle, 240 tests, 4 per box Panther Fusion Enhancer Reagent-X bottle, 240 tests, 4 per box	PRD-04477	15°C to 30°C
Panther Fusion Elution Buffer 2400 Tests Panther Fusion Elution Buffer pack, 1200 tests, 2 per box	PRD-04334	15°C to 30°C
Panther Fusion Reconstitution Buffer I 1920 Tests Panther Fusion Reconstitution Buffer I, 960 Tests, 2 per box	PRD-04333	15°C to 30°C
Panther Fusion Oil Reagent 1920 Tests Panther Fusion Oil Reagent, 960 tests, 2 per box	PRD-04335	15°C to 30°C

[1] Components can also be ordered in the following bundles:
Panther Fusion Universal Fluids Kit, PRD-04430, contains 1 each Panther Fusion Oil and Panther Fusion Elution Buffer.

Materials Required and Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

Material	Cat. No.
Panther System	303095
Panther Fusion Module	PRD-04173
Panther Fusion System	PRD-04172
Aptima Assay Fluids Kit (Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther System Run Kit for Real-Time Assays contains MTUs, waste bags, waste bin covers, and assay fluids	PRD-03455 (5000 tests)
Or Panther System Run Kit (when running TMA assays in parallel with real-time TMA assays) contains MTUs, waste bags, waste bin covers, auto detect*, and assay fluids	303096 (5000 tests)
Panther Fusion Tube Trays, 1008 tests, 18 trays per box	PRD-04000
Liquid Handling (LiHa) Disposable Tips, 1000 µL	10612513 (Tecan)
Aptima Specimen Transfer Kit	301154C

Material	Cat. No.
Aptima penetrable caps (optional)	105668
Replacement non-penetrable caps (optional)	103036A
Replacement extraction reagent bottle caps	CL0040
P1000 pipettor and tips with hydrophobic plugs	—
Bleach, 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution	—
Disposable powderless gloves	—

*Needed only for Panther Aptima TMA assays.

Panther Fusion System Test Procedure

Note: *Refer to the Panther Fusion System Operator's Manual for additional procedural information.*

A. Work Area Preparation

1. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface with clean, plastic-backed absorbent laboratory bench covers.
2. Clean a separate work surface where samples will be prepared using the procedure described in Step A.1.

B. Reagent Preparation

1. Remove the bottles of IC-X, FCR-X and FER-X from storage.
2. Open the bottles of IC-X, FCR-X and FER-X, and discard the caps. Open the TCR door on the upper bay of the Panther Fusion system.
3. Place the IC-X, FCR-X and FER-X bottles in the appropriate positions on the TCR carousel.
4. Close the TCR door.

Note: *The Panther Fusion system adds the IC-X to the FCR-X. After the IC-X is added to the FCR-X, it is referred to as wFCR-X. If the FCR-X and FER-X are removed from the system, use new caps and immediately store according to the proper storage conditions.*

C. Specimen Handling

Note: *Prepare specimens per the Specimen Processing instructions in the Specimen Collection and Storage section before loading specimens onto the Panther Fusion system.*

1. **Do not vortex samples.**
2. Inspect sample tubes before loading into the rack. If a sample tube contains bubbles or has a lower volume than is typically observed, gently tap the bottom of the tube to bring contents to the bottom.

Note: *To avoid a processing error, ensure adequate specimen volume is added to the Aptima Specimen Transfer Tube. When 1 mL of enriched culture specimen is added to the Aptima Specimen Transfer Tube, there is sufficient volume to perform 3 nucleic acid extractions.*

D. System Preparation

For instructions on setting up the Panther Fusion system including loading samples, reagents, assay cartridges and universal fluids, refer to the *Panther Fusion System Operator's Manual.*

Procedural Notes

A. Controls

1. The Panther Fusion GBS Positive Control and the Panther Fusion Negative Control can be loaded in any rack position, in any Sample Bay lane on the Panther Fusion system.
2. Once the control tubes are pipetted and processed for the Panther Fusion GBS assay, they are active for up to 30 days (control frequency configured by an administrator) unless control results are invalid or a new assay cartridge lot is loaded.
3. Each control tube can be tested once.
4. Patient specimen pipetting begins when one of the following two conditions is met:
   a. Valid results for the controls are registered on the system.
   b. A pair of controls is currently in process on the system.

Quality Control

A run or specimen result may be invalidated by the Panther Fusion system if problems occur while performing the assay. Specimens with invalid results must be retested.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the negative assay control and positive assay control must be tested each time a new lot of assay cartridges is loaded on the Panther Fusion system or when the current set of valid controls for an active assay cartridge lot has expired.

The Panther Fusion system is configured to require that assay controls run at an administrator-specified interval of up to 30 days. Software on the Panther Fusion system alerts the operator when assay controls are required and does not start new tests until the assay controls are loaded and have started processing.

During processing, criteria for acceptance of the assay controls are automatically verified by the Panther Fusion system. To generate valid results, the assay controls must pass a series of validity checks performed by the Panther Fusion system.

If the assay controls pass all validity checks, they are considered valid for the administrator-specified time interval. When the time interval has passed, the assay controls are expired by the Panther Fusion system and a new set of assay controls is required prior to starting any new samples.

If any one of the assay controls fails the validity checks, the Panther Fusion system automatically invalidates the affected samples and requires a new set of assay controls be tested prior to starting any new samples.

Internal Control

An internal control is added to each sample during automated specimen processing on the Panther Fusion system. During processing, the internal control acceptance criteria is automatically verified by the Panther Fusion system software. Detection of the internal control is not required for samples that are positive for GBS. The internal control must be detected in all samples that are negative for GBS. Samples that fail to meet that criteria are reported as invalid. Each sample with an invalid result must be retested.

The Panther Fusion system is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther Fusion System Operator's Manual.*

Interpretation of Results

The Panther Fusion system automatically determines the test results for samples and controls. A test result may be negative, positive, or invalid.

Table 1 shows the possible results reported in a valid run with result interpretations.

Table 1: Interpretation of Results

GBS Result	IC Result	Interpretation
Negative	Valid	GBS not detected.
Positive[1]	Valid	GBS detected.
Invalid[2]	Invalid	Invalid. There was an error in the generation of the result. Retest sample.

Ct = cycle threshold, IC = internal control.

[1] Samples with a GBS (FAM) Ct value less than the FAM Ct threshold of 40 are reported as GBS positive.

[2] Samples with a GBS (FAM) Ct value greater than the FAM Ct threshold of 40 and an IC (RED677) Ct value greater than the RED677 Ct threshold of 38 are reported as invalid.

Limitations

A. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. Avoid contamination by adhering to good laboratory practices and to the procedures specified in this package insert.

D. The Panther Fusion GBS assay is for laboratory use only in hospital, clinical, reference, or state laboratory settings. The device is not intended for point of care use.

E. A positive test result does not necessarily indicate the presence of viable organisms. It is, however, presumptive for the presence of Group B *Streptococcus* DNA.

F. Negative results do not preclude the presence of GBS and should not be used as the sole basis for treatment or other patient management decisions.

G. GBS colonization during pregnancy can be intermittent, persistent, or transient. The clinical utility of GBS screening decreases when testing is performed more than 5 weeks prior to delivery.

H. The Panther Fusion GBS assay does not provide susceptibility results. Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.

I. Mutations in primer/probe binding regions may affect detection using the Panther Fusion GBS assay.

J. Results from the Panther Fusion GBS assay as implemented on the Panther Fusion system should be used as an adjunct to clinical observations and other information available to the physician. The test is not intended to differentiate carriers of Group B *Streptococcus* from those with streptococcal disease. Test results may be affected by concurrent antibiotic therapy as GBS DNA may continue to be detected following antimicrobial therapy.

K. Use of the assay for clinical specimen types other than those specified has not been evaluated and performance characteristics are not established.

Panther Fusion System Assay Performance

Expected Values

The performance of the Panther Fusion GBS assay was evaluated in a prospective clinical study of vaginal/rectal swab specimens from antepartum women conducted at three sites in the U.S. Overall, the prevalence of GBS colonization as determined by the Panther Fusion GBS assay was 24.2% (229/947); whereas, the prevalence by conventional culture was 21.4% (203/947), as shown in Table 2.

Table 2: Panther Fusion GBS Assay and Culture Prevalence

Culture Medium	Clinical Site	N	Panther Fusion GBS Assay		Conventional Culture	
			N Positive	% Prevalence	N Positive	% Prevalence
Lim Broth	1	300	65	21.7%	60	20.0%
	2	343	71	20.7%	60	17.5%
	Overall	643	136	21.2%	120	18.7%
Carrot Broth	3	304	93	30.6%	83	27.3%
Combined	Overall	947	229	24.2%	203	21.4%

Reproducibility

Panther Fusion GBS assay reproducibility was evaluated at three US sites using seven panel members. Testing was performed using one lot of assay reagents and six operators (two at each site). At each site, testing was performed two times per day during five non-consecutive days. Each run had three replicates of each panel member. A negative panel member was created using Lim Broth matrix. Positive panel members were created by spiking GBS at 1-2X LoD (limit of detection; low-positive) or 3X LoD (moderate-positive) concentrations of the target analyte into Lim Broth matrix. The agreement with expected result was 100% in the negative and moderate positive panel members and ≥98.9% in low-positive panel members for the three GBS strains evaluated (serotypes III, V and non-hemolytic isolate - NH), as shown in Table 3.

Table 3: Agreement of Panther Fusion GBS Assay Results with Expected Results

Panels			Expected Result	Agreement with Expected Result	
Description	**Composition**	**Concentration (CFU/mL)**	**GBS**	**N**	**% (95%CI)**
GBS III Low Pos	1-2X LoD	262	+	90/90	100 (95.9 - 100%)
GBS III Mod Pos	3X LoD	504	+	90/90	100 (95.9 - 100%)
GBS V Low Pos	1-2X LoD	188	+	89/90	98.9 (94.0 - 99.8%)
GBS V Mod Pos	3X LoD	367	+	90/90	100 (95.9 - 100%)
GBS NH Low Pos	1-2X LoD	523	+	90/90	100 (95.9 - 100%)
GBS NH Mod Pos	3X LoD	900	+	90/90	100 (95.9 - 100%)
Neg	Negative	N/A	-	90/90	100 (95.9 - 100%)

CI = Score confidence interval, LoD = limit of detection, N/A = not applicable, Mod = moderate, Neg = negative, Pos = positive.

The total GBS signal variability measured as %CV ranged from 1.51% to 2.25% in low and moderate positive panel members. Across the sources of variation, excluding within runs, %CV values were ≤1.33%, as shown in Table 4.

Table 4: Signal Variability of the Panther Fusion GBS Assay by Panel Member

Panel Description	N	Mean Ct	Between Sites		Between Operators		Between Days		Between Runs		Within Runs		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
GBS III Low Pos	90	37.1	0.49	1.33%	0.47	1.26%	0.18	0.48%	0.14	0.37%	0.55	1.47%	0.77	2.09%
GBS III Mod Pos	90	36.2	0.45	1.24%	0.41	1.12%	0.15	0.41%	0.05	0.14%	0.42	1.15%	0.66	1.81%
GBS V Low Pos	90	37.3	0.44	1.17%	0.41	1.09%	0.09	0.23%	0.11	0.31%	0.68	1.82%	0.84	2.25%
GBS V Mod Pos	90	36.3	0.31	0.84%	0.31	0.85%	0.26	0.72%	0.08	0.23%	0.48	1.33%	0.61	1.69%
GBS NH Low Pos	90	36.2	0.27	0.75%	0.27	0.74%	0.14	0.40%	0.10	0.28%	0.50	1.37%	0.58	1.61%
GBS NH Mod Pos	90	35.4	0.20	0.57%	0.20	0.55%	0.16	0.45%	0.03	0.08%	0.46	1.31%	0.53	1.51%
Negative	90	31.9	0.32	1.00%	0.30	0.95%	0.06	0.20%	0.16	0.50%	0.26	0.83%	0.56	1.77%

Ct = threshold cycle, CV = coefficient of variation, Pos = positive, SD = standard deviation.

The signal variability as measured as %CV was ≤1.35% between sites/operators, between days/runs, or overall for the Panther Fusion GBS assay positive control and negative control, as shown in Table 5.

Table 5: Signal Variability of the Panther Fusion GBS Assay Controls

Control	N	Mean Ct	Between Sites/ Operators		Between Days/ Runs		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)
Positive	15	31.9	0.17	0.53%	0.10	0.32%	0.22	0.70%
Negative	15	28.3	0.20	0.72%	0.27	0.94%	0.38	1.35%

Ct = threshold cycle, CV = coefficient of variation, SD = standard deviation.

Clinical Performance

A prospective multicenter study was conducted using leftover enriched culture samples (Lim Broth and Carrot Broth) from vaginal/rectal swab specimens collected from antepartum women undergoing routine GBS screening. A total of 947 specimens were tested with both the reference culture method and the Panther Fusion GBS assay, and clinical sensitivity and specificity was determined. The results are shown in Tables 6, 7, and 8.

Table 6: Lim Broth Specimens

Lim Broth		Reference Method		
		Positive	**Negative**	**Total**
Panther Fusion GBS Assay	Positive	120	16[1]	136
	Negative	0	507	507
	Total	120	523	643
Sensitivity		120/120 = 100% (95% CI: 96.9% - 100%)		
Specificity		507/523 = 96.9% (95% CI: 95.1% - 98.1%)		
Positive Predictive Value		120/136 = 88.2% (95% CI: 81.7% - 92.6%)		
Negative Predictive Value		507/507 = 100% (95% CI: 99.3% - 100%)		

CI = confidence interval.

[1] Of 16 false positives, 14 (87.5%) were positive on the Becton Dickinson BD MAX GBS assay.

Table 7: Carrot Broth Specimens

Carrot Broth		Reference Method		
		Positive	**Negative**	**Total**
Panther Fusion GBS Assay	Positive	83	10	93
	Negative	0	211	211
	Total	83	221	304
Sensitivity		83/83 = 100% (95% CI: 95.6% - 100%)		
Specificity		211/221 = 95.5% (95% CI: 91.9% - 97.5%)		
Positive Predictive Value		83/93 = 89.3% (95% CI: 81.3% - 94.1%)		
Negative Predictive Value		211/211 = 100% (95% CI: 98.2% -100%)		

CI = confidence interval.

Table 8: Combined Lim Broth and Carrot Broth Specimens

Lim and Carrot Broth Combined		Reference Method		
		Positive	**Negative**	**Total**
Panther Fusion GBS Assay	Positive	203	26	229
	Negative	0	718	718
	Total	203	744	947
Sensitivity		203/203 = 100% (95% CI: 98.1% - 100%)		
Specificity		718/744 = 96.5% (95% CI: 94.9% - 97.6%)		
Positive Predictive Value		203/229 = 88.7% (95% CI: 83.9% - 92.1%)		
Negative Predictive Value		718/718 = 100% (95% CI: 99.5% - 100%)		

CI, confidence interval.

Analytical Sensitivity

The analytical sensitivity (LoD) of the Panther Fusion GBS assay was determined by testing serial dilutions of 11 GBS serotypes and one non-hemolytic (NH) isolate in Lim Broth negative clinical matrix. Thirty replicates were tested with each of the three reagent lots for a combined total of 90 replicates per dilution. Probit analysis was performed for each reagent lot with the reported 95% LoD based upon the worst estimate, as shown in Table 9. Serotype specific LoD predictions were verified by testing an additional 20 replicates with one reagent lot.

Table 9: GBS Limit of Detection (LoD)

GBS Serotype	95% LoD in CFU/mL (95% CI)
Ia	137.4 (103.7 - 209.7)
Ib	140.5 (100.6 - 234.7)
Ic	136.3 (99.2 - 220.5)
II	179.0 (135.1 - 276.2)
III	168.0 (125.2 - 261.5)
IV	84.0 (63.0 - 130.4)
V	122.3 (92.2 - 186.8)
VI	282.0 (201.9 - 475.8)
VII	250.8 (180.2 - 424.8)
VIII	231.3 (167.3 - 380.9)
IX	301.0 (202.0 - 567.7)
NH	300.2 (212.0 - 523.0)

CFU = colony forming units, CI = confidence interval, NH = non-hemolytic.

Analytical Specificity and Microbial Interference

The analytical specificity of the Panther Fusion GBS assay was evaluated by testing a panel of 110 microorganisms (as listed in Table 10), consisting of viral, bacterial, fungal, parasite, protozoan and yeast strains representing pathogens or flora commonly present in the vaginal/rectal tract or related to the GBS family. Bacteria and yeast were tested at $1x10^6$ CFU/mL, except where noted. Viruses, fungi, parasites, and protozoan were tested at $1x10^5$ PFU/mL, except where noted. Organisms were tested both with and without GBS analyte spiked at a concentration 3X LoD. All of the microorganisms tested were found to have no impact on the performance or analytical specificity of the Panther Fusion GBS assay.

Table 10: Microorganisms and Evaluated Concentrations

Pathogen	Concentration* (CFU/mL or PFU/mL)	Pathogen	Concentration* (CFU/mL or PFU/mL)
Bacillus cereus	$1x10^6$	*Streptococcus anginosus*	$1x10^6$
Yersinia enterocolitica subsp. enterocolitica	$1x10^6$	*Prevotella oralis*	$1x10^6$
Anaerococcus prevotii	$1x10^6$	*Streptococcus canis*	$1x10^6$
Propionibacterium acnes	$1x10^6$	*Lactobacillus delbrueckii subsp. lactis*	$1x10^6$
Clostridium difficile	$1x10^6$	*Corynebacterium sp (genitalium)*	$1x10^6$
Fusobacterium nucleatum	$1x10^6$	*Neisseria gonorrhoeae*	$1x10^6$
Bifidobacterium adolescentis Reuter	$1x10^6$	*Streptococcus pneumoniae* (oral group)	$1x10^6$
Candida albicans (NIH 3147)	$1x10^6$	*Streptococcus mutans* (oral group)	$1x10^6$
Candida glabrata (CBS 138)	$1x10^6$	*Corynebacterium urealyticum*	$1x10^6$
Candida tropicalis	$1x10^6$	*Lactobacillus reuteri*	$1x10^6$
Cryptococcus neoformans	$1x10^5$ *	*Lactobacillus sp.*	$1x10^6$
Klebsiella pneumoniae	$1x10^6$	*Lactobacillus casei*	$1x10^6$
Proteus mirabilis	$1x10^6$	*Lactobacillus acidophilus*	$1x10^6$
Alcaligenes faecalis	$1x10^6$	*Streptococcus gordonii* (oral group)	$1x10^6$
Enterobacter aerogenes	$1x10^6$	*Bulkholderia cepacia*	$1x10^6$
Stenotrophomonas maltophilia	$1x10^6$	*Aeromonas hydrophila*	$1x10^6$
Campylobacter jejuni	$1x10^6$	*Moraxella atlantae*	$1x10^6$
Providencia stuartii	$1x10^6$	*Prevotella bivia*	$1x10^6$
Micrococcus luteus	$1x10^6$	*Pasteurella aerogenes*	$1x10^6$
Staphylococcus haemolyticus	$1x10^6$	*Rhodococcus equi*	$1x10^6$
Enterococcus faecalis	$1x10^6$	*Listeria monocytogenes*	$1x10^6$
Pseudomonas fluorescens	$1x10^6$	*Lactobacillus gasseri*	$1x10^6$
Staphylococcus saprophyticus	$1x10^6$	*Peptoniphilus asaccharolyticus*	$1x10^6$
Proteus vulgaris	$1x10^6$	*Atopobium vaginae*	$1x10^6$
Toxoplasma gondii	$1x10^5$ *	*Bifidobacterium brevis*	$1x10^6$
Enterococcus faecium	$1x10^6$	*Abiotropha defectiva*	$1x10^6$
Escherichia coli	$1x10^6$	*Anaerococcus tetradius*	$1x10^6$
Enterobacter cloacae	$1x10^6$	*Finegoldia magna*	$1x10^6$
Morganella morganii	$1x10^6$	*Peptostreptococcus anaerobius*	$1x10^6$

Table 10: Microorganisms and Evaluated Concentrations (continued)

Pathogen	Concentration* (CFU/mL or PFU/mL)	Pathogen	Concentration* (CFU/mL or PFU/mL)
Shigella flexneri	$1x10^6$	*Anaerococcus lactolyticus*	$1x10^6$
Streptococcus pyogenes (group A)	$1x10^6$	Human herpesvirus 4 (EBV)	$1x10^5$ *
Streptococcus ratti	$1x10^6$	*Bacteroides fragilis*	$1x10^6$
Staphylococcus lugdunensis	$1x10^6$	*Bordetella pertussis*	$1x10^6$
Acinetobacter baumannii	$1x10^6$	*Chlamydia trachomatis*	$1x10^6$
Staphylococcus aureus	$1x10^6$	Human herpesvirus 5 (CMV)	$1x10^5$ *
Staphylococcus epidermidis	$1x10^6$	*Hafnia alvei*	$1x10^6$
Shigella sonnei	$1x10^6$	Trichomonas vaginalis	$1x10^5$ *
Citrobacter freundii	$1x10^6$	Human immunodeficiency virus-1 (HIV-1)	$1x10^5$ *
Enterococcus gallinarum	$1x10^6$	*Moraxella catarrhalis*	$1x10^6$
Acinetobacter lwoffii	$1x10^6$	*Mycoplasma genitalium*	$1x10^6$
Pseudomonas aeruginosa	$1x10^6$	*Prevotella melaninogenica*	$1x10^6$
Streptococcus criceti	$1x10^6$	Rubella Virus	$1x10^5$ *
Haemophilus influenzae	$1x10^6$	*Serratia marcescens*	$1x10^6$
Klebsiella oxytoca	$1x10^6$	*Streptococcus intermedius*	$1x10^6$
Streptococcus bovis (group D)	$1x10^6$	Human Papilloma Virus Type 16 (HPV16)	$1x10^5$ *
Streptococcus parasanguinis	$1x10^6$	Hepatitis B Virus	$1x10^5$ *
Streptococcus equi subsp. equi (group D)	$1x10^6$	Hepatitis C Virus	$1x10^5$ *
Enterococcus durans	$1x10^6$	Herpes Simplex Virus-1 (HSV-1)	$1x10^5$ *
Lactobacillus plantarum	$1x10^6$	Herpes Simplex Virus-2 (HSV-2)	$1x10^5$ *
Streptococcus dysgalactiae	$1x10^6$	Human herpesvirus 3 (VZV)	$1x10^5$ *
Streptococcus constellatus	$1x10^6$	*Arcanobacterium pyogenes*	$1x10^6$
Streptococcus oralis (oral group)	$1x10^6$	*Mobiluncus curtisii subsp. curtisii*	$1x10^6$
Bacillus coagulans	$1x10^6$	*Gardnerella vaginalis*	$1x10^6$
Streptococcus pseudoporcinus	$1x10^6$	*Salmonella enterica subsp. enterica ser. dublin* (group D)	$1x10^6$
Streptococcus mitis (oral group)	$1x10^6$	*Streptococcus acidominus*	$1x10^6$

CFU = colony-forming units, PFU = plaque-forming units.

* Microorganisms evaluated as extracted DNA were tested in copies/mL.

Interference

Amniotic fluid, blood, urine, stool and other potentially interfering endogenous and exogenous substances that may be present in vaginal/rectal specimens were evaluated in the Panther Fusion GBS assay. Concentrations exceeding clinically relevant amounts of the potentially interfering substances were added to Lim Broth clinical negative matrix and tested unspiked or spiked with GBS analyte at a 3X LoD concentration. The substances consisted of topical medications, lubricants, deodorants, laxatives and contraceptives, as shown in Table 11.

All of the substances tested were found to have no impact on the performance of the Panther Fusion GBS assay, at the concentrations tested.

Table 11: Potentially Interfering Substances

Substance	Ingredients	Concentration
Human Amniotic Fluid	N/A	4% v/v
Human Whole Blood EDTA	N/A	4% v/v
Human Whole Blood Na Citrate	N/A	4% v/v
Human Serum	N/A	4% v/v
Human Urine Sample	N/A	4% v/v
Human Fecal Sample	N/A	4% v/v
Topical Hemorrhoid Ointment (Preparation H Cream)	Mineral Oil, Petrolatum, Phenylephrine HCl	3.4% w/v
Anti-Diarrheal Medication (Pepto Bismol)	Bismuth subsalicylate	4% v/v
Personal Lubricant (K-Y Jelly Personal Lubricant)	Glycerine, Methylparaben, Propylparaben	2.2% w/v
Lubricating gel (Aquagel)	N/A	2.1% w/v
Vaginal Anti-itch Cream (OTC) (Vagisil)	Benzocaine, Resorcinol	3.9% w/v
Vaginal Anti-itch Cream (OTC) (Gyno-Daktarin)	Miconazole nitrate	3.8% w/v
Vaginal Antifungal Cream (OTC) (Monistat)	Miconazole nitrate	3.1% w/v
Vaginal Antifungal Gel	*Candida albicans* 27X HPUS, *Candida parapsilosis* 27X HPUS, Pulsatilla 27X HPUS	3.0% w/v
Anti-Diarrheal Caplet (Kaopectate)	Bismuth subsalicylate	1.1% w/v
Deodorant Powder (Vagisil)	Zea Mays Starch, Magnesium Stearate, Sodium Bicarbonate, Aloe Barbadensis Leaf extract, Tocopheryl Acetate, Tricalcium Phosphate, Mineral Oil, Polyoxymethylene Urea, Maltodextrin, Fragrance	1.1% w/v
Deodorant Suppositories (Norforms Suppositories)	PEG-20, PEG-32, PEG-20 Stearate, Benzethonium Chloride, Methylparaben, Lactic Acid, Fragrance, Neutresse (Odor synthesis)	2.1% w/v

Table 11: Potentially Interfering Substances (continued)

Substance	Ingredients	Concentration
Deodorant Spray (FDS)	Isopropyl Myristate, Zea Mays Strach, Magnesium Stearate, Fragrance, Zinc Ricinoleate, Laureth-3, Benzyl alcohol, Mineral Oil (Paraffinum Liquidum, Huile Minérale), Tetrahydroxypropyl Ethylenediamine, Sodium Bicarbonate, Citronellol, Linalool, Propylene Glycol, Butylphenyl Methylpropional, Lanolin Alcohol, Anise Alcohol, Oleyl Alcohol, Benzyl Benzoate, Chamomilla Recutita, Flower extract, Tocopheryl Acetate, Aloe Barbadensis Leaf extract	1.5% w/v
Body Powder (Gold Bond Powder)	Menthol	0.4% w/v
Body Oil	Isopropyl Myristate, sesame seed oil, PEG-40, Sorbitan Peroleate, Propylparaben, BHT, Fragrance	4% v/v
Spermicidal Foam	Nonoxynol-9	2.1% w/v
Oral Laxative (Metamucil Fiber Supplement)	Psyllium husk	2.2% w/v
Grains de Vals (SennosideB)	Sennocide B	0.4% w/v
Oral Laxative (Phillips Milk of Magnesia)	Magnesium hydroxide	7.3% w/v
Stool Softener	Bisacodyl	0.9% w/v
Astroglide Liquid personal lubricant	Glycerin, Propylene Glycol, Polyquaternium 15, Methylparaben, Propylparaben	2.7% w/v
Enema Solution (Fleet enema)	Dinatriumhydrogenphosphat-Dodecahydrat / Natriumhydrogenphosphat-Dihydrat	4% v/v

N/A = not applicable, OTC = over the counter, v/v = volume/volume, w/v = weight/volume.

Carryover/Contamination

The carryover/cross-contamination study was performed with Lim Broth clinical negative samples alternately placed between high positive samples and tested. High positive samples were prepared by spiking GBS at 1 x 10^6 CFU/mL (> 5,000X LoD). A total of ten separate runs with negative samples and positive samples placed in a checkerboard pattern were tested in addition to four runs of negative specimens over two different instruments a combined total of 300 positive and 420 negative samples. There were no false positive results observed for a carryover rate of 0.0%.

Assay Precision

Panther Fusion GBS assay precision was evaluated with a 7-member panel. The panel was tested by three operators on five separate runs per day, using three reagent lots on one Panther Fusion system over 12 non-consecutive days. The panel members are described in Table 12, along with a summary of the agreement with expected results for each target. Table 13 presents

the mean and variability analysis between reagent lots, between operators, between days, between runs and within runs, and overall (total) for Ct.

Table 12: Percent Agreement to the Expected Result

Panel Member	% Positive	% Agreement (95% CI)
GBS III 1-2X LoD	100% (180/180)	100% (97.9 - 100)
GBS III 3X LoD	100% (180/180)	100% (97.9 - 100)
GBS V 1-2X LoD	100% (180/180)	100% (97.9 - 100)
GBS V 3X LoD	100% (180/180)	100% (97.9 - 100)
GBS NH 1-2X LoD	100% (180/180)	100% (97.9 - 100)
GBS NH 3X LoD	100% (180/180)	100% (97.9 - 100)
Negative	0% (0/180)	100% (97.9 - 100)

CI = confidence interval, LoD = limit of detection, NH = non-hemolytic.

Table 13: Signal Variability

Panel Member	Mean Ct	Between Reagent Lots		Between Operators		Between Days		Between Runs		Within Runs		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
GBS III 1-2X LoD	36.3	0.06	0.16%	0.16	0.45%	0.11	0.31%	0.33	0.91%	0.43	1.18%	0.53	1.46%
GBS III 3X LoD	35.4	0.17	0.48%	0.12	0.35%	0.13	0.36%	0.34	0.95%	0.32	0.90%	0.43	1.22%
GBS V 1-2X LoD	36.4	0.13	0.37%	0.13	0.35%	0.17	0.46%	0.29	0.78%	0.50	1.36%	0.55	1.51%
GBS V 3X LoD	35.4	0.13	0.38%	0.11	0.31%	0.12	0.34%	0.28	0.79%	0.41	1.14%	0.46	1.31%
GBS NH 1-2X LoD	35.7	0.23	0.65%	0.12	0.35%	0.14	0.39%	0.31	0.86%	0.38	1.06%	0.46	1.28%
GBS NH 3X LoD	34.8	0.19	0.55%	0.04	0.12%	0.10	0.29%	0.28	0.81%	0.29	0.84%	0.40	1.14%
Negative (IC)	31.5	0.24	0.77%	0.08	0.24%	0.14	0.43%	0.32	1.03%	0.27	0.86%	0.41	1.32%

Ct = threshold cycle, CV = coefficient of variation, LoD = limit of detection, SD = standard deviation.

Bibliography

1. Ohlsson, A., and Shah, V.S. 2009. Intrapartum antibiotics for known maternal Group B streptococcal colonization. Cochrane Database Syst Rev 3:CD007467. http://dx.doi.org/10.1002/14651858.CD007467.pub2.
2. Schuchat, A. 1998. Epidemiology of group B streptococcal disease in the United States: shifting paradigms. Clin Microbiol Rev 11:497–513.
3. Desa, D.J., and Trevenen, C.L. 1984. Intrauterine infections with group B beta-haemolytic streptococci. Br J Obstet Gynaecol 91:237–239.
4. Katz, V., and Bowes, W.A. Jr. 1988. Perinatal group B streptococcal infections across intact amniotic membranes. J Reprod Med 33:445–449.
5. Franciosi, R.A., Knostman, J.D., and Zimmerman, R.A. 1973. Group B streptococcal neonatal and infant infections. J Pediatr 82:707–718.
6. Centers for Disease Control and Prevention. 2002. Prevention of perinatal group B streptococcal disease: revised guidelines from CDC. MMWR 51 (No. RR-11).
7. Centers for Disease Control and Prevention. 2010. Prevention of perinatal group B streptococcal disease: revised guidelines from CDC. MMWR 59 (No. RR-10).
8. Clinical & Laboratory Standards Institute. Document M29: Protection of Laboratory Workers from Occupationally Acquired Infections. CLSI website. https://clsi.org/standards/products/microbiology/documents/m29/. Accessed September 2017.

IVD CE

EC REP
Hologic N.V.
Da Vincilaan 5
1930 Zaventem
Belgium

Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121 USA

Customer Support: +1 800 442 9892
customersupport@hologic.com
Technical Support: +1 888 484 4747
molecularsupport@hologic.com

For more contact information visit www.hologic.com.

AW-17997-001 Rev. 001
2018-08