REF 200400 NeuMoDx™ GBS Test Strip

Rx only

CE IVD For *In Vitro* Diagnostic Use with the commercially marketed **NeuMoDx™ 288 Molecular System**

INSTRUCTIONS FOR USE

For detailed instructions, refer to NeuMoDx™ 288 Molecular System Operator's Manual (P/N 40600108)

INTENDED USE

The **NeuMoDx™ GBS Assay** as implemented on the **NeuMoDx™ 288 Molecular System** (**NeuMoDx™ System**) is a qualitative *in vitro* diagnostic test designed to detect group B *Streptococcus* (GBS) DNA from 18-24 hour Lim broth enrichments of vaginal/rectal swabs from pregnant women. The test incorporates automated DNA extraction to isolate the target nucleic acid from the specimen and real-time polymerase chain reaction (PCR) to detect an 88 bp region of the *pcsB* gene sequence in the *Streptococcus agalactiae* chromosome. Results from the **NeuMoDx™ GBS Assay** can be used as an aid in determining colonization status in antepartum women.

The **NeuMoDx™ GBS Assay** does not provide susceptibility results. Cultured isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.

SUMMARY AND EXPLANATION

A vaginal/rectal swab is collected and transported to the laboratory using standard bacterial swab transport systems containing a non-nutritive transport medium. Appropriate transport media (e.g. Amies or Stuart's) are commercially available. In the lab, the specimen is inoculated into selective broth medium such as Lim broth (Todd-Hewitt broth supplemented with colistin and nalidixic acid). After incubation of inoculated selective broth for 18-24 hours at 37 °C in ambient air or 5% CO_2, an aliquot of the broth is mixed with **NeuMoDx™ Lysis Buffer 4** to begin lysing the sample and is fully processed on the **NeuMoDx™ System** using the **NeuMoDx™ GBS Test Strip** reagents. The **NeuMoDx™ System** automatically extracts the target nucleic acid and amplifies a section of the *pcsB* gene sequence of the GBS chromosome, if present. The **NeuMoDx™ GBS Test Strip** includes a DNA Sample Process Control (SPC1) to monitor for the presence of potential inhibitory substances as well as system or reagent failures that may be encountered during the extraction and amplification processes.

GBS is a Gram-positive bacterium found in 10-35% of healthy, adults. A person who carries GBS but does not show signs of GBS disease is said to be "colonized" with GBS. GBS are commonly found bacteria associated with the human body. Under certain circumstances, GBS can invade the body and cause serious infection; this is referred to as group B *Streptococcal* disease[1].

GBS can cause severe disease in a newborn and is known to be the leading cause of life-threatening bacterial infection in newborns. A number of strains of the pathogen circulate in the community, and approximately 80% of newborn infections are acquired during birth by vertical (mother-to-baby) transmission. Research has shown that GBS colonizes the anogenital mucosa of 25-40% of healthy women. Before active prevention was initiated, an estimated 7,500 cases of neonatal GBS disease occurred annually in the United States[1]. Striking declines in disease incidence coincide with the increased prevention activities in the 1990s[2], and a further reduction occurred following the issuance of the recommendation for universal screening in 2002[3]. Despite the introduction of antibiotic prophylaxis in the US, GBS disease remains the leading infectious cause of morbidity and mortality among newborns in the United States, approximately 2000 cases of newborn infections per year, with estimates of a mortality rate of 0.27 per 1000 live births[4,5,6].

The current standard of care for preventing neonatal GBS disease is screening pregnant women at 35-37 weeks of gestation to determine their GBS colonization status[7]. When GBS testing is performed by culture, it can take up to 48 hours for definitive identification of GBS following the initial $\geq$ 18 hour incubation step. The **NeuMoDx™ GBS Test Strip,** as implemented on the **NeuMoDx™ System**, can provide results for the first 8 specimens within an hour after the initial $\geq$ 18 hour incubation/enrichment step. The **NeuMoDx™ GBS Assay** streamlines and simplifies the testing process by eliminating the need for operator intervention from the time the sample is placed onto the system until results are available.

PRINCIPLES OF THE PROCEDURE

Following the 18 - 24 hour incubation period, the enriched broth is used for detection of the presence of GBS. The **NeuMoDx™ System** will mix 25 µL of the broth with **NeuMoDx™ Lysis Buffer 4** and extraction reagents to begin processing. The **NeuMoDx™ System** automates and integrates DNA extraction and concentration, reagent preparation, and nucleic acid amplification and detection of the target sequence using real-time PCR. The Sample Process Control is also incorporated into the sample process and amplification steps to monitor for the presence of potential inhibitory substances as well as system or reagent failures. No operator intervention is necessary once the specimen is loaded onto the **NeuMoDx™ System**.

The **NeuMoDx™ System** uses a combination of heat, lytic enzyme and extraction reagents to perform cell lysis, DNA extraction and removal of inhibitors. The released nucleic acids are captured by magnetic affinity microspheres. The microspheres, with the bound nucleic acids, are loaded into the **NeuMoDx™ Cartridge** where the unbound, non-DNA components are further washed away with **NeuMoDx™ WASH Solution** and the bound DNA is eluted using **NeuMoDx™ RELEASE Solution**. The **NeuMoDx™ System** then uses the released DNA to rehydrate dried assay reagents containing all the elements necessary for amplification of the GBS-specific target. The dried PCR reagents also contain the components required to amplify a section of the Sample Process Control sequence to enable simultaneous amplification and detection of both target and control DNA sequences. After reconstitution of the dried PCR reagents, the **NeuMoDx™ System** dispenses the prepared PCR-ready mixture into one PCR

chamber (per specimen) of the **NeuMoDx™ Cartridge**. Amplification and detection of the control and target (if present) DNA sequences occur in PCR chamber. The chamber and the cartridge are designed to contain the amplicon following real-time-PCR and essentially eliminate contamination risk post-amplification.

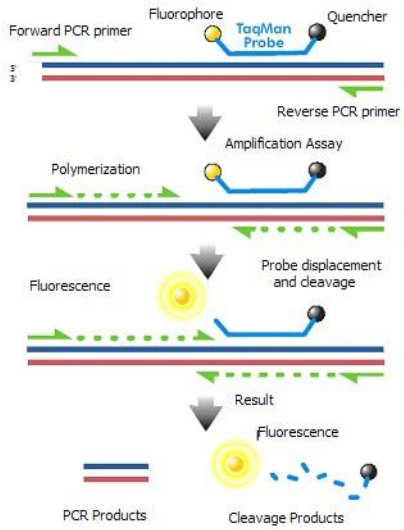

Figure 1:
Mechanism of action of TaqMan® chemistry

The amplified targets are detected in real time using hydrolysis probe chemistry (commonly referred to as TaqMan® chemistry) using fluorogenic oligonucleotide probe molecules specific to the amplicons for their respective targets.

TaqMan® probes consist of a fluorophore covalently attached to the 5'-end of the oligonucleotide probe and a quencher at the 3'-end (Figure 1). While the probe is intact, the fluorophore and the quencher are in proximity, resulting in the quencher molecule quenching the fluorescence emitted by the fluorophore via FRET (Förster Resonance Energy Transfer).

TaqMan® probes are designed such that they anneal within a DNA region amplified by a specific set of primers. As the Taq DNA polymerase extends the primer and synthesizes the new strand, the 5' to 3' exonuclease activity of the Taq DNA polymerase degrades the probe that has annealed to the template. Degradation of the probe releases the fluorophore from it and breaks the close proximity to the quencher, thereby overcoming the quenching effect due to FRET and allowing detecting fluorescence of the fluorophore. The resulting fluorescence signal detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and can be correlated to the amount of target DNA present in PCR.

A TaqMan® probe labeled with a fluorophore (Excitation: 490 nm & Emission: 521 nm) at the 5' end, and a dark quencher at the 3' end, is used to detect GBS DNA. For detection of the Sample Process Control, the TaqMan® probe is labeled with an alternate fluorescent dye (Excitation: 535 nm & Emission: 556 nm) at the 5' end, and a dark quencher at the 3' end. The **NeuMoDx™ System** monitors the fluorescent signal emitted by the TaqMan® probes at the end of each amplification cycle. When amplification is complete, the **NeuMoDx™ System** analyzes the data and reports a final result (POSITIVE / NEGATIVE / INDETERMINATE / UNRESOLVED).

REAGENTS / CONSUMABLES

Material Provided

REF	Contents	Tests per unit	Total tests per case
200400	**NeuMoDx™ GBS Test Strip** *Dried PCR reagents containing GBS-specific TaqMan® probe and primers along with Sample Process Control-specific TaqMan® probe and primers.*	16	384

NeuMoDx™ Reagents / Consumables Required But Not Provided

REF	Contents
100200	**NeuMoDx™ Extraction Plate** *Dried magnetic affinity microspheres, Lytic enzyme, and sample process controls*
400700	**NeuMoDx™ Lysis Buffer 4**
400100	**NeuMoDx ™ WASH Solution**
400200	**NeuMoDx™ RELEASE Solution**
100100	**NeuMoDx ™ Cartridge**
235903	**300 µL Tips compatible with the NeuMoDx™ System**
235905	**1000 µL Tips compatible with the NeuMoDx™ System**

Other Equipment and Materials Required But Not Provided

1. **NeuMoDx™ 288 Molecular System** [REF 500100]
2. Vortex mixer
3. Disposable transfer pipettes
4. Specimen Tubes compatible with the **NeuMoDx™ System** (See *Test Preparation* for details)
5. Specimen Barcode Labels compatible with the **NeuMoDx™ System** (See the *Accessories/Specimen Tube Carriers* section of the *NeuMoDx™ 288 Molecular System Operator's Manual* for details)
6. Lab coat and disposable powder free, nitrile gloves
7. Lim broth
8. Swabs compatible with vaginal/rectal specimen collection and recommended transport media (e.g. Amies or Stuart's)
9. General laboratory incubator

WARNINGS & PRECAUTIONS

- This test is for in vitro diagnostic use with the **NeuMoDx™ System** only.
- Do not use the reagents after the listed expiration date.
- Do not use any reagents if the safety seal is broken or if the packaging is damaged upon arrival.
- Do not use reagents if the protective pouch is open or broken upon arrival.
- Minimum specimen volume is 1 mL of enriched Lim broth; volume less than 1 mL may result in a System error.
- Testing outside of conditions recommended by CDC may produce erroneous results when using the **NeuMoDx™ GBS Assay**.
- Avoid microbial and deoxyribonuclease (DNase) contamination of reagents at all times. The use of sterile DNase-free disposable transferring pipettes is recommended. Use a new pipette for each specimen.
- To avoid contamination, do not handle or break apart any **NeuMoDx™ Cartridge** post-amplification. Do not retrieve post-amplification cartridges from the waste under any circumstances. The **NeuMoDx™ Cartridge** is designed to prevent contamination.
- In cases where open-tube PCR tests are also conducted by the laboratory, care must be taken to ensure that the **NeuMoDx™ GBS Test Strip**, the additional reagents required for testing, and the **NeuMoDx™ System** are not contaminated.
- Clean, powder-free, nitrile gloves should be worn when handling **NeuMoDx™** reagents and consumables. Care should be taken not to touch the top surface of the **NeuMoDx™ Cartridge** or foil seal surface of the **NeuMoDx™ GBS Test Strip** or **NeuMoDx™ Extraction Plate**; handling of the products should be done by touching side surfaces only.
- Safety Data Sheets (SDS) are provided for each reagent.

- Follow instructions in the *General Precautions/Routine Use* section of the *to NeuMoDx™ 288 Molecular System Operator's Manual* for recommended cleaning solutions to be used on the system.
- Do not pipette by mouth. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in *Biosafety in Microbiological and Biomedical Laboratories*[8] and in CLSI Document M29-A3.
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state and local regulations.

STORAGE, HANDLING & STABILITY

- Vaginal/rectal swab specimens from antepartum women for enrichment in Lim broth should be collected, stored, and handled according to the CDC recommended clinical procedure.
- **NeuMoDx™** reagents and consumables are stable in the primary packaging at 18 to 28 °C through the stated expiration date on the immediate product label.
- Do not use reagents past the stated expiration date.
- Do not use any test product if the primary or secondary packaging has been visually compromised.
- A **NeuMoDx™ GBS Test Strip** placed on the worktable of the **NeuMoDx™ System** is stable for 28 days; the **NeuMoDx™ System** software will prompt the removal of the reagents that have been in-use on board the system for longer than 28 days and new test strips will need to be opened and loaded on the **NeuMoDx™ System**.

INSTRUCTIONS FOR USE

Specimen Collection / Transport / Enrichment

1. After collecting the vaginal/rectal specimen using the CDC recommended clinical procedure,[7] transport the specimen to the laboratory in a non-nutritive transport medium such as Amies or Stuart's.
2. If vaginal and rectal swabs are collected separately from the same patient, both swabs can be placed in the same container of transport medium.
3. Label specimens clearly and indicate specimens are for GBS testing; the label should also indicate if antibiotic susceptibility testing is to be performed.
4. Remove swab(s) from transport medium and inoculate a recommended selective broth medium such as Lim broth [Todd Hewitt broth supplemented with colistin and nalidixic acid.]
5. Incubate inoculated selective broth (Lim broth) for 18-24 hours at 37 °C in ambient air or 5% CO_2.
6. Proceed to Test Preparation section.

Test Preparation

1. Apply specimen barcode label to a specimen tube compatible with the **NeuMoDx™ System**.
2. Gently vortex the enriched broth specimen to achieve uniform distribution.
3. Using a transfer pipette, transfer ≥ 1 mL of Lim broth to the barcoded specimen (daughter) tube. Use a different transfer pipette for each specimen. The daughter tube must meet the following tube specifications compatible with the **NeuMoDx™ System** based on Specimen Tube Carrier being used for processing.
   - 32-Tube Carrier: between 11 mm – 14 mm in diameter and between 60 mm and 120 mm in height
   - 24-Tube Carrier: between 14.5 mm – 18 mm in diameter and between 60 mm and 120 mm in height

NeuMoDx™ 288 Molecular System Operation

For detailed instructions, refer to the NeuMoDx™ 288 Molecular System Operator's Manual (P/N 40600108).

1. Populate the system carriers as necessary with the following consumables and use the touchscreen to load carrier(s) into the **NeuMoDx™ System**:
   a. 1000 µL Pipette Tips
   b. 300 µL Pipette Tips
   c. **NeuMoDx™ Cartridge**
   d. **NeuMoDx™ Extraction Plate**
   e. **NeuMoDx™ GBS Test Strip**
   f. **NeuMoDx™ Lysis Buffer 4** (NOTE: *remove foil seal from troughs prior to loading*)
2. Replace **NeuMoDx™ WASH** and **NeuMoDx™ RELEASE Solutions**, and empty Priming Waste as necessary.
3. Empty Biohazardous Waste Container as necessary or prompted by the **NeuMoDx™ System** software.
4. Load the specimen tube(s) into a standard 32-Tube carrier or 24-Tube Carrier, and ensure caps are removed from all specimen tubes.
5. Place the Specimen Tube carrier on the Autoloader shelf and use the touchscreen to load carrier into the system**.** This will initiate processing of test(s).

LIMITATIONS

1. The **NeuMoDx™ GBS Test Strip** can only be used on the **NeuMoDx™ 288 Molecular System.**
2. The performance of the **NeuMoDx™ GBS Assay** has been established with vaginal/rectal specimens collected from antepartum patients using swabs in a non-nutritive transport medium (such as Amies or Stuart's), after enrichment using selective Lim broth. Performance of the **NeuMoDx™ GBS Assay** was validated with Lim broth only. Performance has not been validated with other GBS selective broth enrichment media.
3. The use of the **NeuMoDx™ GBS Assay** with other clinical sources has not been assessed and performance characteristics of this test are unknown for other specimen types.
4. Because detection of group B *Streptococcus* is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
5. Erroneous test results could occur from improper specimen collection, handling, storage, technical error, or sample mix-up. In addition, false negative results could occur because the number of organisms in the specimen is below the analytical sensitivity of the test.
6. Testing is limited to use by personnel trained on the use of the **NeuMoDx™ 288 System.**

7. If the Sample Process Control does not amplify and the **NeuMoDx™ GBS Assay** result is Negative, an invalid result (Indeterminate or Unresolved) will be reported and the test should be repeated.
8. 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.
9. 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.
10. GBS colonization during pregnancy can be intermittent, persistent, or transient. The clinical utility of GBS screening decreases when testing is performed more than five weeks prior to delivery.
11. The NeuMoDx GBS test does not provide susceptibility results. Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.
12. While there are no known strains/isolates of GBS lacking the *pcsB* gene, the occurrence of such a strain could lead to an erroneous result using the **NeuMoDx™ GBS Test Strip**.
13. Mutations in primer/probe binding regions may affect detection using the **NeuMoDx™ GBS Test Strip**.
14. Results from **NeuMoDx™ GBS Assay** 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.
15. Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination of specimens.

RESULTS

Expected Values - Prevalence

Approximately 10–40% of pregnant women are colonized with GBS. Culture screening of both the vagina and rectum for GBS late in gestation (generally 35-37 weeks), during prenatal care, can detect women who are likely to be colonized with GBS at the time of delivery. During the clinical method comparison study, 1193 residual Lim broth samples were enrolled and tested across three geographically diverse laboratories in the United Sates. The overall prevalence of GBS in the study, based on the gold standard culture identification results provided as the reference method for all included samples, was 21.9% (261/1193) with a 95% CI of (19.6%-24.3%), as calculated using the 95% score confidence interval method per CLSI guideline EP12-A2.[9] Actual prevalence rates may vary among geographical locations based on local patient populations.

NeuMoDx™ 288 Molecular System

Available results may be viewed or printed from the 'Results' tab in the Results window on the **NeuMoDx™ System** touchscreen.

Test results are automatically generated by the **NeuMoDx™ System** software. A test result may be reported as Negative, Positive, Indeterminate or Unresolved based on the amplification status of the target and sample processing control. Results are reported based on the decision algorithm in Table 1.

Table 1. NeuMoDx™ GBS Assay Decision Algorithm

Result	GBS C_t	Sample Process Control (SPC1) C_t
Positive	9 < **C_t** < 37 And EP > 3000	N/A
Negative	N/A **OR** C_t < 9 **OR** > 37	25 < **C_t** < 35 **And** EP > 2000
Indeterminate	N/A SYSTEM ERROR NOTED	N/A SYSTEM ERROR NOTED
Unresolved	Not detected	Not detected

EP = End Point Fluorescence (after baseline correction)

Quality Control

Clinical Laboratory Improvement Amendments (CLIA) regulations specify that the laboratory is responsible for having control procedures that monitor accuracy and precision of the complete analytical process, and must establish the number, type, and frequency of testing control materials using verified performance specifications for an unmodified, FDA-cleared or approved test system (42 CFR Part 493.1256).

1. External control materials will not be provided by NeuMoDx Molecular, Inc.; appropriate controls must be chosen and validated by the laboratory.
   Recommended positive control: 10 µL of AcroMetrix™ GBS Positive Control (Thermo Fisher Scientific REF 960041) diluted in 1 mL Lim broth.
   Recommended negative control: 1 mL of Lim broth without inoculation.
2. The primers and probe for specific for Sample Process Control 1 (SPC1) are included in each **NeuMoDx™ GBS Test Strip**. This Sample Process Control allows the system to monitor the efficacy of the DNA extraction and PCR amplification processes.

3. A positive test result reported for a negative control sample indicates a specimen contamination problem. Please refer to NeuMoDx™ *288 Molecular System Operator's Manual* for Troubleshooting tips.

4. A negative result reported for a positive control sample may indicate there is a reagent or instrument related problem.

Invalid Results
If a test performed on the **NeuMoDx™ System** fails to produce a valid result, it will be reported as either Indeterminate or Unresolved based on the type of error that occurred.

An Indeterminate result will be reported if a System error is detected during sample processing. In the event an Indeterminate (IND) result is reported, a retest is recommended.

An Unresolved result will be reported if no target is detected and there is no amplification of the Sample Process Control, which indicates possible reagent failure or the presence of inhibitors. In the event an Unresolved (UNR) result is reported, a retest is recommended.

PERFORMANCE CHARACTERISTICS

Clinical Performance
Performance characteristics were determined during a prospective clinical method comparison study conducted at three (3) geographically diverse laboratory locations to evaluate the comparative performance of the of the **NeuMoDx™ GBS Assa**y as implemented on the **NeuMoDx™ 288 Molecular System** compared to conventional culture methods recommended by the Center for Disease Control (CDC) to identify GBS from subcultures of enriched Lim broth. Specimens eligible for enrollment were collected from pregnant women by health care providers for routine standard of care screening purposes recommended by the CDC between 35-37 weeks gestation.

The collected vaginal / rectal swab specimens were transported to the various laboratories in appropriate transport medium and then inoculated into a selective Lim broth medium by laboratory personnel in preparation to undergo an 18 – 24 hour incubation period. Following the incubation period and routine care testing, the residual Lim broth samples were sub-cultured to a sheep blood agar plate as recommended by the 2010 published CDC procedures for processing clinical specimens for culture of GBS. The agar plates were incubated for up to 48 hours and inspected for organisms suggestive of GBS. Suspected colonies were Gram-stained and the Gram-positive cocci colonies were tested for catalase production; Gram positive cocci colonies that tested negative for catalase production were worked-up for further identification by a streptococcal grouping latex agglutination test to determine the presence of GBS. Clinical performance is based on 1193 specimens with complete, valid, and compliant results included in the study and summarized in the tables below. The lower and upper limits of the presented 95% confidence interval (CI) were calculated using the 95% score confidence interval method.

Table 2. NeuMoDx™ GBS Assay Clinical Performance Summary

Clinical Site Summary		Culture / Reference Method		
		Positive	**Negative**	***Total***
NeuMoDx™ GBS	Positive	253	37	*290*
	Negative	8	895	*903*
	Total	*261*	*932*	*1193*

Sensitivity = 96.9%
95% CI (94.1 – 98.4)

Specificity = 96.0%
95% CI (94.6-97.1)

Table 3. Site Specific Clinical Performance of the NeuMoDx™ GBS Assay

Site	n	Sensitivity (95% CI) [a]	Specificity (95% CI) [a]	Prevalence [b] (95% CI) [a]
A	351	92.4% 73/79 (84.4-96.5)	96.7% 263/272 (93.8-98.3)	22.5% 79/351 (15.1-22.2)
B	400	98.4% 62/63 (91.5-99.7)	94.4% 318/337 (91.4-96.4)	15.8% 63/400 (10.8-17.0)
C	*442*	99.2% 118/119 (95.4-99.9)	97.2% 314/323 (94.8-98.5)	26.9% 119/442 (18.2-24.7)
Total	*1193*	*96.9%* *253/261* *(94.1-98.4)*	*96.0%* *895/932* *(94.6-97.1)*	*21.9%* *261/1193* *(19.6-24.3)*

[a] The lower and upper limits of the presented 95% confidence interval (CI) were calculated using the 95% score confidence interval method.

[b] Prevalence calculations based on reference method results obtained by following the CDC-recommended procedures for processing clinical specimens for culture of group B *Streptococcus*. (Published 2010)

Sensitivity

The Analytical Sensitivity of the **NeuMoDx™ GBS Assay** using the **NeuMoDx™ GBS Test Strip** was characterized by testing five different levels of GBS (ATCC BAA-611 serotype V) prepared from five independent clinical negative pools on the **NeuMoDx™ 288 Molecular System**. The study was performed over non-consecutive days across multiple systems with each system processing ten replicates at each level per day. A unique lot of each of the following: **NeuMoDx™ GBS Test Strip, NeuMoDx™ Extraction Plate** and **NeuMoDx™ Lysis Buffer 4** was tested on each System. Detection rates are depicted in Table 4. The LoD was determined to be 500 CFU/mL.

Table 4. Positive percent detection rates for samples used to determine LoD of the NeuMoDx™ GBS Assay

GBS CFU/mL	Number of Valid Tests	Number of Positives	Number of Negatives	Detection Rate
1000	60	60	0	100%
500*	60	60	0	100%
200	60	53	7	88%
100	60	35	25	58%
0	60	0	60	0%

*equivalent to 20 CFU/test

The **NeuMoDx™ GBS Assay** as implemented using the **NeuMoDx™ GBS Test Strip** detected all major serotypes of group B S*treptococcus,* including the four most clinically relevant. The twelve different strains of GBS bacteria spanning the serotypes that were tested using the **NeuMoDx™ GBS Test Strip** are shown in Table 5.

Table 5. GBS Serotypes Tested

GBS Serotype	GBS Strain	ATCC/BEI#	Concentration (CFU/mL) with 100% Detection
Ia	A909	ATCC: BAA-1138	1500
Ib	H36b	ATCC: BAA-1174	1000
II	MNZ933	BEI: NR-43896	400
III	MNZ938	BEI: NR-43897	400
Ic	CDC SS700	ATCC: 27591	800
IV	2011201884	ATCC: BAA-2673	800
VI	2010228816	ATCC: BAA-2671	800
VII	4832-06	ATCC: BAA-2670	800
VIII	5030-08	ATCC: BAA-2669	800
IX	7509-07	ATCC: BAA-2668	800
Non-hemolytic	NCTC 8181	ATCC: 13813	800
TX Clinical Isolate 2012	SGBS030	BEI: NR-44144	800

Analytical Specificity and Cross-reactivity

Analytical specificity was demonstrated by screening 136 organisms common to the urogenital and digestive tract, as well as species phylogenetically related to GBS for cross-reactivity on the **NeuMoDx™ 288 Molecular System** using the **NeuMoDx™ GBS Test Strip**. Organisms were prepared in pools of 5-6 and tested at a high concentration (bacteria 6 – $9x10^6$ CFU/mL; viruses $1x10^6$ – $1x10^7$ copies/mL). None of the organisms screened demonstrated cross-reactivity when implementing the **NeuMoDx™ GBS Assay**. The organisms tested are shown in Tables 6.

Table 6. Pathogens Used to Demonstrate Analytical Specificity

Bacteria, Yeast and Parasites		
Streptococcus pyogenes	*Salmonella enterica* (serovar Minnesota)	*Cryptococcus neoformans*
Streptococcus salivarius	*Alcaligenes faecalis*	*Candida glabrata*
Streptococcus sanguinis	*Staphylococcus saprophyticus*	*Achromobacter xerosis*
*Moraxella (*Branhamella) *catarrhalis*	*Eikenella corrodens*	*Rhodospirillum rubrum*
Neisseria gonorrhoeae	*Enterococcus avium*	*Neisseria subflava*
Streptococcus pyogenes	*Micrococcus luteus*	*Pseudomonas putida*
Streptococcus mitis	*Citrobacter freundii*	*Bacillus subtilis*
Lactococcus lactis	*Gemella haemolysans*	*Corynebacterium xerosis*
Listeria monocytogenes	*Kingella kingae*	*Mycobacterium smegmatis*
Morganella morganii	*Rahnella aquatilis*	*Legionella pneumophila*
Plesiomonas shigelloides	*Bacillus cereus*	*Moraxella lacunata*
Proteus vulgaris	*Aeromonas hydrophila*	*Streptomyces griseus*
Salmonella enterica (serovar Typhi)	*Enterobacter cloacae*	*Gardnerella vaginalis*
Staphylococcus aureus	*Brevibacterium linens*	*Clostridium perfringens*
Staphylococcus epidermidis	*Candida parapsilosis*	*Peptostreptococcus anaerobius*
Streptococcus mutans	*Lactobacillus brevis*	*Bifidobacterium adolescentis*
Yersinia enterocolitica	*Deinococcus radiodurans*	*Derxia gummosa*
Providencia stuartii	*Pseudomonas protegens*	*Veillonella parvula*
Pseudomonas aeruginosa	*Acinetobacter calcoaceticus*	*Mycoplasma pneumoniae*
Acinetobacter lwoffii	*Lactobacillus acidophilus*	*Bacteroides fragilis*
Proteus mirabilis	*Vibrio parahaemolyticus*	*Acinetobacter baumannii*
Klebsiella pneumoniae	*Corynebacterium genitalium*	*Corynebacterium,*strain HFH0082
Aerococcus viridans	*Enterococcus faecalis*	*Enterobacter aerogenes*
Enterococcus faecium	*Salmonella enterica*	*Klebsiella oxytoca*
Neisseria lactamica	*Lactobacillus jensenii*	*Escherichia coli*
Neisseria meningitidis	*Lactobacillus delbrueckii*	*Streptococcus canis*
Streptococcus pneumoniae	*Serratia marcescens*	*Streptococcus dysgalactiae*
Kingella denitrificans	*Candida albicans*	*Streptococcus oralis*
Haemophilus influenzae	*Candida tropicalis*	*Streptococcus uberis*
Neisseria perflava	*Chromobacterium violaceum*	*Streptococcus suis*
Moraxella osloensis	*Candida krusei*	
Neisseria meningitidis Sero C	*Saccharomyces cerevisiae*	
Neisseria meningitidis Sero A	*Corynebacterium urealyticum*	**Viruses**
Streptococcus anginosus (Grp C)	MRSA	CMV*
Streptococcus bovis	*Chlamydia trachomatis*	EBV (HHV-4)
Streptococcus intermedius	*Bifidobacterium breve*	HSV1*
Neisseria meningitidis M158 group D	*Mobiluncus mulieris*	HSV2*
Neisseria flavescens	*Propionibacterium acnes*	VZV (HHV 3)*
Streptococcus parasanguinis	*Campylobacter jejuni*	HPV-16*
Lactobacillus casei	*Haemophilus ducreyi*	JC virus*
Lactobacillus lactis	*Mycoplasma hominis*	BK virus
Haemophilus influenzea type B	*Mycoplasma genitalium*	HHV-6A
Salmonella newport	*Trichomonas vaginalis*	HHV-6B
Shigella flexneri	*Pseudomonas fluorescens*	HHV-7
Shigella sonnei	*Enterococcus dispar*	HHV-8
Enterococcus durans	*Ureaplasma urealyticum*	
Enterococcus sp. *(ATCC® 202155™)*	*Chlamydia pneumoniae**	

*Tested at 10 ng/ml

Interfering Substances - Commensal Organisms

The **NeuMoDx™ GBS Assay** was tested for interference in the presence of non-target organisms (co-habiting in the urogenital tract) by evaluating the performance of the assay at low levels of GBS on the **NeuMoDx™ 288 Molecular System**. The same panel of 136 organisms (Table 6) used for assessing cross-reactivity was used for this study. The organisms were pooled into groups of 5-6 in clinical negative Lim broth and spiked with 1200 CFU/mL cultured GBS. Testing validated detection of group B *streptococcus* in all of the pools tested. No interference due to commensal organisms was observed.

Endogenous and Exogenous Substances Encountered in GBS Clinical Specimens

The performance of the **NeuMoDx™ GBS Assay** was assessed on the **NeuMoDx™ 288 Molecular System** in the presence of exogenous and endogenous interfering substances which may typically be encountered in GBS clinical specimens. Each of the endogenous and exogenous substances listed below in Table 7 were added to pooled clinical negative Lim broth samples containing GBS at 1200 CFU/mL or 4000 CFU/mL. The 20 exogenous and 6 endogenous substances that were tested for interference using the **NeuMoDx™ GBS Test Strip** resulted in no adverse effect on detection of GBS at either level tested further demonstrating the robustness of the **NeuMoDx™ GBS Assay**.

Table 7. Exogenous and Endogenous Interfering Agents tested

Exogenous Substances			Endogenous Substances
Monistat® Cream	Dulcolax® Suppositories	K-Y™ Jelly	Human Amniotic Fluid
Yeast Gard Advanced™ (Douche)	Fleet® Enema	McKesson Gel	Human Whole Blood
Metamucil® Fiber Supplement	Preparation H® Cream	Contraceptive Foam	Human Urine
Ex-lax® (Chocolate Pieces)	Vagisil™ Powder	Moisturizing Lotion	Human Fecal Sample
Phillips'® Milk of Magnesia	Norforms® Suppositories	Neutrogena® Body oil	Mucus
Pepto-Bismol™	FDS® Deodorant Spray	Gold Bond® Powder	Human Genomic DNA
Kaopectate®	New Mama Bottom Spray		

Precision

Qualitative testing was performed on the **NeuMoDx™ 288 Molecular System** using the **NeuMoDx™ GBS Test Strip** where 2 runs per day were performed across 3 systems over a period of 12 non-consecutive days. This within-lab precision testing included 2 reagent lots and was performed by 2 operators. A run was defined as three replicates tested for each of the five different levels shown in Table 8 (True Negative, Low Negative, Moderate Negative, Low Positive and Moderate Positive) for a total of 15 specimens per run per system. Specimens were prepared by spiking cultured GBS into pooled, screened negative clinical remnant Lim broth. For each run performed, a positive and a negative external control were processed in addition to the 15 specimens. A total of 72 runs and 1224 tests were performed in this study, including the external controls. Table 9 shows comparison across instruments. Table 10 shows precision across operators.

Table 8. Within Lab Precision Panel

Panel Member	Level Tested	GBS (CFU/mL)
Moderate Positive (MP)	3-4x LoD	1600
Low Positive (LP)	1-2x LoD	600
Moderate Negative (MN)	> 10-fold dilution of 1x LoD	40
Low Negative (LN)	>100-fold dilution of 1x LoD	4
True (Blank) Negative (TN)	0	0

Table 9. Qualitative Results from Within-Lab Precision Study (Across Instruments)

Level	Instrument 1	Instrument 2	Instrument 3	Overall
	Percent Positive	Percent Positive	Percent Positive	Percent Positive
MP	100% (72/72)	100% (72/72)	100% (72/72)	100% (216/216)
LP	100% (72/72)	95.8% (69/72)	97.2% (70/72)	97.7% (211/216)
	Percent Negative	Percent Negative	Percent Negative	Percent Negative
MN	77.7% (56/72)	86.1% (62/72)	83.3% (60/72)	82% (178/216)
LN	97.2% (70/72)	100% (72/72)	98.6% (71/72)	98.6% (213/216)
TN	100% (72/72)	100% (72/72)	100% (72/72)	100% (216/216)

Table 10. Quantitative GBS Parameter Analysis from Within Lab Precision (Across Operators)

Level	First Operator					Second Operator					Combined Data Set				
	Detected Pos/Total	% Positive	Ave Ct	Std Dev	% CV*	Detected Pos/Total	% Positive	Ave Ct	Std Dev	% CV	Detected Pos/Total	% Positive	Ave Ct	Std Dev	% CV
MP	108/108	100.0%	31.61	0.54	1.7%	108/108	100.0%	32.22	0.51	1.6%	216/216	100.0%	31.91	0.61	1.9%
LP	106/108	98.1%	34.16	0.68	2.0%	105/108	97.2%	34.39	0.72	2.1%	211/216	97.7%	34.27	0.71	2.1%
MN	20/108	18.5%	35.00	0.53	1.5%	18/108	16.7%	35.28	0.40	1.1%	38/216	17.6%	35.10	0.49	1.4%
LN	2/108	1.9%	35.49	0.12	0.3%	1/108	0.9%	35.03	N/A		3/216	1.4%	35.33	0.28	0.8%
TN	0/108	0.0%	N/A			0/108	0.0%	N/A			0/216	0.0%	N/A		

%CV: The coefficient of variation, 100* standard deviation/Ave Ct.

Inter-Lab Reproducibility

The reproducibility of the **NeuMoDx™ GBS Assay** as implemented on the **NeuMoDx™ 288 Molecular System** using the **NeuMoDx™ GBS Test Strip** was evaluated at 3 different testing sites by testing 5 replicates of a 4-member panel over 5 days, which generated a total of 75 replicates per panel member. Panel samples were prepared by spiking cultured GBS into pooled, negative clinical Lim broth to create Low Negative, Low Positive and Moderate Positive panel members, whereas the True (Blank) Negative samples contained no GBS. Concentrations of the panel members correspond to the same levels listed in Table 8 above used for Precision (minus the Moderate Negative sample). A positive and a negative external control were also processed on each day of testing.

Overall, there were 4 invalid results obtained during the Reproducibility study – one replicate of each of the 4 concentrations yielded an "Indeterminate" and all occurred on the same day of testing (Day 2) at Site B. Upon repeat testing, 2 of the 4 samples yielded a valid, correct result; the remaining two samples yielded an "Indeterminate" result a second time before yielding a valid, correct result. The percent agreement with the expected result for the panel members for all sites combined is presented in the Table 11 below.

Table 11: Inter-Lab Reproducibility Performance Summary of the NeuMoDx™ GBS Assay

Panel Member Concentration	Site 1 (A)	Site 2 (B)	Site 3 (D)	Total Agreement *(CI 95%)* [a]
Moderate Positive	25/25	25/25	25/25	***100% (75/75) (95.1 – 100)***
Low Positive	24/25	25/25	24/25	***97.3% (73/75) (90.8 – 99.3)***
Low Negative	25/25	25/25	24/25 [b]	***98.7% (74/75) (92.8 – 99.8)***
Blank Negative	25/25	25/25	25/25	***100% (75/75) (95.1 – 100)***

[a] The lower and upper limits of the presented 95% confidence interval (CI) were calculated using the 95% score confidence interval method.

[b] The Low Negative sample concentration is anticipated to be detected as positive ~5% of the time.

Carry-over and Cross-contamination

Potential sample carry-over and cross-contamination studies were performed on the **NeuMoDx™ 288 Molecular System** using the **NeuMoDx™ GBS Test Strip**. The two-part study first evaluated the impact on GBS negative samples by being interspersed with samples containing high GBS target (at $1x10^7$ CFU/mL). The positive and negative samples were loaded such that each negative sample was adjacent to a high positive sample. The second part of this study processed all negative samples immediately following a run which had processed all high GBS concentration samples. No contamination was seen in negative samples integrated with high level samples, or in negative samples that followed samples with high concentrations of GBS demonstrating the lack of any carry over and/or cross-contamination.

Effectiveness of Control

The efficacy of the sample process control included in the **NeuMoDx™ GBS Test Strip** to report any process step failures or inhibition affecting **NeuMoDx™ GBS Assay** performance was assessed on the **NeuMoDx™ 288 Molecular System**. The conditions that were tested are representative of critical process step failures that could potentially occur during sample processing and *may not be detected* by the onboard sensors that are monitoring the performance of the **NeuMoDx™ System.** This was evaluated by simulating failure of various sample process flow steps to mimic a potential system error and by spiking sample with a known inhibitor to observe the effect of inefficient inhibitor mitigation on detection of the Sample Process Control (see table 12). In instances where the processing errors did not adversely impact the performance of the sample process control (NO WASH/NO WASH BLOWOUT), the test was repeated with positive GBS samples (at 400 CFU/mL) to confirm the process error had NO adverse effect on the detection of GBS Target as well. Table 12 summarizes the results of the efficacy of control verification test.

Table 12. Effectiveness of Control Data Summary

Condition	Expected Result	Observed Result
Normal Processing	Negative	Negative
Normal Processing + Inhibitor	Unresolved	Unresolved
No Wash Solution	Unresolved or Negative	Negative
No Wash Blowout	Unresolved or Negative	Negative
No Release Solution	Indeterminate	Indeterminate
No PCR Master Mix Reagents	Indeterminate	Indeterminate

On-board Sample Stability

Samples with different collection dates were processed on the **NeuMoDx™ 288 Molecular System** at "Time 0" and "Time 24" in order to determine the on-board sample stability for the **NeuMoDx™ GBS Assay**. Both clinical GBS positive and negative samples were processed initially and then left on the system worktable for 24 hours before being processed a second time. 100% concordance was observed between the results obtained from the initial test (Time 0) and test performed 24 hours later (Time 24) for the 23 GBS negative samples tested (Table 13). After 24 hours, all but one of the positive samples generated a positive result for a 95.8% concordance with the expected result.

Table 13. On-board Sample Stability Data Summary

		Confirmed Positive Samples (Samples A)		Confirmed Negative Samples (Samples B)	
		# Positive	# Negative	# Positive	# Negative
Test 1	Time 0	23	0	0	23
Test 2	Time 24	22	1*	0	23
% Concordance		**95.8**		**100**	

* *One sample was initially identified as positive at Time 0; further evaluation concluded the sample was falsely identified as positive, due to a low level of GBS DNA or non-viable cellular material as there was no GBS growth in culture reported by the reference laboratory.*

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TRADEMARKS

SYMBOLS

SYMBOL	MEANING
Rx only	Prescription Use Only
	Manufacturer
IVD	*In Vitro* Diagnostic Medical Device
EC REP	EC Representative
REF	Catalog Number
LOT	Batch Code
	Use By
	Temperature Limitation
	Humidity Limitation
	Do Not Reuse
CONTROL	Control
	Contains Sufficient for "n" Tests
	Consult Instructions for Use
	Caution
	Biological Risks (Potentially Biohazardous Material)
CE	CE Mark

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