Group B Streptococcus Real-time PCR Kit, IVD

REF MD04941, 96 reactions

For professional* in vitro *diagnostic use only

CE IVD

EN

Instructions for Use

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1. Introduction

Streptococcus agalactiae, also known as Group B *Streptococcus* (GBS), is the pathogen causing the most serious bacterial infections in newborns. This bacterium colonizes the gastrointestinal and the lower genital tract in 10 to 40% of pregnant women and there is a risk that the baby may become infected during delivery. GBS infection in newborns can lead to sepsis, pneumonia, and meningitis. To prevent GBS infection in newborns, late in pregnancy (between 35 and 37 weeks of gestation), pregnant women are routinely screened for GBS. A positive test for GBS, will require the administration of antibiotics during labour to reduce the risk of infection to the baby. However, women who give birth to preterm infants usually do not have the opportunity to be screened for GBS, so presence of this bacterium in labour is uncontrolled. Thus, in these cases, there is an increased risk of serious infections in newborns leading to the administration of antibiotics during childbirth, which has been associated with the emergence of resistant bacterial strains. Recent studies revealed that up to two-thirds of newborns who developed early GBS septicemia were born to mothers whose prenatal screening performed using routine microbiological culture methods indicated that they were GBS negative. Hence, although microbiological culture is considered the standard method for detecting GBS, it has limitations related with its lowest sensitivity and the time to result period that takes up to 72 hours. Rea-time PCR assay represents a fast, powerful, and robust method that can be used to identify the presence of Group B *Streptococcus*.

2. Intended Use

NZYtech' Group B Streptococcus Real-time PCR Kit, IVD, is a molecular test based on real-time PCR technology, intended for the rapid detection and qualitative diagnosis of specific nucleic acids of GBS, from vaginal and/or perianal swabs collected in a transport kit containing Liquid Amies. This test is intended to identify GBS colonization in antepartum women. There are no contraindications to using NZYtech' Group B Streptococcus Real-time PCR Kit, IVD. Testing must be performed by specialized and qualified laboratory technicians, especially in real-time PCR technique and molecular *in vitro* diagnostics experience. The kit should only be used as indicated in this user manual.

3. Principles of the Assay

NZYtech' Group B Streptococcus Real-time PCR Kit, IVD, provides the set of reagents, enzymes, and oligonucleotides (primers and probes) for the qualitative detection of the GBS genome, using real-time PCR (see equipment specification requirements in **Section 6**). The kit's target sequence was previously identified as a good genetic marker for GBS. NZYtech' Group B Streptococcus Real-time PCR Kit, IVD, was designed to have a broad detection profile while remaining specific for GBS detection. In addition, the set of oligonucleotides was specifically designed for the detection of this organism and does not show significant homology with other genomes, which reflects the high specificity and detection sensitivity of the test. As such, the kit was designed to be specific to GBS and to avoid the detection of other organisms causing similar infections. The internal control, included in the kit, validates the efficacy of the extraction process as well as the absence of PCR inhibitors potentially present in the human biological samples. Periodically, NZYtech revisits the GBS target gene sequence and, if necessary, can provide a new version of this kit. Additionally, the kit includes two external controls (a positive control and a negative control) as described below. The positive control consists of a nucleic acid fragment that contains the target sequence detected by the kit.

In this kit, the qualitative determination of DNA is based on real-time PCR technique, a reference methodology in laboratory diagnosis. It is a highly sensitive and specific methodology to accurately detect the presence of this organism. The principle of NZYtech' Group B Streptococcus Real-time PCR Kit, IVD, consists in the use of isolated and purified DNA through an extraction system to search the presence of bacterial DNA. Extracted DNA is subjected to a PCR amplification, in a single reaction, using a set of highly specific primer/probe sets exploiting the TaqMan® principle. In the presence of GBS DNA, the TaqMan® probe specifically binds to conserved regions of the specific GBS gene which is flanked by a specific primers pair. A second primers/probe set acts as an internal control, detecting the human β-actin gene (ACTB). Detection of the internal control validates the efficacy of the extraction process, and it also allows confirming whether the PCR reaction was compromised by the presence/absence of inhibitors in clinical samples. To allow identifying the amplification of the two specific targets in a single reaction, probes specific for GBS and for human β-actin are differently labelled with FAM™ and HEX™ reporter dyes, respectively. Thus, this kit consists of a duplex assay where the GBS target is detected in the optical channel FAM and the human target gene is detected in the optical channel VIC (see **Section 9.2**). These oligonucleotides/probe sets are provided in optimized concentrations to ensure that human DNA, even when present at extremely high concentrations, does not limit the efficiency of GBS primers/probe sets.

4. Kit Composition

NZYtech' Group B Streptococcus Real-time PCR Kit, IVD, provides a comprehensive set of reagents sufficient to perform 96 Real-time PCR reactions in a single step.

KIT COMPONENT		**VOLUME** (PER VIAL)	**NUMBER OF TUBES**	**CAP COLOUR**
GBS MMix	NZYSupreme qPCR Probe Master Mix (2x)	1050 µL	1	Neutral
GBS PPMix	GBS/ACTB Primer & Probe Mix (10x)	205 µL	1	Brown
GBS POS	GBS/ACTB Positive Control	105 µL	1	Red
NTC	No-Template Control	105 µL	1	Neutral

5. Storage, Stability and Handling Conditions

NZYtech' Group B Streptococcus Real-time PCR Kit, IVD, is shipped refrigerated. Upon receipt of the kit, all components should be immediately stored at -85 °C to -15 °C. When in use, kit components should be promptly placed in the freezer after use to minimize the exposure time to room temperature. In addition:

- Minimise the number of freeze-thaw cycles by storing in working aliquots. If appropriate, kit components may be aliquoted into smaller volumes after thawing. The kit is stable through a minimum of 6 freeze-thaw cycles.
- The GBS PPMix component should be stored protected from light. Particularly, do not expose GBS MMix to direct sunlight after combining with GBS PPMix.
- If the package that protects the kit arrives damaged, please contact NZYtech.
- Beware of the expiry date indicated on the packaging. NZYtech does not recommend using the kit after the expiry date. On this date, the kit must be discarded following the disposal instructions in **Section 8.2**.

6. Materials and Instrumentation Required but Not Provided

- Real-time PCR Instrument that detects FAM™ and VIC™ (at emission wavelengths of 520 and 556 nm, respectively). See in **Section 11** the equipment models for which the kit was validated.
- Equipment and consumables for isolating DNA of biological/clinical samples.
- RNase/DNase-free Real-time PCR plasticware: PCR tubes, strips, caps, 96-well plates, and adhesive films.
- Pipettes and filter tips (RNase/DNase-free).
- Cooling block.
- Disposable gloves.
- Vortex and centrifuge.

7. Sample Collection and Preparation

The kit is designed to detect DNA extracted from vaginal and/or perianal swabs. Different factors, such as the biological sample collection procedure, transport, storage, and sample processing time, are critical to ensure sample integrity and achieving optimal results. Vaginal and/or perianal specimens should be collected using a unique swab (ESwab®, Copan). Specimens should be tightly sealed in proper tubes or containers, correctly labelled, and then promptly transported to the laboratory. Collected samples should be tested as soon as possible. Inappropriate sample collection, handling and/or transport of specimens may result in a false result. Extracted nucleic acids constitute the starting material for the assay with NZYtech' Group B Streptococcus Real-time PCR Kit, IVD. NZYtech recommends the use of one of the following magnetic bead technology-based nucleic acid purification kits: NZY Mag Viral RNA/DNA Isolation Kit, IVD (MD0488, NZYtech) or chemagic Pathogenic NA gDNA Kit H96 (PerkinElmer). In addition, nucleic acids extraction can be performed using silica-based spin columns, particularly the NZY Tissue gDNA Isolation kit (MB135, NZYtech). These three kits have been validated for the extraction of GBS clinical samples and subsequent detection using Group B Streptococcus Real-time PCR Kit, IVD. Silica-based DNA extraction methods can be more prone to contamination than automated extraction systems due to the manual handling of samples. Please ensure that cross-contaminations did not occur and that DNA samples are suitable in terms of purity, concentration, and nucleic acid integrity. Since ethanol is a strong inhibitor of real-time PCR, it is necessary to eliminate this component before elution of nucleic acids during the extraction process. NZYtech' kit contains an internal control that targets human DNA co-purified with bacterial GBS DNA. Human DNA is amplified with the set of oligonucleotides (primers and probe) from the human β-actin gene. The introduction of internal control is useful in assessing the efficiency of DNA extraction and isolation and/or in detecting the presence of potential inhibitors during sample processing.

8. Precautions and Warnings

Carefully follow the procedures and guidelines provided in this handbook to ensure that the test is performed correctly. Before using the test, check the product's integrity, namely the amount and type of kit components and their correct labelling. As in any analytical testing procedure, good laboratory practices are essential. Any deviation from good laboratory practices may result in assay failure or cause erroneous results. Due to high sensitivity of the kit, special care must be taken to keep reagents and PCR amplification mixes free from contamination.

8.1. Safety Information

Before using the kit, please consult the Safety Data Sheet (SDS) that is available on NZYtech website (www.nzytech.com). This kit detection should be performed only by staff trained in the relevant technical and safety procedures in appropriately equipped laboratories. International and national guidelines on laboratory biosafety should be followed in all circumstances.

8.2. Handling and Procedural Requirements

- Only for professional *in vitro* diagnostic use.
- Do not use this kit after the expiration date.
- Do not use the test components if the kit sealing is damaged.
- Do not interchange reagents of different production lots.
- No reagents from other manufacturers should be used along with the reagents of this kit.
- DNase/RNase-free disposable plasticware and pipettes should be used in all procedures.
- Sample preparation, the reaction set up and amplification should be performed in different working areas. The order of tasks in the lab should be unidirectional. Always wear disposable gloves in each area and change them before entering a different area. If possible, change your coat.
- Select specific materials and equipment for each individual work area and do not transfer them from one area to another.
- The extraction of nucleic acids using silica columns can be more prone to cross-contamination than extraction performed with automated extraction systems.
- Always use NTC (No-template Control) provided in the kit.
- Biological samples must be handled as if they are infectious following proper biosafety precautions.
- Positive control contains high copy number templates; it should be opened and processed away from test samples and kit components to avoid cross-contamination.

- Handle post-amplification plates with care and dispose them immediately after the end of the testing; plates should always be discarded into a proper biohazard container after use. Do not open post-amplification reaction tubes/plates to avoid amplicon contamination.
- At the end of each test, clean work surfaces and equipment with a DNA/RNA remover.
- Residues of chemicals and preparations are generally considered hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations.
- All results should be interpreted by a healthcare professional in the context of the patient's medical history and clinical symptoms.
- This test cannot exclude diseases caused by other pathogens.
- A negative result for any PCR test does not conclusively rule out the possibility of infection.
- Follow good laboratory practices, wear protective clothing, permanently wear disposable powder-free gloves, and use goggles and a mask. Do not eat, drink, or smoke in the working area.

9. Testing Procedure

Please read the instructions for use carefully before performing the assay. Beware that all pipetting steps and plate set-up should be performed following good Real-time PCR practices. After the plate is poured start immediately the real-time PCR protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection. Before the experiment, start by gently mixing the reaction tubes provided with the finger and centrifuge for five seconds to collect contents at the bottom of the tube. Place tubes on ice. **We strongly recommend pipetting GBS POS last to avoid cross-contaminations.**

9.1. Reaction set-up

1. Prepare a Real-time PCR mix sufficient for the number of tests to be performed with a 5% additional volume for pipetting losses. Proceed according to the table below that specifies the volumes for 1 and *n* tests (where *n* corresponds to the total number of reactions):

COMPONENT	**1 TEST VOLUME (µL)**	***n* TESTS * VOLUME + 5% (µL)**
GBS MMIX **	10	*n* x 10.5
GBS PPMIX	2	*n* x 2.1
FINAL VOLUME	**12**	***n* x 12.6**

** To calculate the total number of reactions needed for each assay, count the number of samples, and add two more, to include the Negative and the Positive controls, respectively.*
*** Please notice that a precipitate in the bottom of the master mix tube may be observed, in particular after multiple freeze/thaw cycles. To ensure optimal performance, please make sure all components are thawed and resuspended before use. In this case do not spin the master mix before pipetting.*

2. Pipette 12 µL of the Real-time PCR mix into individual wells according to your real-time PCR experimental plate set-up.
3. For the negative control, add 8 µL of NTC instead of the DNA template into the negative control well. The final volume should be 20 µL.
4. For the biological samples, add 8 µL of each DNA sample into the sample wells, according to your experimental plate set-up. The final volume in each well should be 20 µL.
5. For the positive control, add 8 µL of GBS POS instead of the DNA template into the positive control wells. The final volume should be 20 µL.
6. Cover and seal the plate with an appropriate optical adhesive film or caps before proceeding with the Real-time PCR and detection steps.
7. Place the reaction plate in the real-time PCR instrument and run the real-time PCR protocol according to the section below.

9.2. Programming the real-time PCR instrument

The table below displays a standard protocol optimized on a few platforms. However, these conditions may be adapted and validated to suit different machine-specific protocols.

CYCLES	**TEMPERATURE**	**TIME**	**STEP**
1	95 °C	3 min	Polymerase activation
45	95 °C	5 s	Denaturation
	60 °C	30 s	Annealing/Extension*

** Depending on the Real-time PCR instrument select suitable detection channels. Fluorogenic data should be collected during this step through channels FAM and HEX.*

The fluorescent dyes used in this kit and their correspondent detection channels are the following:

Fluorescent Dyes & Detection Channels

TARGETS	**FLUORESCENT DYE**	**DETECTION CHANNELS**
GBS specific gene	FAM™	FAM
Human β-actin (ACTB) gene	HEX™	HEX/VIC/JOE
GBS POS	FAM™ & HEX™	FAM & HEX/VIC/JOE

Group B Streptococcus Real-time PCR Kit, IVD, was validated for the following Real Time PCR Systems: Applied Biosystems™ QuantStudio 5, Applied Biosystems™ 7500 FAST, Applied Biosystems™ StepOne Plus, Roche Lightcycler® 96 Instrument, Bio-Rad® CFX Opus Real-Time PCR and Bio-Rad® CFX96 Touch Real-Time PCR. If other equipment is used, the user should validate the kit using previously characterised samples (both positive and negative).

10. Data Analysis

10.1. Run Validation Criteria

Before analysing results, we recommend consulting the user manual of the respective real-time PCR device. Then verify that the real-time PCR test is valid. Thus, for each plate, please confirm if the results for Positive and Negative controls performed as expected, according to the following criteria:

Negative control (no template reaction): no amplification is detected. Sample contamination may have occurred if the negative control has amplification curves (FAM and HEX) with a sigmoidal shape. Repeat the test following good Real-time PCR practices.

Positive control: the amplification curves of FAM (GBS) and HEX (ACTB) curves are positive. Positive control is expected to amplify at Ct < 32, in both channels FAM and HEX. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

If the controls are according to the expected, the test is **valid**. Please proceed with the interpretation of the results for the tested samples.

If any of the controls do not exhibit the expected performance, the assay was compromised or executed improperly and should be considered **invalid**.
Please, repeat the test. If the problem persists, contact the manufacturer.

10.2. Test Results Interpretation

NZYtech' Group B Streptococcus Real-time PCR Kit, IVD, uses the following Ct cut-off values for results interpretation:

CT VALUE	RESULTS INTERPRETATION
Ct ≤38	Detected (+) → **POSITIVE**
Ct >38	Not Detected (-) → **NEGATIVE**

GBS is detected if the FAM amplification curve displays a sigmoidal shape with a Ct≤38, regardless of what result is obtained for the ACTB (HEX) assay. The presence or absence of a signal in the HEX channel is not significant for the validity of the test.

GBS is not detected if FAM curve does not amplify (Ct>38), while the ACTB (HEX) assay displays a positive sigmoidal curve with Ct≤45.

The **test is invalid** if the GBS and ACTB assays are negative. The test should be repeated with nucleic acid re-purified from the sample.

The following table summarises the interpretation of principal results (evaluate the overall shape of the amplification curves; **only sigmoidal amplification curves are indicative of true amplification**; **+**: amplification curve detected; **-**: no amplification curve).

GBS (FAM)	ACTB (HEX)	NEGATIVE CONTROL	POSITIVE CONTROL	RESULTS INTERPRETATION
+	+	-	+	**GBS → POSITIVE**
+	- [1]	-	+	**GBS → POSITIVE**
-	+	-	+	**GBS → NEGATIVE**
-	-	-	+	**INVALID TEST [2]**

[1] *A high concentration/load of detectable GBS DNA in the sample can lead to a reduced or absent internal control signal on the HEX channel.*
[2] *Repeat DNA extraction and run the Real-time PCR test again.*

Note: *Interpretation of results must account for the possibility of false negative and false positive results.*

- False negative results may be caused by:
  - Unsuitable collection, handling and/or storage of samples.
  - Sample degradation.
  - Presence of Real-time PCR inhibitors.
  - Mutations in the genome of the pathogenic organism.
  - Failure to follow procedures in this handbook.
  - Use of unvalidated extraction kits or real-time PCR platforms.
- False positive results may be caused by:
  - Cross-contamination with the positive control due to its unsuitable handling.
  - Unsuitable handling of samples containing high concentration of GBS DNA. Due to the high susceptibility of the Real-time PCR method for cross contaminations special care should be taken during DNA isolation.
  - Unsuitable handling of amplified product (post-amplification plate).

Negative results do not preclude GBS infection, and the test result should not be used as the sole basis for treatment or other patient management decisions. In addition, this test cannot rule out diseases caused by other pathogens.

11. Performance Evaluation

This kit performance was validated for the equipment specified in **Section 9.2** (see above). If other equipment is used, the user should validate the kit using previously characterised samples (both positive and negative).

11.1. Expected Results

A typical amplification plots observed for a clinical GBS positive sample is presented in Figure 1. In situations where the sample contains high amounts of GBS DNA, the VIC channel curve, corresponding to the human β-actin target, may be absent or exhibit an atypical shape.

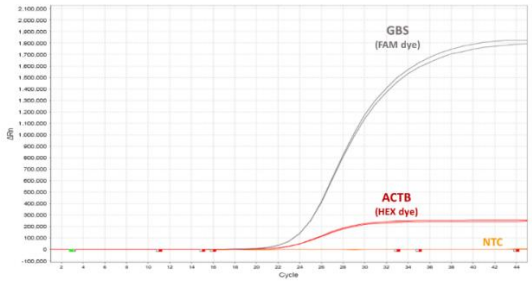

Figure 1. Detection of GBS and human β-actin (ACTB) targets in a clinical positive sample. Grey curve: detection of the GBS target sequence through the FAM channel. Red curve: detection of the human β-actin target (ACTB) through the VIC channel. Orange curve: No-template control (NTC).

11.2. Limit of Detection (LoD) - Analytical Sensitivity

The analytical sensitivity was defined as the lowest concentration of analyte that could be reliably detected with 95% confidence. This was assessed by testing GBS nucleic acids at different copy numbers, spiked into DNA extracted from negative vaginal/perianal samples, using three different kit batches following typical testing reaction conditions. Tests were repeated twice a day over two days, producing 48 replicates for each concentration tested. Together, the data revealed that NZYtech' Group B Streptococcus Real-time PCR Kit, IVD detects 5 copies/reaction or 0,250 copies/µL of GBS DNA with a confidence ≥95%. Thus, the tentative Limit of Detection (LoD) was determined to be 250 copies/mL for GBS. All assays were performed using the Applied Biosystems™ QuantStudio 5 Real Time PCR instrument and the analysis was performed using the instrument's software.

The kit LoD was re-evaluated by two different operators, using three kit lots, and three alternative nucleic acids isolation methods namely, NZY Mag Viral RNA/DNA Isolation Kit, IVD (MD0488, NZYtech), NZY Tissue gDNA Isolation kit (MB135, NZYtech) and chemagic Pathogenic NA gDNA Kit H96 (PerkinElmer), in three experiments with a total of 48 tests for each isolation kit. Data show that when nucleic acids are extracted using NZY Mag Viral RNA/DNA Isolation Kit, IVD (MD0488, NZYtech) or NZY Tissue gDNA Isolation kit (MB135, NZYtech) the LoD is 250 copies/mL and that when nucleic acids extraction is performed using chemagic Pathogenic NA gDNA Kit H96 (PerkinElmer) the LoD is 500 copies/mL.

The ability of the Group B Streptococcus Real-time PCR Kit, IVD, to detect the pathogen at different concentrations (from 5 x 10^5 to 5 copies per reaction) is presented in Figure 2.

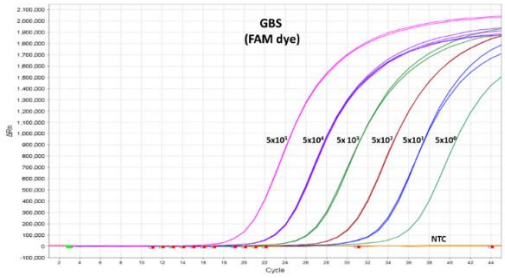

Figure 2. Sensitivity of the NZYtech' Group B Streptococcus Real-time PCR Kit, IVD. Amplification plot (cycle number versus fluorescence - ΔRn) of 1:10 serial dilutions of the GBS DNA, from 5 x 10^5 copies to 5 copies per reaction through the FAM channel. NTC, No-template control (negative control).

11.3. Analytical Specificity

11.3.1. Cross-Reactivity (Exclusivity) and Specificity

Inclusivity and cross-reactivity were evaluated *in silico* by analysing all oligonucleotide probes and primers included in the kit against pathogens related to GBS and normal pathogens that cause infection with similar symptoms, respectively. Assay primers and probes were screened against published genome sequences. Upon *in silico* analysis the assay design was found to specifically detect GBS and exhibited no reactivity with non-related species.

In vitro cross-reactivity assays (exclusivity) were performed to confirm that Group B Streptococcus Real-time PCR Kit, IVD, does not react with other colonizing and pathogenic microorganisms commonly found in human vaginal/perianal specimens. This study was performed using a commercial panel of vaginal pathogens (NATtrol™ Vaginal Panel®, ZeptoMetrix®) and genomic DNA (DSMZ) of the selected microorganisms. Assays were performed using genomic DNA of the following 29 microorganisms: *Atopobium vaginae Z242, Bacillus cereus ATCC 14579, Bifidobacterium breve ATCC 15700, BVAB2 recombinant, Candida albicans Z006, Candida glabrata 14/73657, Candida krusei Z009, Candida tropicalis ATCC 13803, Chlamydia trachomatis ATCC VR-902B, Clostridium perfringens VPI No.5, Enterobacter cloacae ATCC 13047, Enterococcus avium R 6566, Enterococcus faecium E980, Escherichia coli K-12, Fusobacterium nucleatum ATCC 25586, Gardnerella vaginalis CCUG 792,*

Klebsiella oxytoca 1028, Lactobacillus acidophilus, Lactobacillus crispatus Z246, Mesomycoplasma lagogenitalium ATCC 700289, Mycoplasma hominis ZK-CU2, Neisseria gonorrhoeae ATCC 19424, Proteus mirabilis ATCC 12453, Pseudomonas aeruginosa BWKH 8, Staphylococcus aureus BWKH 152, Staphylococcus epidermidis K2-4, Streptococcus pneumoniae ATCC 33400, Streptococcus pyogenes ATCC 12344 and *Trichomonas vaginalis strain Z070.* All tests were performed in triplicate using three kit batches. None of the pathogens tested yielded a false-positive Real-time PCR signal. *Streptococcus agalactiae* Z019 was also tested, and as expected it yielded a true-positive Real-time PCR signal.

11.3.2. Microbial Interference Studies

Microbial interference studies were performed to determine if NZYtech' Group B Streptococcus Real-time PCR Kit, IVD reacts with other pathogens and/or microorganisms that are likely to be encountered in the clinical samples, generating a false negative test result. Negative vaginal/perianal specimens were spiked with GBS nucleic acids at 3x LoD, and with a high genomic DNA concentration of each potentially inhibitory organism. The assay was performed using three kit batches and following typical testing reaction conditions. The results showed that no interference was observed with the following 24 microorganisms: *Bacillus cereus ATCC 14579, Bifidobacterium breve ATCC 15700, Candida albicans Z006, Candida glabrata 14/73657, Candida tropicalis ATCC 13803, Chlamydia trachomatis ATCC VR-902B, Clostridium perfringens VPI No.5, Enterobacter cloacae ATCC 13047, Enterococcus avium R 6566 Enterococcus faecium E980, Escherichia coli K-12, Fusobacterium nucleatum ATCC 25586, Gardnerella vaginalis CCUG 792, Klebsiella oxytoca 1028, Lactobacillus acidophilus, Mesomycoplasma lagogenitalium ATCC 700289, Mycoplasma hominis ZK-CU2, Neisseria gonorrhoeae ATCC 19424, Proteus mirabilis ATCC 12453, Pseudomonas aeruginosa BWKH 8, Staphylococcus aureus BWKH 152, Staphylococcus epidermidis K2-4, Streptococcus pneumoniae ATCC 33400* and *Streptococcus pyogenes ATCC 12344.*

11.3.3. Interfering Substances

The performance of the NZYtech' Group B Streptococcus Real-time PCR Kit, IVD was evaluated with 25 potentially interfering substances that may be present vaginal/perianal specimens. The assays were performed using negative vaginal/perianal specimens spiked with GBS positive specimens at 3x LoD and 30x LoD. Potentially interfering substances were tested at concentrations that represent the highest levels expected in artificial vaginal/perianal samples based on the literature review. All tests were performed in triplicate using three kit batches and compared to data obtained with a control test containing no interferents. The table below resumes the data collected under these experiments. Data revealed that none of the substances tested interfered with the sensitivity of detection of GBS by the Group B Streptococcus Real-time PCR Kit, IVD.

POTENTIAL INTERFERENT	ACTIVE INGREDIENTS	FINAL CONCENTRATION IN THE SAMPLE	INTERFERENCE YES (Y) OR NO (N)
Antacids (Gaviscon®)	Sodium alginate (250 mg) + Sodium bicarbonate (133,5 mg) + Calcium carbonate (80 mg)	5 mg/mL	N
Anti-Diarrheal Medication (Imodium®)	Loperamide hydrochloride (2 mg)	10 mg/mL	N
Laxatives (Bekunis®)	Senna (105 mg) + bisacodyl (5 mg)	10 mg/mL	N
Anti-Haemorrhoid Creams/Ointments(Scheriproct®)	Prednisolone (1,9 mg/g) + Cinchocaine (5 mg/g)	10% w/v	N
Antacids (Kompensan® Trieffect)	Sodium aluminum dihydroxide carbonate (340 mg) and dimethicone (30 mg)	5 mg/mL	N
Antimicrobials (Gino-Canesten®)	Clotrimazole (10 mg/g)	10% w/v	N
Anti-Diarrheal Medication (UL-250®)	Saccharomyces boulardii (250 mg)	10 mg/mL	N
Laxatives (Microlax®)	sodium citrate dihydrate (450 mg) + sodium lauryl sulfoacetate (45 mg)	5% v/v	N
Antimicrobials (Lomexin®)	Fenticonazole nitrate	10% w/v	N
Medicine (Progeffik®)	Progesterone	5 mg/mL	N
Anti-Fungal	Flucytosine	5 mg/mL	N
Anti-Fungal	Amphotericin B (20 µg/mL)	10% v/v	N
Seminal fluid	-	5% v/v	N*
Whole blood	Glucose, Hormones, Enzymes, Ions, Iron, etc	10% v/v	N
Urine	-	10% v/v	N
Stool	-	1% w/v	N*
Washing products	Saponified fat + silicates	10 mg/mL	N
Topical Products (Lauroderme®)	Zinc oxide (23 mg/g) + Salicylic acid (2 mg/g)	10 mg/mL	N
Topical Products (Bepanthene®)	Dexpantenol (50 mg/g)	10% w/v	N
Topical Products (Halibut®)	Zinc oxide (150 mg/g)	10% w/v	N
Topical Products (ClimaCare®)	Hyaluronic acid + Lactic acid	10% v/v	N
Topical Products (WOMAN ISDIN®)	Glycerin (11%), Glyceryl polyacrylate, Polyacrylic acid	10% v/v	N
Washing products (BETADINE® Vaginal)	Povidone iodine	10% v/v	N*
Washing products	Lactic acid	10% v/v	N
Absolute ethanol	Alcohol	5% v/v	N

* Higher concentrations of this interferent can reduce kit's sensibility or lead to false negative results.

11.4. Precision

Assay precision for NZYtech' Group B Streptococcus Real-time PCR Kit, IVD was determined by the repeated testing of positive samples representing two bacterial load levels, 3x LoD and 30x LoD copies per reaction, spiked into DNA extracted from negative vaginal/perianal samples, using 3 different kit batches, and following typical testing reaction conditions. Precision was evaluated by measuring Cq average, Cq coefficient of variation and % of replicate detection, as described below for each case. The data is resumed in the table displayed below.

Precision of NZYtech' Group B Streptococcus Real-time PCR Kit, IVD

VARIABLE		GBS (COPIES/REACTION)	
		3x LOD	30x LOD
REPEATABILITY	n	12	12
	Mean Cq	35.23	31.26
	Coefficient of Variation (%)	2.01	0.95
	% Replicate Detection	100	100
DAILY REPRODUCIBILITY	n	48	48
	Mean Cq	34.85	31.25
	Coefficient of Variation (%)	2.40	1.10
	% Replicate Detection	100	100
LOT-TO-LOT REPRODUCIBILITY	n	72	72
	Mean Cq	35.03	31.45
	Coefficient of Variation (%)	2.42	1.34
	% Replicate Detection	100	100
OPERATOR REPRODUCIBILITY	n	36	36
	Mean Cq	35.35	31.64
	Coefficient of Variation (%)	2.09	1.25
	% Replicate Detection	100	100
INTER-INSTRUMENT REPRODUCIBILITY	n	60	60
	Mean Cq	35.20	31.69
	Coefficient of Variation (%)	2.18	1.02
	% Replicate Detection	100	100

11.4.1. Repeatability

Repeatability was assessed by one operator by analysing 12 replicates of each sample (3x LoD and 30x LoD copies per reaction), accounting for a final number of 24 tests performed per target.

11.4.2. Daily Reproducibility

Daily reproducibility was assessed by one operator by analysing 48 replicates of each sample (3x LoD and 30x LoD copies per reaction), for 4 days, with 12 replicates of each concentration per day (a total of 96 assays were performed).

11.4.3. Lot-to-lot Reproducibility

Reproducibility between lots was assessed by one operator through the analysis of 72 replicates of each sample (3x LoD and 30x LoD copies per reaction) using 3 different kit batches with 24 replicates per batch.

11.4.4. Operator Reproducibility

Operator reproducibility was assessed by testing 36 replicates of each sample (3x LoD and 30x LoD copies per reaction), by three different operators with 12 replicates per operator.

11.4.5. Inter-instrument Reproducibility

Inter-instrument reproducibility was measured by one operator through the testing 12 replicates of each sample (3x LoD and 30x LoD copies per reaction), in six different Real-time PCR instruments:

REAL-TIME PCR EQUIPMENT' MANUFACTURER	REAL-TIME PCR PLATFORM MODEL
Applied Biosystems®	7500 Fast
	StepOne Plus
	QuantStudio™ 5
Roche®	LightCycler 96™ instrument
Bio-Rad®	CFX Opus 96 Real-Time PCR
	CFX96 Touch Real-Time PCR

11.5. Clinical evaluation

The clinical performance of NZYtech' Group B Streptococcus Real-time PCR Kit, IVD was evaluated using 186 vaginal and/or perianal swab samples obtained from pregnant women and previously characterized using routine microbiological culture. Nucleic acids were extracted using NZY Mag Viral RNA/DNA Isolation Kit, IVD (MD0488, NZYtech) and then amplified using Group B Streptococcus Real-time PCR Kit, IVD. Data revealed that 93.1% of clinical sensitivity (PPA) and 97,0% of clinical specificity (NPA) agreements were achieved for all positive and negative samples tested, respectively.

		COMPARATOR ASSAY - MICROBIOLOGIC CULTURE		
		GBS Positive	**GBS Negative**	**Total**
GROUP B STREPTOCOCCUS REAL-TIME PCR KIT, IVD	**GBS Positive**	81	3*	84
	GBS Negative	6	96	102
	Total	87	99	186

PPA (Positive percent agreement): 93.1% for Group B *Streptococcus*
NPA (Negative percent agreement): 97.0% for Group B *Streptococcus*

* A possible explanation for those negative culture samples is loss of bacterial viability during specimen collection and/or transport where the nonviable bacterial DNA remained available for amplification. In addition, this difference can be justified by the fact that Real-time PCR technique is more sensitive than culture methods.

Furthermore, comparison of the clinical performance of the NZYtech' Group B Streptococcus Real-time PCR Kit, IVD and a real-time PCR comparator test was also performed. Data revealed that 98.8% of clinical sensitivity (PPA) and 98.1% of clinical specificity (NPA) agreements were achieved for all positive and negative samples tested, respectively.

		COMPARATOR ASSAY – REAL-TIME PCR KIT		
		GBS Positive	**GBS Negative**	**Total**
GROUP B STREPTOCOCCUS REAL-TIME PCR KIT, IVD	**GBS Positive**	82	2	84
	GBS Negative	1	101	102
	Total	83	103	186

PPA (Positive percent agreement): 98.8% for Group B *Streptococcus*
NPA (Negative percent agreement): 98.1% for Group B *Streptococcus*

Additionally, the clinical performance of the kit was evaluated when using two alternative nucleic acids extraction methods, namely NZY Tissue gDNA Isolation kit (MB135, NZYtech) and chemagic Pathogenic NA gDNA Kit H96 (PerkinElmer). For NZY Tissue gDNA Isolation kit (MB135, NZYtech) data revealed a 100% PPA and a 100% NPA when compared with the detection of nucleic acids extracted using NZY Mag Viral RNA/DNA Isolation Kit, IVD (MD0488, NZYtech). For chemagic Pathogenic NA gDNA Kit H96 (PerkinElmer) data revealed a 95.8% PPA and a 100% NPA. Overall, results show high sensitivity and specificity to detect GBS using NZYtech' Group B Streptococcus Real-time PCR Kit, IVD.

12. Quality Control

All components of NZYtech' Group B Streptococcus Real-time PCR Kit, IVD, are tested following the protocols described above. The duplex Real-time PCR system allows the detection of targets described for the identification of GBS DNA as well as human ACTB DNA. Positive amplifications are observed for target genes, positive and internal controls through FAM and HEX, according to respective primers/probe set reporter dyes.

13. Technical Support

For Technical support, please contact our dedicated technical support team by Phone: +351 (0) 21 364 35 14 or Email: info@nzytech.com.

14. Trademarks and Disclaimers

All trademarks that appear in this manual are the property of their respective owners.

15. Explanation of Symbols

IVD	*In vitro* diagnostic medical device		Consult instructions for use
REF	Catalogue number		Manufacturer
LOT	Batch code		Use by
	Temperature limitation		Sufficient for
CONTROL +	Positive control		Keep away from the sun light (primer/probe mix)
CONTROL -	Negative control		

16. Conformity Declaration

Product Name: Group B Streptococcus Real-time PCR Kit, IVD

Catalogue Number: MD04941

Intended use: *Streptococcus agalactiae* qualitative detection.

Classification: Others (not covered by Annex II or not intended to self-testing) according to the EC Directive 98/79/EC.

Manufacturer: NZYtech - Genes & Enzymes,

Estrada do Paço do Lumiar, Campus do Lumiar

Edifício E, R/C,

1649-038, Lisboa

Portugal

We, NZYtech, Lda – Genes & Enzymes, hereby declare that this product, to which this declaration of conformity relates, is in conformity with the following standards and other normative documents ISO 9001:2015 and ISO 13485:2016, following the provisions of the 98/79/EC Directive and of the Regulation (EU) 2017/746 on *in vitro* diagnostic medical devices as transposed into the national laws of the Member States of the European Union.

The product technical file is maintained at NZYtech, Estrada do Paço do Lumiar, Campus do Lumiar - Edifício E, R/C, 1649-038 Lisboa, Portugal.

Joana Brás, PhD

Technical Director

17. References

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