DNA-TECHNOLOGY

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CE IVD

For professional use only

Streptococcus agalactiae REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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R1-P012-S3/4EU
R1-P012-23/4EU
R1-P012-UA/9EU

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1. INTENDED USE

The **Streptococcus agalactiae REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Streptococcus agalactiae REAL-TIME PCR Detection Kit** is intended for *Streptococcus agalactiae* (group B streptococcus) DNA detection in human biological samples (blood, phlegm, urine, scrapes from respiratory tract, urogenital and gastrointestinal tracts, faeces or meconium, bioptates, cerebrospinal fluid), washings from catheters and endotracheal tubes and bacterial cultures by polymerase chain reaction with detection in real-time.

Indications for the use: screening of symptomless carriers of *Streptococcus agalactiae* or presence of symptoms of infectious disease caused by *Streptococcus agalactiae*.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **Streptococcus agalactiae REAL-TIME PCR Detection Kit.**

The **Streptococcus agalactiae REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and research practice.

Potential users: personnel qualified in molecular diagnostics methods and working in the clinical and diagnostic laboratory.

It is necessary to apply the kit only as directed in this instruction for use.

2. METHOD

The implemented PCR method is based on amplification of a target DNA sequence. The process of amplification includes repeating cycles of thermal DNA denaturation, annealing of primers with complementary sequences and their extension by DNA-polymerase.

To increase the sensitivity and specificity of amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin. The polymerase chain reaction starts only when paraffin is melted. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

The PCR-mix contains target-specific probes, each of them bearing reporter fluorescent dyes and quencher molecules. When specific product is formed, DNA probes are disintegrated and the quencher molecule stops affecting the fluorescent dye. Thus the level of fluorescence increases and it is detected by special devices. As a result of probe activation fluorescence increases proportionally to target sequence amplification. The amount of disintegrated probes increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The PCR-mix includes the internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probe used for the detection of the *Streptococcus agalactiae* product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the fluorescent dye Hex. Table 1 shows the detection channels of amplification products.

Table 1– Detection channels of amplification products

Fam (Green)	**Hex (Yellow)**	**Rox (Orange)**	**Cy5 (Red)**	**Cy5.5 (Crimson)**
Streptococcus agalactiae	IC	-	-	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **Streptococcus agalactiae REAL-TIME PCR Detection Kit** (see the catalogue at https://www.dna-technology.com to see available supply options). The current version of the software is available for download at https://www.dna-technology.com/software.

The **Streptococcus agalactiae REAL-TIME PCR Detection Kit** is also approved for use with Rotor-Gene Q (Qiagen) real-time thermal cycler.

3. CONTENT

The detailed description of content is represented in Tables 2-4.

Table 2. **The Streptococcus agalactiae REAL-TIME PCR Detection Kit** content, package S (standard), strips for R1-P012-S3/4EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under white waxy fraction	960 µL (20 µL in each tube)	6 8-tube strips
Taq-polymerase solution	Colorless transparent liquid	500 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	1 tube
Positive control	Colorless transparent liquid	130 µL	1 tube
Strip's caps	6 8-caps		

Table 3. The **Streptococcus agalactiae REAL-TIME PCR Detection Kit** content, package S (standard), tubes for R1-P012-23/4EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under white waxy fraction	960 µL (20 µL in each tube)	48 tubes
Taq-polymerase solution	Colorless transparent liquid	500 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	1 tube
Positive control	Colorless transparent liquid	130 µL	1 tube

Table 4. The **Streptococcus agalactiae REAL-TIME PCR Detection Kit** content, package U, for R1-P012-UA/9EU

Reagent	Description	Total volume	Amount
PCR-mix	Colorless transparent liquid	600 µL	1 tube
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	30 µL	1 tube
PCR-buffer	Colorless transparent liquid	600 µL	1 tube
Positive control	Colorless transparent liquid	130 µL	1 tube

All components are ready to use and do not require additional preparation for operation.

The kit is intended for single use and designed for 48 tests (package S) including no more than 46 experimental samples, negative control and positive control samples. The kit in the package U is intended for 96 samples and requires no less than 5 samples in a single run (3 experimental samples, positive and negative controls) or on using a dosing device it is possible to run 96 tests simultaneously (94 experimental samples, negative control and positive control samples).

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs, single-use sterile flasks and sterile containers to collect clinical material;
- Sterile tubes containing transport medium: "DNA-Technology" made **PREP-RAPID** (REF P-001/1EU, not applicable to male urethral smears) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent or sterile physiological saline solution for the transportation of the sample.
- For blood collection: 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example, salt of EDTA at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant.

Please use only salt of EDTA or sodium citrate as an anticoagulant, since other substances can provide PCR inhibition.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 50-65 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU), **PREP-RAPID** (REF P-001/1EU, not applicable to male urethral smears) and **PREP-MB RAPID** (REF P-116-N/4EU, P-116-A/8EU) extraction kits are recommended);
- Physiological saline solution 0.9% NaCl (Sterile);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Freezing chamber (in case of using package U REF R1-P012-UA/9EU);
- Vortex mixer;
- Vortex rotor for strips (in case of using package in strips REF R1-P012-S3/4EU);
- Tube rack for 1.5 mL tubes;

- PCR tube rack for 0.2 mL tubes or strips;
- 0.2 mL PCR tubes (in case of using package U REF R1-P012-UA/9EU);
- Single channel pipettes (dispensers covering 1.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 10 µL, 20 µL, 200 µL, 1000 µL);
- DTstream M1 dosage instrument (only for automated dosing using detection kit in the package U REF R1-P012-UA/9EU);
- Device for tray sealing DTpack ("DNA-Technology", LLC) (only for automated dosing using detection kit in the package U REF R1-P012-UA/9EU);
- Centrifuge for microtrays (only for automated dosing using detection kit in the package U REF R1-P012-UA/9EU);
- Polymer thermal seal for microtray sealing (only for automated dosing using detection kit in the package U REF R-P012-UA/9EU);
- PCR microtray (only for automated dosing using detection kit in the package U REF R1-P012-UA/9EU);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

- Real-time PCR thermal cycler.

Software:

The most recent version of the DT thermal cyclers software can be downloaded from https://www.dna-technology.com/software.

The OS supported: all versions of Windows starting from 7.

5. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 months from the date of production.

All components of the **Streptococcus agalactiae REAL-TIME PCR Detection Kit** except TechnoTaq MAX polymerase (package U) must be stored at temperatures from 2 °C to 8 °C during the storage period.

TechnoTaq MAX polymerase must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period. The excessive temperature and light can be detrimental to product performance.

The kit has to be transported in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions of the kit components.

Transportation of the kit, except the TechnoTaq MAX polymerase, is allowed in termobox with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but no more than 5 days and should be stored at temperatures from 2 °C to 8 °C immediately on receipt.

It is allowed to transport the TechnoTaq MAX polymerase in termobox with ice packs by all types of roofed transport at temperatures up to 25 °C but no more than 5 days and should be stored at temperatures from minus 18 °C to minus 22 °C immediately on receipt.

Shelf-life of the kit following the first opening of the primary container:

- components of the kit, except TechnoTaq MAX polymerase, should be stored at temperatures from 2 °C to 8 °C during the storage period;

- TechnoTaq MAX polymerase should be stored at temperatures from minus 18 °C to minus 22 °C during the storage period;
- PCR-mix for amplification should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period.

The kit stored in under undue regime should not be used.

An expired the **Streptococcus agalactiae REAL-TIME PCR Detection Kit** should not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

The conformity of the **Streptococcus agalactiae REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **Streptococcus agalactiae REAL-TIME PCR Detection Kit**.

6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular diagnostics and the rules of work in the clinical and diagnostic laboratory are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial DNA synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of

amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be exclusively employed for this specific purpose. Remove PCR waste only in a closed form. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Do not open the tubes after amplification. Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

Emergency actions

Inhalation: Inhalation of the PCR-mix contained within this kit is unlikely, however care should be taken.

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

Do not use the kit:

- When the transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are NOT anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

7. SAMPLES

The **Streptococcus agalactiae REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from the blood, phlegm, urine, scrapes from respiratory tract, urogenital and gastrointestinal tracts, faeces or meconium, bioptates, cerebrospinal fluid, washings from catheters and endotracheal tubes and bacterial cultures.

Sampling, sample processing procedures and storage are carried out in accordance with the instructions to the DNA extraction kit from biological material.

Interfering substances

The presence of PCR inhibitors in a sample may cause controversial (uncertain) results. The sign of PCR inhibition is the simultaneous absence of internal control and specific product of amplification.

PCR inhibitors are the presence of hemoglobin, bilirubin, cholesterol, triglycerids, mucus and medicines (IRS-19, nasonex, pinosol, chlorhexidine, indometacin in suppositories, rhinofluimucil, octenisept) in the DNA sample as a result of incomplete removal during the extraction of DNA from a biomaterial sample, as well as the presence of isopropyl alcohol and methyl acetate in the DNA sample as a result of incomplete removal of washing solutions during sample preparation.

Maximal concentration of interfering compounds that do not inhibit PCR are the following:

Biomaterial	Interfering compound	Concentration
Endogenous compounds		
Blood, urine, scrapes from gastrointestinal tract, faeces or meconium	bilirubin	684 µmoL/L
Blood, scrapes from gastrointestinal tract, faeces or meconium, cerebrospinal fluid	cholesterol	13 mmoL/L
Blood, phlegm, urine, scrapes prom respiratory tract, urogenital and gastrointestinal tracts, faeces or meconium, bioptates, cerebrospinal fluid, washings from catheters and endotracheal tubes	hemoglobin	0.35 mg/mL
Blood, faeces or meconium, scrapes from gastrointestinal tract	triglycerids	37 mmoL/L
Phlegm, scrapes from respiratory and urogenital tracts	mucus	20%
Exogenous compounds		
Blood, washings from catheters	EDTA	up to 2.0 mg/mL
Phlegm, scrapes from respiratory tract	IRS-19	5%
Phlegm, scrapes from respiratory tract	nasonex	2%
Phlegm, scrapes from respiratory tract	pinosol	2%
Phlegm, scrapes from respiratory and urogenital tracts	chlorhexidine	5%
Faeces or meconium	indometacin in suppositories	5%
Phlegm, scrapes from respiratory tract	rhinofluimucil	5%
Phlegm, scrapes from respiratory and urogenital tracts	octenisept	2%
Blood, phlegm, urine, scrapes prom respiratory tract, urogenital and gastrointestinal tracts, faeces or meconium, bioptates, cerebrospinal fluid, washings from catheters and endotracheal tubes, bacterial cultures	isopropil alcohol	10%
Blood, phlegm, urine, scrapes prom respiratory tract, urogenital and gastrointestinal tracts, faeces or meconium, bioptates, cerebrospinal fluid, washings from catheters and endotracheal tubes, bacterial cultures	methylacetate	10%

General requirements

PCR analysis is a direct method, so taking of biological material must be carried out from the location of the infectious process. The decision about analyzing the location of sampling is done by a physician according to anamnesis and clinical picture.

The quality of taking a sample of biomaterial, its storage, transportation and pre-processing have a great importance for obtaining correct results.

Incorrect sample taking can lead to invalid results and the need for resampling.

ATTENTION! Before DNA extraction pre-processing of samples is needed.

Sample collection

ATTENTION! Pretreatment, sampling and storage of the material is carried out in accordance with the user manual for DNA extraction kit.

Peripheral blood

Peripheral blood sampling is carried out in vacuum plastic tube. It may be 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL. After taking the material, it is necessary to mix the blood with anticoagulant inverting the tube 2 – 3 times.

ATTENTION! It is not allowed to use heparin as an anticoagulant.

Phlegm

Sample taking is made in amount no less than 1.0 mL into single-use graduated sterile flacks with wide neck and screwing caps with volume no less than 50 mL.

Urine

The first portion of morning urine in the amount of 20–30 mL is selected for the analysis. The urine is taken into a special dry sterile container with volume of up to 60 mL, equipped with a hermetical screw-cap. After the urine collection, container is tightly screwed and marked.

Scrapes from respiratory tract, gastrointestinal and urogenital tracts

Sample taking is made with special sterile single-use tools – probes, cytobrushes and swabs depending on the source of biological material according to established procedure.

ATTENTION! In case of pregnancy the use of cytobrushes for genitourinary smears sampling is contraindicated.

NOTE. - Before sampling procedure, it is necessary to remove the mucus with a cotton tampon.

The taking of the scrapes is carried out in tubes with transport medium intended by the manufacturer for transportation and storage of samples for PCR.

Order of taking:

1. Open the tube.
2. Take biological material with a sterile swab.
3. Put the swab into the tube with transport medium and rinse it thoroughly for 10-15 seconds. Avoid spraying of solution.
4. Remove swab from solution, press it to the wall of tube and squeeze the rest of the liquid. Throw out the swab.
5. Close the tube tightly and mark it.

Faeces or meconium

Samples of faeces with mass (volume) 1.0-3.0 g (1.0-3.0 mL) are transferred to a sterile dry flack by a single-use filtered pipette tip or single-use shovel. After sample collection the flack is tightly closed with a lid and marked.

Bioptates

Place a tissue biopsy sample in a 1.5 mL tube with transport medium for transportation and storage of biomaterial. Close the tube and mark it.

Cerebrospinal fluid

Sampling of cerebrospinal fluid is carried out by single-use needles in single-use 1.5 mL tubes in volume no less than 500 µL according to the established procedure. After sample collection the tube is tightly closed and marked.

Bacterial cultures

Sample taking from liquid and solid media is made with single-use microbiological loop or spreader. Place a sole colony of bacterial cells or 100 µL of liquid medium in single-use tube with volume 1.5-2.0 mL with 500 µL of sterile physiological saline solution. Close the tube tightly and mark it.

Washings from parts of intravenous catheters

Cut with sterile scissors 5-10 mm of a catheter tip and place it into a 1.5 mL tube of Eppendorf type. Close the tube and mark it. Washings from catheters are made in a laboratory during sample preparation.

Washings from endotracheal tubes

Sample taking is made in single-use 50 mL tubes. After sample taking close the tube tightly and mark it.

Transportation and storage of samples

ATTENTION! Samples are stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

Blood, cerebrospinal fluid

Blood and cerebrospinal fluid samples are allowed to transport and store:

- At room temperature from 20 °C to 25 °C no more than 2 hours;
- At temperature from 2 °C to 8 °C no more than 6 hours from taking the material.

ATTENTION! The whole blood cannot be frozen.

Phlegm

Phlegm samples can be transported and stored:

- At room temperature from 18 °C to 25 °C no more than 6 hours;
- At temperature from 2 °C to 8 °C no more than 3 days.

Native or preprocessed urine samples

Native or preprocessed urine samples can be transported and stored:

- At temperature from 2 °C to 8 °C no more than 1 day;
- At temperature from minus 18 °C to minus 20 °C no more than one week;
- At temperature minus 70 °C – 6 months.

ATTENTION! Only one freezing-unfreezing of the material is allowed.

Scrapes from respiratory tract, gastrointestinal and urogenital tract

Scrapes from respiratory tract, gastrointestinal and urogenital tract, exudates must be transported and stored according to the instructions for DNA extraction kits or transport medium.

Native faeces or meconium samples

Native faeces samples can be transported and stored:

- At room temperature from 18 °C to 25 °C no more than 6 hours;
- At temperature from 2 °C to 8 °C no more than 3 days.

Biopsy samples, parts of intravenous catheters, smears and washings from endotracheal tubes, bacterial cultures

Biopsy samples, parts of intravenous catheters, smears and washings from endotracheal tubes, bacterial cultures can be transported and stored:

- At temperature from 2 °C to 8 °C no more than 1 day;
- At temperature from minus 18 °C to minus 20 °C no more than one week;
- At temperature minus 70 °C – 6 months.

ATTENTION! Only one freezing-unfreezing of the material is allowed.

Sample preparation

Blood

Preparation of blood samples are made according to the instructions for DNA extraction kits.

Phlegm

Method 1

1. Put approximately 500 µL of biological sample into sterile 1.5 mL tube and close it tightly.
2. Add to the sample an equal volume of 10% triple-substituted sodium phosphate x12H_2O and mix intensively.
3. Incubate the mixture for 18–24 hours at 37 °C, then neutralize with 1M HCl (down to pH 6.8–7.4).
4. Centrifuge the tube for 20 minutes at RCF(g) 900.
5. Take out the supernatant into the 5% solution of chloramine for disinfection.
6. Add 500 µL of distilled water to precipitate, mix by pipetting and put to the new 1.5 mL tube.
7. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
8. Remove the supernatant, leaving the volume of precipitate + liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

Method 2

1. Add mucolysin to the sampling container in the 5:1 ratio (5 parts of mucolysin to 1 part of phlegm), referring to container calibrations.
2. Close the lid of the container, mix the content and incubate for 20–30 minutes at room temperature, shake the container every 2-3 minutes.

Urine

1. Transfer 1.0 mL of the sample to the 1.5 mL tube.
2. Centrifuge the tube at PCF(g) 16000 for 10 minutes.
3. Remove the supernatant completely.
4. Add 1.0 mL of sterile physiological saline solution to the precipitate.
5. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
6. Remove the supernatant, leaving the volume of precipitate + liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

Scrapes from respiratory tract, gastrointestinal and urogenital tract, bacterial cultures from liquid and solid media

1. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
2. Remove the supernatant, leaving the volume of precipitate + liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

Faeces or meconium (preparation of the suspension)

1. Put approximately 100-200 mg (µL) of faeces into the 1.5 mL tube with 1.0 mL of sterile physiological saline solution.
2. Vortex the tube for 5-10 seconds.
3. Further preparation of samples is made according to the instructions for DNA extraction kits.

Bioptates

1. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
2. Remove the supernatant, leaving the volume of precipitate + liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

Cerebrospinal fluid

1. Transfer 500 µL of biomaterial into 1.5 mL tube with an automatic dispenser with filtered pipette tip.
2. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
3. Remove the supernatant, leaving the volume of precipitate + liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

Washings from endotracheal tubes

1. Transfer 1.0 mL of biomaterial into 1.5 mL tube with an automatic dispenser with filtered pipette tip.
2. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
3. Remove the supernatant, leaving the volume of precipitate + liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

Fragments of intravenous catheters (only with PREP-NA extraction kit)

1. Add in the tube with a fragment of catheter 100 µL of distilled water or sterile physiological saline solution.
2. Vortex the tube for 3-5 seconds and spin down the drops for 3-5 seconds in vortex.
3. Add in the tube 300 µL of lysis buffer from **PREP-NA** extraction kit.
4. Vortex the tube for 3-5 seconds and spin down the drops for 3-5 seconds in vortex.
5. Termostate the tube on 65 °C for 15 minutes.
6. Spin down the drops for 3-5 seconds in vortex and transfer the supernatant in a new tube.

Further processing of the samples should be done according to instructions for DNA extraction kits used starting from the step of precipitation buffer adding.

8. PROCEDURE

DNA extracting from biological material

DNA extraction is carried out in accordance with the instruction to the extraction kit. **PREP-NA**, **PREP-GS**, **PREP-RAPID** and **PREP-MB RAPID** extraction kits are recommended. It is allowed to use any kits of reagents registered as a medical device and recommended by manufacturers for the extraction of DNA from the corresponding types of biomaterial.

ATTENTION! Independently of DNA extraction kit used, a negative control sample should go through all stages of DNA extraction. Physiological saline solution or negative control sample from an extraction kit can be used as a negative control in volumes as indicated.

Assay procedure

8.1 Preparing PCR for package S

ATTENTION! The reagents and tubes should be kept away from direct sun light.

ATTENTION! When using package S (R1-P012-S3/4EU), strips, strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips of the other kits!

8.1.1 Mark the required number of tubes with paraffin sealed PCR-mix for each test sample, positive control (C+) and negative control (C-).

Example: to test 4 samples, mark 4 tubes for samples, 1 tube for “C-” and 1 tube for “C+”. The resulting number of tubes is 6.

8.1.2 Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

8.1.3 Add 10 µL of Taq-polymerase solution into each tube. Avoid paraffin layer break.

8.1.4 Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene Q thermal cycler). Close the tubes.

8.1.5 Vortex the tubes with samples, “C+” and “C-” for 3-5 seconds and spin down drops for 1-3 seconds.

ATTENTION! In case of using **PREP-GS DNA Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g) 16000 for one minute to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

In case of using **PREP-MB RAPID DNA Extraction Kit**, after vortexing put the tubes with the DNA preparation in magnetic rack. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 3-5 seconds in a vortex mixer.

ATTENTION! Open the cap of the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA sample to prevent contamination. In case of using tubes in strips, close the strip before proceeding to the next strip to prevent contamination. Close the tubes/strips tightly. Use filter tips.

8.1.6 Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the “C+”, “C-” tubes. Avoid paraffin layer break.

8.1.7 Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into corresponding tube. Add 5.0 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

8.1.8 Spin tubes/strips for 3-5 seconds (when using the Rotor-Gene Q thermal cycler, spin is not required).

8.1.9 Set the tubes/strips into the Real-time Thermal Cycler.

8.1.10 Launch the operating software for DT instrument[1]. Add corresponding test[2], specify the number and ID’s of the samples, positive and negative control samples. Specify the position of the tubes/strips in the thermal unit (see 8.1.9) and run PCR. See Table 5.

For Rotor-Gene Q the program is contained in the Table 6.

[1] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[2] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

Table 5. The PCR program for DTlite and DTprime Thermal Cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80	0	30	1		Cycle
	94	1	30			
2	94	0	30	5		Cycle
	64	0	15		v	
3	94	0	10	45		Cycle
	64	0	15		v	
4	94	0	5	1		Cycle
5	10[1]			Holding		Holding
[1] – holding at 25°C is allowed						

Table 6. The PCR program for Rotor-Gene Q thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	300 sec	
Cycling 2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 3	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement			

8.2 Preparing PCR for package U, manual dosing

ATTENTION! The reagents and tubes should be kept away from direct sun light.

8.2.1 Mark the required number of 0.2 mL tubes for each test sample, positive control (C+) and negative control (C-).

Example: to test 4 samples, mark 4 tubes for samples, 1 tube for “C-” and 1 tube for “C+”. The resulting number of tubes is 6.

8.2.2 Vortex the tube with PCR-mix for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

8.2.3 Add to each tube 6.0 µL of PCR-mix.

8.2.4 Vortex the tube with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

ATTENTION! TechnoTaq MAX polymerase should be got out from the freezer immediately prior to use.

8.2.5 Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase. Add into the one tube:

6.0 x (N+1) µL of PCR-buffer,

0.3 x (N+1) µL of TechnoTaq MAX polymerase,

N is a quantity of the samples to be tested taking to account “C-”, “C+”.

Example: for simultaneous testing of 4 samples, “C-” and “C+” in one PCR run, mark 6 tubes (4 tubes for samples to be tested, 1 tube for “C+” and 1 tube for “C-”). Prepare the mixture of PCR-buffer and Taq-polymerase for 7 (6+1) tubes. Mix 42 µL of PCR-buffer and 2.1 µL of TechnoTaq MAX polymerase.

8.2.6 Vortex the tube with the mixture of PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

ATTENTION! Mixture of PCR-buffer and TechnoTaq MAX polymerase must be prepared immediately prior to use.

8.2.7 Add 6.0 µL of PCR-buffer and TechnoTaq MAX polymerase mixture into each tube with PCR-mix.

ATTENTION! Follow the steps listed in pp. 8.2.8 – 8.2.13 within two hours after addition of PCR-buffer and TechnoTaq MAX polymerase mixture to PCR-mix.

8.2.8 Vortex the tubes with samples, “C+” and “C-” for 3-5 seconds and spin down drops for 1-3 seconds.

ATTENTION! In case of using **PREP-GS DNA Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g) 16000 for one minute to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

In case of using **PREP-MB RAPID DNA Extraction Kit**, after vortexing put the tubes with the DNA preparation in magnetic rack. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 3-5 seconds in a vortex mixer.

ATTENTION! Open the cap of the tube, add DNA sample (or control sample), then close the tube before proceeding to the next tube to prevent contamination. Close the tubes tightly. Use filter tips.

8.2.9 Add 6.0 µL of DNA sample into corresponding tubes. Do not add DNA into the “C+”, “C-” tubes.

8.2.10 Add 6.0 µL of negative control (C-) which passed whole DNA extraction procedure into corresponding tube. Add 6.0 µL of positive control sample (C+) into corresponding tube.

8.2.11 Spin tubes for 3-5 seconds (when using the Rotor-Gene Q thermal cycler, spin is not required).

8.2.12 Set the tubes into the Real-time Thermal Cycler.

8.2.13 Launch the operating software for DT instrument[3]. Add corresponding test[4], specify the number and ID’s of the samples, positive and negative control samples. Specify the position of the tubes in the thermal unit (see 8.2.12) and run PCR. See Table 7.

Amplification program for Rotor-Gene Q thermocycler is contained in the Table 8.

[3] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[4] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

Table 7. The PCR program for DTlite and DTprime Thermal Cyclers for package U

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80	0	5	15		Cycle
	94	0	5			
2	94	5	00	1		Cycle
3	94	0	30	5		Cycle
	64	0	15		√	
4	94	0	10	45		Cycle
	64	0	15		√	
5	94	0	5	1		Cycle
6	10[1]			Holding		Holding
[1] – holding at 25°C is allowed						

Table 8. The PCR program for Rotor-Gene Q thermal cycler for package U

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	300 sec	
Cycling 2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 3	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement			

8.3 Preparing PCR for package U, using DTStream

ATTENTION! The reagents and tubes should be kept away from direct sun light.

8.3.1 Vortex the tube with PCR-mix for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

8.3.2 Vortex the tube with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

ATTENTION! TechnoTaq MAX polymerase should be got out from the freezer immediately prior to use.

8.3.3 Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase according to the user manual for dosing device DTstream.

8.3.4 Vortex the tube with the mixture PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, the spin in vortex for 1-3 seconds to collect the drops.

8.3.5 Vortex the tubes with DNA samples, “C-” and “C+” for 3-5 seconds and spin in vortex for 1-3 seconds to collect the drops.

ATTENTION! In case of using **PREP-GS DNA Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g) 16000 for one minute to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

ATTENTION! In case of using **PREP-MB RAPID DNA Extraction Kit**, vortex the tubes for 3-5 seconds on a vortex mixer, put the tubes with the DNA preparation in magnetic rack and transfer the supernatant containing the isolated DNA to new tubes. If, after DNA extraction, the supernatant containing the isolated DNA was already transferred to new tubes, centrifugation is carried out for 3-5 seconds on a vortex mixer.

8.3.6 Set the tubes with PCR-mix, the mixture of PCR-buffer and TechnoTaq MAX polymerase, DNA samples, positive and negative controls and PCR microtray on the DTstream working table and conduct dosage of the components according to DTstream user manual.

8.3.7 After the end of dosing program on DTstream put the PCR microtray without shaking on the working table of DTpack sealing device.

8.3.8 Run the process of sealing of PCR microtray according to the user manual of DTpack sealing device.

8.3.9 Centrifuge the microtray on RCF(g) 500 for 30 seconds.

8.3.10 Set the PCR microtray into the Real-time Thermal Cycler.

8.3.11 Launch the operating software for DT instrument[5]. Add corresponding test[6], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes in the thermal unit (see 8.3.10) and run PCR. See Table 7.

9. CONTROLS

The **Streptococcus agalactiae REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the *Streptococcus agalactiae* genome. It is produced with genetic engineering techniques and characterized by automatic DNA sequencing. The PCR-mix from the kit includes the Internal control (IC). IC is an artificial plasmid intended to assess the quality of PCR performance.

ATTENTION! A negative control sample should go through all stages of DNA extraction. Physiological saline solution or negative control sample from an extraction kit can be used as a negative control sample in volumes indicated in supplied instructions.

The test result is considered valid when:

- Positive result for the specific product is present, in this case the internal control is not taken into account.
- Positive result for the specific product is absent and for internal control is present.

The test result is considered invalid when a positive result for the specific product and for internal control is absent.

If positive control (C+) has not positive result for the specific product, it is necessary to repeat the whole test. It may be caused by inhibitors, operation error or violation of storage and handling requirements.

In case of obtaining positive result for negative control sample all results of the current PCR run are considered false. In this case conduction of special procedures against possible contamination is required.

[5] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[6] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

10. DATA ANALYSIS

Registration of the results is carried out automatically during amplification by the software provided with detecting thermocycler.

After the end of the run the software registers “+” or “-” in the table with results. In this case a conclusion can be formed.

For samples with negative results on both detection channels in the “result” table will be “invalid” conclusion (invalid result). In this case amplification, or DNA extraction, or collecting of clinical material are required to be repeated (performed sequentially).

For negative and positive controls the results must match those contained in Table 9.

Table 9. The results of the test for positive and negative control samples

Detection channel		**Result**	**Result interpretation**
Fam	**Hex**		
Positive control sample			
Cp is specified	Is not considered	+	Positive result. The results are valid
Negative control sample			
Cp is not specified	Cp is specified	-	Negative result. The results are valid

Principles of result interpretation are contained in the table in Table 10.

Data registration and analysis of results are carried out automatically. This appendix contains general principles of interpretation.

Table 10. Principles of result interpretation

Detection channel		**Result**	**Interpretation of the result**
Fam	**Hex**		
Analyzed samples			
Cp is specified	Is not considered	+	DNA of *Streptococcus agalactiae* is detected
Cp is not specified	Cp is specified	-	DNA of *Streptococcus agalactiae* is not detected
Cp is not specified	Cp is not specified	Invalid	Invalid result*

* In this case amplification, or DNA extraction, or collecting of clinical material are required to be repeated (performed sequentially).

In case of results for negative control sample different from those in Table 9, the results of all series are considered invalid. In this case decontamination procedures are required.

In case of results for positive control sample different from those in Table 9, it is required to repeat amplification for all series.

11. SPECIFICATIONS

a. Analytical specificity

In investigated samples containing *Streptococcus agalactiae* DNA detecting termocycler records positive results of target sequences amplification.

In investigated samples not containing *Streptococcus agalactiae* DNA detecting termocycler records negative results of target sequences amplification and positive signal of internal control.

The absence of non-specific positive results was shown for high concentration of DNA of the following closely related or potentially present in the biomaterial microorganisms: *Streptococcus bovis, Streptococcus sanguinis, Escherichia coli, Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus anginosus, Staphylococcus aureus, Burkholderia spp., Klebsiella pneumoniae, Proteus spp., Morganella morganii, Pseudomonas aeruginosa, Enterobacter cloacae, Acinetobacter spp., Enterococcus spp.* (10^5-10^6 CFU/mL), and human DNA in concentration more than 740 ng on sample.

Absence of inhibition was shown for samples with high concentration of non-specific DNA and low concentration of *Streptococcus agalactiae* DNA.

b. Analytical sensitivity

Detection threshold is 5 copies of DNA on amplification tube. Detection threshold was established by a series of dilution of laboratory control sample (LCS).

Detection threshold in an analyzed sample depends on DNA extraction kit and final elution volume, for example, for scrapes from vagina in 500 µL of transport medium:

Kits for DNA extraction/elution volume, µL			
PREP-NA/ 50	**PREP-GS/ 100**	**PREP-MB RAPID/ 300**	**PREP RAPID/ 500**
50 copies/sample	100 copies/sample	300 copies/sample	500 copies/sample

c. Diagnostic characteristics

Biomaterial	**Sensitivity (95% CI)**	**Specificity (95% CI)**
Blood	100.0 (39.76-100.0)	100.0 (91.40-100.0)
Phlegm	100.0 (47.82-100.0)	100.0 (91.19-100.0)
Scrapes from respiratory tract	100.0 (54.07-100.0)	100.0 (88.06-100.0)
Washings endotracheal tubes	100.0 (59.04-100.0)	100.0 (59.04-100.0)
Washings from catheters	100.0 (15.81-100.0)	100.0 (39.76-100.0)
Faeces or meconium	100.0 (54.07-100.0)	100.0 (88.06-100.0)
Bacterial cultures	100.0 (80.49-100.0)	100.0 (87.66-100.0)
Urine	100.0 (82.35-100.0)	100.0 (88.78-100.0)
Scrapes from gastrointestinal tract	100.0 (78.20-100.0)	100.0 (88.43-100.0)
Scrapes from urogenital tract	100.0 (63.06-100.0)	100.0 (90.51-100.0)
Bioptates*	100.0 (69.15-100.0)	100.0 (69.15-100.0)
Cerebrospinal fluid*	100.0 (69.15-100.0)	100.0 (69.15-100.0)
Streptococcus agalactiae	**100.0 (96.6-100.0)**	**100.0 (98.6-100.0)**
Note:	* - synthetic samples	

ATTENTION! The claimed specifications are guaranteed when DNA extraction is performed with **PREP-RAPID** (REF P-001/1EU), **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU), **PREP-MB RAPID** (REF P-116-N/4EU, P-116-A/8EU) extraction kits.

12. TROUBLESHOOTING

Table 11. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error PCR inhibition Violation of storage and handlingrequirements	Repeat whole test Dispose current batch
C-	+	Contamination	Dispose current batch Perform decontamination procedures
IC	Invalid	PCR inhibition	Repeat whole test Resample

If you face to any undescribed issues contact our customer service department regarding quality issueswith the kit:

Phone: +7(495) 640.16.93

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

13. QUALITY CONTROL

"DNA-Technology Research&Production", LLC declares that the above mentioned products meet the provision of the Council Directive 98/79/EC for *in vitro* Diagnostic Medical Devices. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of IVDD products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our official representative in EU by quality issues of **Streptococcus agalactiae REAL-TIME PCR Detection Kit**.

Technical support:

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14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
VER	Version	CONTROL +	Positive control
EC REP	Authorized representative in the European Community		Caution

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