DNA-TECHNOLOGY

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RUO

For research use only

GBS LAMP Kit

INSTRUCTION FOR USE

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REF L2-P012-N3/4EU

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1. INTENDED USE

The **GBS LAMP Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **GBS LAMP Kit** is intended for *Streptococcus agalactiae* (group B streptococcus) DNA detection in human biological samples (urogenital scrape, rectal swab) by loop-mediated isothermal amplification.

Populational and demographical aspects: women in labor. There are no contradictions for use of the **GBS LAMP Kit.**

The **GBS LAMP Kit** can be used in research practice.

Potential users: personnel qualified in molecular research methods.

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

2. METHOD

The implemented loop-mediated isothermal amplification method is based on DNA amplification catalyzed by Bst DNA polymerase from thermophilic bacterium *Bacillus stearothermophilus* at a constant temperature, which consists of annealing of primers with complementary sequences, subsequent amplicons synthesis with single chain displacement and elongation of polynucleotide chains with loop primers.

DNA probes, each containing a fluorescent label and a fluorescence quencher, are introduced into the reaction mixture for amplification. When a specific product is formed, the DNA probe is destroyed and the quencher stops affecting the fluorescent label, which leads to an increase in the fluorescence level that is recorded by specialized equipment. The number of destroyed probes (and hence the fluorescence level) increases in proportion to the number of specific amplicons formed.

Amplification mixture includes internal control (IC) which is designed for quality assessment of amplification. DNA probe used for specific DNA amplification product detection includes fluorescent dye Fam. DNA probe used for internal control amplification product detection includes fluorescent dye Hex. Detection channels of amplification products are shown in Table 1.

Since Bst DNA polymerase is not a thermostable enzyme, isothermal amplification reaction does not imply primary DNA denaturation step (heating at temperatures exceeding 63 °C). For primary denaturation, heat the DNA-containing tubes according to the instruction for use. No preliminary heating may be an inhibiting factor and lead to unreliable results.

Table 1. Detection channels of amplification products

Fam	Hex/Vic	Rox	Cy5	Cy5.5
Streptococcus agalactiae DNA	IC	-	-	-

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

The **GBS LAMP Kit** is also approved for use with CFX96 (Bio-Rad) and Applied Biosystems QuantStudio 5 (Life Technologies Pte. Ltd.) real-time thermal cyclers.

The analysis consists of the following stages: DNA extraction (sample preparation), isothermal DNA amplification with real-time results detection using GBS LAMP Kit. Hands-on time (without sample preparation): from 30 minutes.

3. CONTENT

The detailed description of content is represented in Table 2.

Table 2. The **GBS LAMP Kit** content, package N with sample preparation for L2-P012-N3/4EU

Reagent	Description	Total volume	Amount
Lysis solution	Colorless transparent liquid	20 mL (400 µL in each tube)	50 tubes
Neutralizing solution	Colorless transparent liquid	400 µL	1 tube
LAMP-mix	Colorless or pink transparent liquid	1.3 mL	1 tube
Bst DNA polymerase	Colorless transparent viscous liquid	65 µL	1 tube
Positive control	Colorless transparent liquid	80 µL	1 tube
Negative control	Colorless transparent liquid	80 µL	1 tube

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

The kit is designed for 48 tests (no more than 16 runs), including the analysis of test samples, positive controls and negative controls.

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 biomaterial.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- High speed centrifuge for 1.5 mL tubes (RCF(g) at least 12,000);
- Solid-state thermostat with pressure lid maintaining temperature of 90 °C;
- Electric laboratory aspirator with trap flask;
- RNase and DNase free single-use aspirator filter tips, volume 200 µL;
- Refrigerator;
- Vortex mixer;
- RNase and DNase free 1.5 mL microfuge tubes with caps;
- Tube rack for 1.5 mL tubes;
- Single channel pipettes (dispensers covering 2.0-1,000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL; 1,000 µL);
- Pipette rack;
- Powder-free surgical gloves;
- Container for used pipette tips, tubes and other consumables;

- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Vortex mixer;
- Vortex rotor for 0.2 mL strips (if using strips);
- Refrigerator;
- Freezing chamber;
- Tube rack for 1.5 mL tubes;
- Tube rack for 0.2 mL tubes;
- Tube rack for 0.2 mL strips (if using strips);
- Single channel pipettes (dispensers covering 0.5-1,000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 10 µL; 20 µL; 200 µL; 1,000 µL);
- Pipette rack;
- RNase and DNase free 0.5 mL tubes;
- RNase and DNase free 0.2 mL amplification tubes/strips with caps and strip caps;
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves.

Post-Amplification – Amplification detection area

- Real-time thermal cycler

Software:

The most recent version of the DT thermal cyclers software can be downloaded from http://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 **GBS LAMP Kit** except Bst DNA polymerase shall be stored at temperatures from 2 °C to 8 °C throughout the shelf life of the kit. LAMP-mix shall be stored at temperatures from 2 °C to 8 °C and out of light throughout the shelf life of the kit. Bst DNA polymerase shall be stored in a freezer at temperatures from minus 18 °C to minus 22 °C during the storage period.

LAMP-mix must be stored at temperatures from 2 °C to 8 °C and out of light throughout the shelf life of the kit. The excessive temperature and light can be detrimental to product performance.

Transportation of the kit is carried out in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermobox corresponding to the storage conditions of the components included in the kit.

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

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

Kits transported with violation of temperature conditions shall not be used.

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

- components of the kit, except Bst DNA polymerase, shall be stored at temperatures from 2 °C to 8 °C throughout the shelf life of the kit;
- Bst DNA polymerase shall be stored at temperatures from minus 18 °C to minus 22 °C throughout the shelf life of the kit;
- LAMP-mixshall be stored at temperatures from 2 °C to 8 °C and out of light throughout the shelf life of the kit.

The kit stored under undue regime should not be used.

An expired **GBS LAMP 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 **GBS LAMP 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 **GBS LAMP Kit**.

6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular research are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the analysis 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 analysis. 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 LAMP-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 **GBS LAMP Kit** is designed to detect DNA extracted from the urogenital, rectal swabs.

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

7.1. Interfering substances and factors of isothermal amplification inhibition

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

Inhibitors that may be present in a DNA sample include the following endogenous and exogenous interfering substances: whole blood, hemoglobin, mucus, NaCl, chelating agents (e.g. EDTA salts). Inhibition was expressed as a change in accumulation curves in the presence of whole blood and hemoglobin. In the presence of other inhibitors, there was a shift of the curve with an increase in the reaction time.

Table 3. Concentrations of interfering substances that inhibited amplification

Biomaterial	Interfering compound	Concentration per sample/reaction
Endogenous and exogenous substances		
Urogenital scrapes (swabs)	Mucus	>20% (in a sample)
	Whole blood	>10% (in a sample)
	NaCl	>200 mM (in a sample)
	Hemoglobin	>70 µg (in amplification reaction)
	Na_2-EDTA	>0.4 мМ (in amplification reaction)
Rectal scrapes (swabs)	Mucus	>20% (in a sample)
	Whole blood	>10% (in a sample)
	NaCl	>200 mM (in a sample)
	Hemoglobin	>70 µg (in amplification reaction)
	Na_2-EDTA	>0.4 mM (in amplification reaction)

Since Bst DNA polymerase is not a thermostable enzyme, isothermal amplification reaction does not imply primary DNA denaturation step (heating at temperatures exceeding 63 °C). For primary denaturation, heat the DNA-containing tubes according to the instruction for use. No preliminary heating may be an inhibiting factor and lead to unreliable results. Time for running amplification (after sample heating) is no more than 15 minutes.

7.2. General requirements

Loop-mediated isothermal amplification (LAMP) analysis is a direct laboratory diagnostic method, therefore human biomaterial sample intake must be performed from the spot where infectious process is localized. The decision to study the spot is based on medical history and aspects of the disease.

To obtain correct results, the quality of biomaterial sample intake is of high importance, as well as its storage, transport, and pretreatment.

If biomaterial shall be sampled from several biotopes, repeat the procedure using new swab and new tube for each sample.

Incorrect sampling may lead to unreliable results and therefore to the necessity for resampling.

At biomaterial preparation stage use RNase and DNase free filter tips.

7.3. Sample collection

Use dry swab for biomaterial sampling! The contact of solutions with skin, eyes and mucous membranes **shall be omitted**. If the contact occurred, wash the affected area with water immediately and obtain medical attention.

Sample intake shall be performed using special medical devices with registration certificate in accordance with the procedure established depending on the type of biomaterial.

1. Using cytobrushes is not allowed during pregnancy.
2. Before obtaining epithelial scrape from posterior vaginal fornix and cervical canal remove free-flowing vaginal secretions with a sterile swab.

7.4. Method limitations

- for urogenital scrapes — local application of medications;
- for rectal scrapes — application of rectal suppositories, laxatives, medications containing high percentage of iron, colposcopy less than 48 hours prior to the analysis.

Biomaterial sample shall be taken into single-use 1.5 mL plastic tubes with 400 µL of lysis solution.

After taking sample, transfer swab to the tube with lysis solution and wash it thoroughly in the liquid for 10-15 seconds. Avoid splashing of liquid.
Take the swab out of the solution and press it to the inner wall of the tube above the solution level using rotational movements to wring out the excess liquid. Remove the swab from the tube and dispose of it.

Close the cap of the tube tightly and mark the tube.

7.5. Transportation and storage of samples

Samples should be stored:

- at 20 °C to 25 °C – for no longer than 48 hours;
- at 2 °C to 8 °C – for no longer that one week.

Avoid repeated freezing and thawing of samples.

8. PROCEDURE

8.1. DNA extraction from biological material

It is necessary to prepare negative control and carry it through all stages of sample preparation simultaneously with DNA extraction from biomaterial. To do this, use sample that does not contain biomaterial, e.g. lysis solution. In case of singular tests (no more than 3 test samples) it is allowed to use negative control included in the GBS LAMP kit at the amplification preparation stage. In this case, additional sample preparation is not required.

8.1.1. General requirements

8.1.1.1. Use RNase and DNase free single-use filter tips. Change tip after each solution removal from tube. When working with aspirator, use RNase and DNase free tips without filter.

8.1.1.2. When adding solution to a tube containing biomaterial, introduce solution carefully, without touching walls of the tube. If touching the wall, change the tip.

8.1.1.3. To prevent contamination, only open the cap of the tube where solution is going to be introduced or from which supernatant is going to be removed, and close it before proceeding to the next tube. It is not allowed to work with several open tubes simultaneously.

8.1.1.4. Treat test samples and negative control (C-) the same way according to this instruction.

8.1.2. DNA extraction procedure

8.1.2.1. Vortex tubes with test samples and negative control thoroughly for 10-30 seconds.

8.1.2.2. Spin down the drops from tube caps on vortex for 10-30 seconds.

8.1.2.3. Incubate the tubes at 90 °C for 5 minutes.

Tube caps may open during incubation. Please use programmable thermostats with pressure lid (e.g. Gnome thermostat manufactured by DNA-Technology R&P, LLC).

It is recommended to use a program with active final cooling for thermostat, or to take the tubes out carefully from thermostat and let them cool down to room temperature (18 °C – 25 °C).

8.1.2.4. Centrifuge the tubes at RCF(g) 12,000–16,000 for 1 minute

8.1.2.5. Vortex the tube with neutralizing solution for 1–3 seconds. Spin down the drops on vortex for 1–3 seconds.

8.1.2.6. Add 8.0 µL of neutralizing solution (2.0 µL of neutralizing solution per 100 µL of sample) into each tube without touching the walls of the tube.

8.1.2.7. Vortex tubes thoroughly for 10 seconds and spin on vortex for 1–3 seconds.

The obtained DNA preparation can be stored at 2 °C – 8 °C for no longer than one month and at minus 18°C and lower for no longer than 6 months.

8.2. Preparing and performing amplification

When performing the following actions, tubes with LAMP-mix must be protected from direct sunlight!

8.2.1. Mark the necessary amount of single-use 0.2 mL amplification tubes/strips for test samples, negative control (C-) and positive control (C+).

The amount of reagents is calculated for no more than 16 runs provided variable number of test samples, one negative control and one positive control in each run.

Example: To test 4 test samples, mark 6 tubes: 4 tubes for test samples, 1 tube for C- and one tube for C+.

8.2.2. Vortex the tubes with LAMP-mix and Bst DNA polymerase for 3–5 seconds and spin on vortex for 1–3 seconds.

Take Bst DNA polymerase out of the freezer immediately before use.

8.2.3. Prepare a mixture with Bst DNA polymerase. Mix in a separate single-use tube:

- 20 × (N+1) µL of LAMP-mix;
- 1,0 × (N+1) µL of Bst DNA polymerase,

where N is the number of marked tubes considering C- and C+.

Example:

To test 4 test samples (6 marked tubes), prepare a mixture with Bst DNA polymerase for 7 (6+1) tubes, i.e. 140 µL of LAMP-mix + 7.0 µL of Bst DNA polymerase.

8.2.4. Vortex the prepared mixture with Bst DNA polymerase for 3–5 seconds and spin on vortex for 1–3 seconds.

Prepare the mixture with Bst DNA polymerase immediately before use.

8.2.5. Add 20 µL of the mixture into each tube including (C- and C+). Close the tubes/strips.

Note - If preparation for amplification is performed immediately after DNA extraction, the time of reaction mixture preparation (after warming up samples) **should not exceed 15 minutes.**

If amplification is performed after a period of storage of DNA samples, the tubes with DNA preparation, negative control and positive control must be **thermostated at 70 °C for 10 minutes**. After thermostating, spin down the drops on vortex for 1-3 seconds.

To prevent contamination, only open the cap of the tube where the DNA sample is going to be introduced, and close it before proceeding to the next tube. In case of working with strips close strip cap after introducing samples before proceeding to the next strip. Close tubes/strips tightly. Introduce DNA samples and controls with filter tips.

8.2.6. Add 5.0 µL of DNA preparation into the corresponding marked tubes. Do not introduce DNA into the “C-” and “C+” tubes.

8.2.7. Add 5.0 µL of negative control that passed through DNA extraction stage into the “C-” tube.

Note - If the number of test samples does not exceed 3, it is allowed to use negative control from GBS LAMP kit.

8.2.8. Add 5.0 µL of positive control into the “C+” tube.

8.2.9. Spin all the tubes/strips on vortex for 3–5 seconds.

8.2.10. Set all the tubes into the detecting thermal cycler heat block.

8.2.11. For DT series thermal cyclers:

Launch the thermal cycler software. Upload the corresponding test during the first amplification[1]. For further runs create a corresponding assay protocol: specify the number of samples and sample IDs, including negative and positive controls, mark the position of the tubes in the heat block matrix in accordance with their positioning and run amplification. When choosing test, the program shown in Table 4 shall be displayed.

8.2.12. For CFX96 and Applied Biosystems QuantStudio 5 thermal cyclers[2]:

Run amplification considering the volume of reaction mixture which amounts to 25 µL, according to amplification programs shown in Tables 5 and 6 respectively.

Table 4 – Amplification program for DTprime and DTlite detection thermal cyclers

Step	Temperature, °C	min	s	Number of cycles	Optical measurement
1	63	0	30	50	√
√- optical measurement					

Table 5 – Amplification program for CFX96 detection thermal cyclers

Step	Temperature, °C	Time (min:s)	Number of cycles
1	63√	0:30	50
√- optical measurements (Plate Read), set fluorescence measurement on the necessary detection channels (Fam, Hex) at 63°C			

Table 6 – Amplification program for Applied Biosystems QuantStudio 5 detection thermal cyclers

step	Temperature, °C	Time min:s	Number of cycles
PCR	63√	00:30	50
√- data collection for the necessary fluorophores (Fam, Vic (Hex)) is on			

[1] - The test for DT series thermal cyclers is created by entering test parameters or is provided by the kit manufacturer.

[2] - If using CFX96 or Applied Biosystems QuantStudio 5 thermal cyclers, the test is created in the cycling mode.

9. CONTROLS

The **GBS LAMP 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 LAMP-mix from the kit includes the internal control (IC). IC is an artificial plasmid intended to assess the quality of PCR performance.

A negative control should go through all stages of DNA extraction.

The test result is considered valid when:

- Positive result for the specific product is present, in this case the internal control is not considered.
- 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 run are considered false. In this case conduction of special procedures against possible contamination is required.

10. DATA ANALYSIS

Registration of fluorescence signal is performed by thermal cycler automatically during amplification.

Detection and recording of results are performed by thermal cycler automatically.

10.1. Recording of reaction results on DTprime and DTlite thermal cyclers is performed automatically by the software delivered together with the thermal cycler.

10.2. If using Applied Biosystems QuantStudio 5 (Life Technologies Pte. Ltd.) thermal cyclers, amplification data may be obtained by different methods such as base threshold (Ct) or relative threshold (Crt). Amplification results do not depend on the chosen method and may be recorded and interpreted using both Ct and Crt.

10.3. If using CFX 96 (Bio-Rad) thermal cyclers, use regression type analysis (Cq Determination Mode: Regression) and exclude the first 5 cycles from the analysis (Analyze Date from Cycle 5 to 50).

10.4. The results are interpreted according to Table 7. The results of the run are valid if the conditions for the internal controls' result interpretation are fulfilled.

10.5. Unreliable result may be due to the presence of inhibitors in the DNA preparation obtained from biomaterial; wrong performance of analysis protocol; noncompliance with amplification temperature mode etc. In this case a repeated analysis with the same DNA preparation, or repeated DNA extraction and amplification, or resampling (performed sequentially) shall be performed.

Table 7. Interpretation of amplification results

Analysis of results		
Detection channel		**Result interpretation**
Fam, Cp, Cq, Ct or Crt	Hex/Vic, Cp, Cq, Ct or Crt	
Test samples		
Specified	Not considered	***Streptococcus agalactiae* DNA is detected**
Not specified	**Specified**	*Streptococcus agalactiae* DNA is not detected
Not specified	**> 40**	Unreliable result
Negative control		
Not specified	**≤ 40**	**Negative result** Results of the run are valid
Positive control		
Specified	Not considered	**Positive result** Results of the run are valid

10.6. If the results obtained for negative control do not match those specified in Table 7, the results of the whole run are considered invalid. In case the values of indicator cycles (Cp, Cq, Ct or Crt) are present on Fam detection channel, special measures are necessary to identify and eliminate possible contamination. Systematic obtaining of indicator cycles' (Cp, Cq, Ct or Crt) values >40 on Hex detection channel may indicate the decrease in Bst DNA polymerase activity.

10.7. If the results obtained for positive control do not match those specified in Table 7, a repeated amplification run of the whole batch of samples is necessary.

11. SPECIFICATIONS

a. Analytical specificity

For human biological samples containing *S. Agalactiae* DNA, thermal cycler software shall register positive amplification results for specific product (*S. agalactiae* genome fragment) on Fam detection channel during amplification.

For human biological samples not containing *S. Agalactiae* DNA, thermal cycler software shall register negative amplification results for specific product (*S. agalactiae* genome fragment) and positive amplification result for internal control during amplification.

During the analytical specificity study, the absence of nonspecific positive amplification results was shown when examining in high concentration the DNA of closely related microorganisms or microorganisms potentially present in unknown samples: *Acinetobacter spp., Acinetobacter baumannii, Achromobacter ruhlandii, Achromobacter xylosoxidans, Burkholderia spp., Citrobacter freundii, Citrobacter koseri, Chlamydophila pneumonia, Moraxella catarrhalis, Mycoplasma pneumonia, Mycoplasma hominis, Ureaplasma parvum, Ureaplasma urealyticum, Morganella morganii, Legionella pneumophila, Haemophilus spp., Haemophilus influenza, Stenotropomonas maltophilia, Staphylocoddus spp., Serratia marcescens, Streptococcus pyogenes (GAS), Cryptococcus rolland, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis, Klebsiella pneumonia, Enterococcus spp., Enterococcus faecalis, Candida albicans, Gardnerella vaginalis, Atopobium vaginale, Trichomonas vaginalis*, as well as human DNA in concentration of >250 ng per amplification reaction.

The absence of competitive inhibition was shown when samples containing nonspecific DNA at high concentration and S. agalactiae DNA at low concentration were examined.

b. Analytical sensitivity

Detection threshold is 5 copies of *Streptococcus agalactiae* DNA per amplification tube, which equals to 1,000 copies in 1.0 mL of sample. Detection threshold was established by a series of dilution of laboratory controls (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:

Biomaterial	Obtained preparation volume, µL	Analytical sensitivity, copies/mL
- Urogenital scrape - Rectal swab	400	1,000

a. Diagnostic characteristics

Biomaterial	Diagnostic sensitivity	Diagnostic specificity
Urogenital scrape	100% (92.29-100)	100% (92.29-100)
Rectal swab	100% (88.43-100)	100% (88.43-100)
All biomaterial	**100% (95.26-100)**	**100% (95.26-100)**

b. Within-batch and between-batch precision

The assessment of within-batch and between-batch precision of the reagent kit was performed by comparing the results obtained after examining 30 biomaterial samples.

Within-batch precision: 100% (95% CI 88.43%-100%).

Between-batch precision: 100% (95% CI 88.43%-100%).

12. TROUBLESHOOTING

Table 8. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error PCR inhibition Violation of storage and handlingrequirements	Repeat the whole test Dispose of the current batch
C-	+	Contamination	Dispose of the 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

http://dna-technology.com

13. QUALITY CONTROL

“DNA-Technology Research&Production”, LLC declares that the abovementioned 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.

Technical support:

E-mail: hotline@dna-technology.ru,

http://www.dna-technology.com

Manufacturer: "DNA-Technology Research & Production", LLC,

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E-mail: info@dna-technology.com

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14. KEY TO SYMBOLS

	Date of manufacture		Consult instructions for use
	Temperature limit		Catalogue number
	Contains sufficient for <n> tests		Manufacturer
	Use by date		Keep away from sunlight
	Batch code		Positive control
	Version		Caution

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