DNA-TECHNOLOGY

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RUO

For research use only

BacScreen Pneumo 2 REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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REF

R1-P459-S3/9ER
R1-P459-23/9ER
R1-P459-UA/9ER

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1. INTENDED USE

The **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT). The **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit** is designed to detect DNA of bacteria (*Streptococcus pneumoniae, Chlamydophila pneumoniae, Mycoplasma pneumoniae* and *Haemophilus influenzae*) in human biological material (nasopharyngeal, oropharyngeal swabs; bronchoalveolar lavage; endotracheal, nasopharyngeal aspirate; phlegm; pleural fluid) by real-time PCR.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit.**

The **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit** can be used in research practice.

Potential users: qualified personnel trained in molecular research methods.

Apply the kit only as directed in this instruction for use.

2. METHOD

Method: polymerase chain reaction (PCR) with detection of the results in real time; qualitative multiplex analysis.

The implemented PCR method is based on amplification of a target DNA sequence. The amplification process consists of a series of repeated cycles of temperature denaturation of DNA, annealing of primers with complementary sequences, and subsequent completion of the polynucleotide chains with Taq polymerase.

To increase the sensitivity and specificity of the amplification reaction, hot start is used. For package S, hot start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin. The PCR starts only when paraffin is melted. Hot start for package U is provided by blocking the activity of Taq polymerase with antibodies. The enzyme activates only after preheating the PCR mix at 94°C. This prevents the nonspecific annealing of primers on the DNA target at low temperatures during the initial heating of the test tube.

DNA probes, each containing a fluorescent label and a fluorescence quencher, are introduced into the PCR mix. 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. The number of hydrolyzed probes (and hence the fluorescence level) increases in proportion to the number of specific amplicons formed. The fluorescence level is measured at each amplification cycle in real time.

PCR mix includes internal control (IC) intended for quality assessment of polymerase chain reaction in each tube.

The DNA probe used for detection of *Streptococcus pneumoniae* DNA amplification product includes fluorescent tag Fam. The DNA probe used for detection of *Haemophilus influenzae* DNA amplification product includes fluorescent tag Rox. The DNA probe used for detection of *Chlamydophila pneumoniae* DNA amplification product includes fluorescent tag Cy5. The DNA probe used for detection of *Mycoplasma pneumoniae* DNA amplification product includes fluorescent tag Cy5.5. The DNA probe used for detection of the amplification product of IC includes fluorescent dye Hex.

The use of several fluorescent dyes reduces the number of tubes in the test by allowing simultaneous detection of all targets in the same tube.

Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam	Hex	Rox	Cy5	Cy5.5
Streptococcus pneumoniae	IC	*Haemophilus influenzae*	*Chlamydophila pneumoniae*	*Mycoplasma pneumoniae*

The automatic analysis available on DNA-Technology made instruments: DTlite, DTprime or DTprime II real-time thermal cyclers for **BacScreen Pneumo 2 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 **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit** is also approved for use with CFX96 (Bio-Rad) real-time thermal cyclers.

3. CONTENTS

The **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit** content is represented in Tables 2-4.

Table 2. The **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit** content, package S (strips) for R1-P459-S3/9ER

Reagent	Description	Total volume	Amount
Paraffin-sealed PCR mix	Colorless or pink transparent liquid under waxy white fraction	20 µL in each	tubes, 12 strips of 8
Taq polymerase solution	Colorless transparent liquid	500 µL in each	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL in each	2 tubes
Positive control[1]	Colorless transparent liquid	130 µL	1 tube
Strip caps	12 strips of 8		

Table 3. The **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit** content, package S (tubes) for R1-P459-23/9ER

Reagent	Description	Total volume	Amount
Paraffin-sealed PCR mix	Colorless or pink transparent liquid under waxy white fraction	20 µL in each	96 individual tubes
Taq polymerase solution	Colorless transparent liquid	500 µL in each	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL in each	2 tubes
Positive control[1]	Colorless transparent liquid	130 µL	1 tube

Table 4. The **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit** content, package U for R1-P459-UA/9ER

Reagent	Description	Total volume	Amount
PCR mix	Colorless or pink transparent liquid	600 µL	1 tube
TechnoTaq MAX polymerase	Colorless transparent liquid	30 µL	1 tube
PCR buffer	Colorless transparent liquid	600 µL	1 tube
Positive control[1]	Colorless transparent liquid	130 µL	1 tube

All components are ready to use.

The **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit** in package S is designed for 96 tests (no more than 24 runs), which includes analysis of test samples, negative and positive controls.

The **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit** in package U is designed for 96 tests with at least 5 samples per run (3 test samples, negative control and positive control).

[1] - marking as C+ is allowed

4. ADDITIONAL REAGENTS AND EQUIPMENT REQUIRED

Table 5. Equipment, reagents and consumables required

Equipment, reagents and consumables	Package S		Package U, dosing	
	strips	tubes	manual	automated[2]
UV PCR cabinet	●	●	●	●
Real-time detecting thermal cycler[1]	●	●	●	●
Vortex mixer[3]	●	●	●	●
Vortex rotor for 0.2 mL strips	●	-	-	-
Refrigerator or cooling chamber	●	●	●	●
Freezing chamber	-	-	●	●
Tube rack for 1.5 mL tubes	●	●	●	●
Tube rack for 0.2 mL tubes	-	●	●[4]	-
Tube rack for 0.2 mL strips	●	-	-	-
Single channel pipettes (dispensers covering 0.5-10; 2.0-20; 20-200; 200-1,000 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 1.5 mL microfuge tubes with caps	-	-	●	●
RNase and DNase free 0.2 mL PCR tubes or 96-well PCR plate[2]	-	-	●	●[5]
Powder-free surgical gloves	●	●	●	●
Container for used pipette tips, tubes and other consumables	●	●	●	●
DTstream dosing instrument, version 12M1 or 15M1	●[6]	-	-	●
RNase and DNase free filter pipette tips (volume 200 µL) for DTstream, or similar	●[6]	-	-	●
DTpack plate sealing device	-	-	●[7]	●
Centrifuge for PCR plates (RCF(g) at least 100)	-	-	●[7]	●
Polymer thermal film for PCR plate sealing	-	-	●[7]	●
384-well PCR plate	-	-	-	●[8]
Transport medium (if necessary), the following are recommended: – **STOR-F** transport medium for biomaterial samples				
Physiological saline solution 0.9% NaCl (sterile)				
NA extraction reagent kits[9], the following are recommended: – **PREP-NA;** – **PREP-NA PLUS**; – **PREP-RAPID**; – **PREP-OPTIMA**; – **PREP-MB-RAPID II**.				
Notes: [1] – hereinafter – detecting thermal cycler; the required parameters are indicated below [2] – not used for DTlite detecting thermal cycler [3] – DTspin laboratory shaker (DNA-Technology, Russia) is recommended [4] – only if using tubes [5] – only PCR plates [6] – in case of automated dosing [7] – only PCR plates [8] – for 384-well PCR plates, DTprime 5X* and DTprime II 5X* thermal cyclers are validated [9] – the choice of DNA extraction kit is determined by biomaterial type				

Equipment, reagents and consumables	Package S		Package U, dosing	
	strips	tubes	manual	automated[2]
Symbol definition: ●: required -: not required				

The following detecting thermal cyclers are validated for work with the **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit**:

- DTprime in DTprime 5M* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime;
- DTprime II in DTprime II 5M* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime II;
- DTprime in DTprime 5X* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime 5X*;
- DTprime II in DTprime II 5X* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime II 5X*;
- DTlite in DTlite 5S* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTlite;
- CFX96 (Optical Reaction Module CFX96) (manufactured by Bio-Rad Laboratories, USA), hereinafter – CFX96.

For the use of detecting thermal cyclers other than those listed above, please consult the reagent kit manufacturer for consultation.

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. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of manufacture.

5.1. Storage conditions

5.1.1. Package S

- All components of **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit** must be stored at temperatures from 2°C to 8°C over the storage period.
- Paraffin-sealed PCR mix must be stored away from light over the storage period.

5.1.2. Package U

- All components of **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit**, except for TechnoTaq MAX polymerase, must be stored at temperatures from 2°C to 8°C over the storage period.
- PCR mix must be stored away from light over the storage period.
- TechnoTaq MAX polymerase must be stored in a freezer at temperatures ranging from minus 22°C to minus 18°C over the storage period.

WARNING! Protect components from light and excessive temperatures, as they are detrimental to product performance.

5.2. Transport conditions

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

5.2.1. **Package S**

- It is allowed to transport the kit in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermoboxes from 2°C to 25°C for no longer than 5 days.

5.2.2. **Package U**

- It is allowed to transport the kit, except for TechnoTaq MAX polymerase, in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermoboxes from 2°C to 25°C for no longer than 5 days.
- It is allowed to transport TechnoTaq MAX polymerase in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermoboxes up to 25°C for no longer than 5 days.

WARNING! Reagent kits transported with violation of temperature conditions must not be used.

5.3. Shelf-life of the kit following the first opening of the primary container

5.3.1. **Package S**

- All components of the kit must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C over the storage period.
- Paraffin-sealed PCR mix must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C and out of light over the storage period.

5.3.2. **Package U**

- All components of the kit, except for TechnoTaq MAX polymerase, must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C over the storage period.
- PCR mix must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C and out of light over the storage period.
- TechnoTaq MAX polymerase must be stored in a freezer at temperatures from minus 22°C to minus 18°C over the storage period.

WARNING! The kits stored under undue regime must not be used.

An expired **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit** must not be used.

We strongly recommend following the current instructions for use in order to obtain accurate and reliable results.

The manufacturer guarantees the conformity of **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit** to the technical documentation if the storage, transportation and handling requirements are fulfilled.

6. WARNINGS AND PRECAUTIONS

- Molecular biology procedures, such as nucleic acid extraction, PCR-amplification and detection require qualified staff to avoid the risk of erroneous or unreliable results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.
- Wear powder-free single-use surgical gloves. Wear work clothes and personal protective equipment while working with pathogenic microorganisms. The work clothes and personal protective equipment must be suitable for work to be performed and comply with health and safety requirements.

- Avoid any direct contact with the biological samples, reagents and materials used to carry out the test. Avoid producing spills or generating aerosols. Do not eat/drink components of the kit. Do not inhale gas/fumes/vapor/aerosols produced by the components of the kit. Avoid contact with eyes.
- 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 only be used for one purpose. The pipettes must be of positive displacement type or be used with aerosol barrier pipette tips. The tips employed must be sterile, free from DNases and RNases and 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 to be utilized in a single session.
- Handle and dispose of all biological samples, reagents and materials used to carry out the assay as potentially infectious[2, 3]. Any material being exposed to biological samples must be treated with disinfecting solution for at least 30 minutes or autoclaved for 1 hour at 121°C before disposal.
- All of the liquid solutions are designed for single use and cannot be used more than once in amplification reactions.
- Only use the reagents provided in the kit and those recommended by the manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits.
- All laboratory equipment and tools, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, gloves, etc., as well as reagents must 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 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. Never introduce amplification products in the area designed for extraction/preparation of amplification reactions.
- Do not open the tubes after amplification. Avoid producing accidental spills of the amplification products. Dispose of all PCR waste materials (tubes, tips etc.) only in a closed form in a specialized sealed container with disinfectant solution. Waste materials must be removed in accordance with laboratory internal procedures, and with national and international standards.
- Working surfaces, as well as rooms where NA extraction and PCR are performed, must be disinfected with bactericidal irradiators (UVGI) for 30 minutes before and after the assay. All surfaces in the laboratory (test tube racks, equipment, tools, etc.) must be treated with disinfecting solution daily.

Emergency actions

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

Skin Contact: If any component of this kit comes into contact with the skin and causes discomfort, remove any contaminated clothing. Rinse the affected area with plenty of soap and water. If pain or irritation occurs, seek medical attention.

Ingestion: If any component of this kit is ingested, rinse the mouth with plenty of potable water. If irritation or discomfort occurs, seek medical attention.

[2] - All oligonucleotide components are produced by artificial synthesis in compliance with internal quality control protocol. They do not contain blood or products of blood processing.

[3] - Positive control is produced using artificial DNA synthesis technology, it does not contain parts of infectious agents.

Do not use the kit:

- If the transportation and storage conditions have been violated;
- If the appearance of the reagents does not correspond to the product documentation;
- If the packaging of the kit components is breached;
- After the expiry date.

Adverse health effects are **NOT** anticipated from routine use of this kit in compliance with the current instruction for use.

7. SAMPLES

BacScreen Pneumo 2 REAL-TIME PCR Detection Kit is designed to detect DNA extracted from nasopharyngeal, oropharyngeal swabs; bronchoalveolar lavage; endotracheal, nasopharyngeal aspirate; phlegm; pleural fluid.

7.1. General requirements

PCR analysis refers to direct methods of laboratory research; therefore, the collection of biological material must be carried out from the site of infection localization. The decision on studying the localization site shall be taken by the physician according to the collected anamnesis and clinical picture of the disease.

The quality of sampling, sample storage, transport and pretreatment are of great importance for obtaining correct results. Incorrect sampling may lead to unreliable results and, therefore, to the necessity for repeated sampling.

If biomaterial from several biotopes is required, repeat the procedure using new swab each time you collect biomaterial.

Use RNase and DNase free filtered tips during biomaterial preparation and NA extraction.

To prevent contamination, only open the cap of the tube you are working with (adding sample/reagent, removing supernatant) and close it before proceeding to the next tube. It is not allowed to work with several tubes with open caps simultaneously.

7.2. Interfering substances

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

PCR inhibitors are the following endogenous and exogenous interfering substances: hemoglobin and drugs in the DNA sample as a result of incomplete removal during DNA extraction from biomaterial sample, as well as isopropyl alcohol and methyl acetate in the DNA sample as a result of incomplete removal of washing solutions during sample preparation.

In order to assess possible drug interference, those that could potentially be present in residues in human biological samples taken from the required biotopes (chlorhexidine bigluconate, Miramistin®, Nasol Baby, Otrivin Baby, Lasolvan®, Pinosol®, RINOFLUIMUCIL®, Tizin® Classic, Tantum® Verde Spray, Hexoral® Solution, Berodual®, Pulmicort® inhalation suspension) were selected.

Maximum concentrations of common interfering substances that do not inhibit PCR are listed in Table 6.

Table 6. Maximum concentration of interfering substances

Biomaterial	Interfering substance	Concentration of interfering substance
Endogenous substances		
Nasopharyngeal, oropharyngeal swab; bronchoalveolar lavage; endotracheal, nasopharyngeal aspirate; phlegm; pleural liquid	Hemoglobin	0.35 mg/mL in DNA sample
Exogenous substances		
Nasopharyngeal, oropharyngeal swab; bronchoalveolar lavage; endotracheal, nasopharyngeal aspirate; phlegm; pleural liquid	Isopropyl alcohol	100 µL/mL of DNA sample
	Methyl acetate	100 µL/mL of DNA sample
Nasopharyngeal, oropharyngeal swab; phlegm	Chlorhexidine bigluconate	10% of biomaterial sample volume
	Miramistin®	10% of biomaterial sample volume
	Nasol Baby	20% of biomaterial sample volume
	Otrivin Baby	5.0% of biomaterial sample volume
	Lasolvan®	20% of biomaterial sample volume
	Pinosol®	20% of biomaterial sample volume
	RINOFLUIMUCIL®	20% of biomaterial sample volume
	Tizin® Classic	20% of biomaterial sample volume
	Tantum® Verde Spray	5.0% of biomaterial sample volume
	Hexoral® Solution	20% of biomaterial sample volume
	Berodual®	20% of biomaterial sample volume
	Pulmicort® inhalation suspension	20% of biomaterial sample volume

To decrease the number of interfering substances, please observe the rules of biomaterial collection. If a big amount of PCR inhibitors is suspected in the sample, we recommend the NA extraction methods allowing to remove the as fully as possible from the sample. We do not recommend express NA extraction methods.

7.3. Sample collection

WARNING! Before DNA extraction, preparation of biological material samples is needed.

Method limitations[4]: local application of medications (sprays, drops, creams, ointments) less than 24 hours before the test. When using aerosols and other inhalation forms of medications in the treatment of bronchial asthma, the biomaterial for the test should be taken no earlier than three hours after inhalation or meal.

7.3.1 Nasopharyngeal, oropharyngeal swabs

Material is taken using special authorized medical devices according to the procedure established depending on the source of biological material.

WARNING! Material is taken into tubes with “PREP-RAPID” reagent using a dry probe! It is necessary to exclude contact of the solution with skin, eyes and mucous membranes.

[4] - if it does not contradict the requirements of the DNA extraction kits used

Biomaterial is taken in accordance with the NA extraction kit instruction for use.

7.3.2 Bronchoalveolar lavage; endotracheal, nasopharyngeal aspirate; pleural fluid

Bronchoalveolar lavage is collected during bronchoscopy according to the standard procedure algorithm.

The material is taken into disposable, tightly screwed tubes with a volume of up to 50 mL.

At least 500 µL of bronchoalveolar lavage and pleural fluid or at least 1.0 mL of nasopharyngeal or endotracheal aspirate are collected into the tube.

After taking the material, close the tube tightly and mark it.

7.3.3 Phlegm

Material is taken into disposable graduated sterile wide-mouth vials with screw caps with a volume of at least 50 mL in an amount of at least 1.0 mL.

Close and vial tightly and mark it.

7.4. Transport and storage of samples

Transport and storage conditions for nasopharyngeal and oropharyngeal smears are indicated in the instructions for use of recommended DNA extraction reagent kits/sets or transport media used for transport and storage of samples.

It is allowed to transport and store biomaterial samples according to Table 7 (if it does not contradict the requirement to the used DNA extraction kits or transport media).

WARNING! Avoid repeated freezing and thawing of samples.

Table 7. Transport and storage conditions for biomaterial samples before DNA extraction

Biomaterial	Transport and storage temperature	Time before DNA extraction
Bronchoalveolar lavage Nasopharyngeal, endotracheal aspirate Pleural fluid	from 2°C to 8°C	up to 24 hours
	from minus 22°C to minus 18°C	up to 7 days
Nasopharyngeal, oropharyngeal swabs	from 2°C to 8°C	up to 24 hours
	from minus 22°C to minus 18°C	up to 1 month
Phlegm	from 18°C to 25°C	up to 6 hours
	from 2°C to 8°C	up to 3 days

7.5. Sample preparation for DNA extraction

7.5.1. Nasopharyngeal, oropharyngeal swabs

Biomaterial preparation (if necessary) is carried out according to the instructions for the used NA extraction kits.

7.5.2. Phlegm

WARNING!

1. If using **PREP-NA** and **PREP-NA PLUS** extraction kits for NA extraction from phlegm, use mucolysin for phlegm pretreatment according to the instruction for use to NA kits.
2. If using **PREP-MB-RAPID II** extraction kit for NA extraction from phlegm, pretreat the phlegm according to this instruction (7.5.2.1–7.5.2.6).

7.5.2.1. Add mucolysin to the vial with phlegm sample in 5:1 ratio (5 parts mucolysin to one part phlegm) using the vial's graduation marks.

7.5.2.2. Close the vial, shake it and incubate at room temperature (18°C–25°C) for 20–30 minutes, shaking the vial occasionally.

Note. It is allowed to extract NA from 100 µL of phlegm pretreated with mucolysin without centrifugation. In this case, steps 7.5.2.3–7.5.2.6 are not performed, the sample is ready for NA extraction.

7.5.2.3. Transfer 500–1,000 µL of diluted phlegm into a 1.5 mL plastic tube. Close the tube tightly.

7.5.2.4. Centrifuge the tube at RCF(g) 2,000 for 3 minutes.

7.5.2.5. Remove supernatant, leaving approximately 100–200 µL in the tube (precipitate + liquid fraction).

7.5.2.6. Mix the contents of the tube thoroughly (resuspend the precipitate) on vortex and centrifuge on vortex for 3–5 seconds.

The sample is ready for DNA extraction.

7.5.3. Bronchoalveolar lavage, pleural fluid, nasopharyngeal and endotracheal aspirates

WARNING!

1. If using **PREP-NA** and **PREP-NA PLUS** extraction kits for NA extraction, pretreat biomaterial according to the instruction for use to NA kits.
2. If using **PREP-MB-RAPID II** extraction kit for NA extraction, pretreat biomaterial according to this instruction (7.5.3.1–7.5.3.4).

7.5.3.1. Transfer 500–1,000 µL of biomaterial into a 1.5 mL plastic tube.

7.5.3.2. Centrifuge the tube at RCF(g) 2,000 for 3 minutes.

7.5.3.3. Remove supernatant, leaving approximately 100–200 µL in the tube (precipitate + liquid fraction).

7.5.3.4. Mix the contents of the tube thoroughly (resuspend the precipitate) on vortex and centrifuge on vortex for 3–5 seconds.

Sample is ready for DNA extraction.

WARNING! In case mucus is visible in bronchoalveolar lavage or nasopharyngeal and endotracheal aspirate samples, pretreat the sample with mucolysin in 1:1 ratio. In this case, pretreatment is performed in the same way as phlegm pretreatment (see 7.5.2.1–7.5.2.6).

Note. For bronchoalveolar lavage and pleural fluid, NA extraction is allowed from 100 µL of stirred sample without pretreatment.

8. PROCEDURE

8.1 DNA extraction from biological material

For NA extraction, we recommend to use reagent kits/sets that are intended for the corresponding types of biomaterial for the purpose of subsequent DNA testing by PCR, for example, **PREP-RAPID**, **PREP-NA**, **PREP-NA PLUS**, **PREP-OPTIMA**, **PREP-MB-RAPID II** (see Table 8).

Table 8. Reagent kits/sets recommended for NA extraction and further testing using **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit**

Reagent kit	Biomaterial	Minimal eluate volume, µL
PREP-NA	Nasopharyngeal, oropharyngeal swabs; bronchoalveolar lavage; endotracheal, nasopharyngeal aspirate; phlegm; pleural fluid	50
	Nasopharyngeal, oropharyngeal swabs **(shortened method according to Annex A)**	

PREP-NA PLUS	Nasopharyngeal, oropharyngeal swabs; bronchoalveolar lavage; endotracheal, nasopharyngeal aspirate; phlegm; pleural fluid	100
	Nasopharyngeal, oropharyngeal swabs **(shortened method according to Annex A)**	
PREP-RAPID	Nasopharyngeal, oropharyngeal swabs	500
PREP-OPTIMA	Nasopharyngeal, oropharyngeal swabs	500
PREP-MB-RAPID II	Nasopharyngeal, oropharyngeal swabs; bronchoalveolar lavage; endotracheal, nasopharyngeal aspirate; phlegm; pleural fluid	100

Nucleic acids are extracted in accordance with the instructions for use of the reagent kit(s) used or in accordance with Annex A (in case of using **PREP-NA**/**PREP-NA PLUS** reagent kits).

WARNING! Simultaneously with NA extraction from biological material, it is necessary to prepare a negative control and run it through all stages of sample preparation. For this purpose, it is recommended to use physiological solution or negative control included in the NA extraction reagent kit in the volume specified in the instructions for use of the corresponding kit.

8.2 PCR, package S

WARNING!

1. The reagents and tubes must be kept away from direct sunlight.
2. When using package S, strips, strictly observe the completeness of the strips and caps. Do not use strip caps from other kits!
3. For package S, strips, automated dosing using DTstream is available.

8.2.1. Mark one tube/strip tube with paraffin-sealed PCR mix for each test sample, positive control (“C+”) and negative control (“C-”).

WARNING! Reagent quantities are calculated for no more than 24 runs assuming a variable number of test samples, 1 negative control and 1 positive control per run.

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

8.2.2. Shake the tube with Taq polymerase solution on vortex for 3–5 seconds and centrifuge on vortex for 1–3 seconds.

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

8.2.4. Add one drop (~20 µL) of mineral oil into each tube. Cover the tubes/strips loosely with caps.

8.2.5. Shake the tubes with samples and “C+” on vortex for 3–5 seconds and centrifuge on vortex for 1–3 seconds.

WARNING!

1. For DNA and negative control, perform the recommendation for the use of DNA preparation from the instruction to the NA extraction reagent kit.
2. If using **PREP-NA** and **PREP-NA PLUS** DNA extraction kits, shake the tubes with DNA preparation and negative control on vortex for 3–5 seconds and centrifuge on vortex for 1–3 seconds.
3. To prevent contamination, before introducing DNA only open the cap of the tube you are working with and close it before proceeding to the next one. In case of using strips, close the strip cap before proceeding to the next strip. Close the tubes/strips tightly. Use filter tips.

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

8.2.7. Add 5.0 µL of negative control (“C-”) which passed whole DNA extraction procedure into the corresponding marked tube. Avoid paraffin layer break.

8.2.8. Add 5.0 µL of positive control sample (“C+”) into the corresponding marked tube. Avoid paraffin layer break.

8.2.9. Centrifuge the tubes/strips on vortex for 1–3 seconds.

8.2.10. Set the tubes/strips into the real-time thermal cycler.

8.2.11. For DT thermal cyclers:

Launch the operating software for DT instrument[5]. Add corresponding test[6], specify the number and IDs of the samples, positive and negative controls. Specify the position of the tubes/strips in the thermoblock and run PCR. See Table 9.

8.2.12. For CFX96 thermal cyclers:

Run PCR considering the PCR mix volume of 35 µL. See Table 10.

Table 9. The PCR program for DTlite, DTprime and DTprime II thermal cyclers for package S

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		√	
3	94	0	10	45		Cycle
	64	0	15		√	
4	94	0	5	1		Cycle
5	25[1]		…	Holding		Holding
√ – optical measurement [1] – holding at 10°C is allowed						

Note. When combined with reagent kits for detection of nucleic acids of pathogens of human acute respiratory viral infections by RT-PCR (manufactured by DNA-Technology, Russia) it is allowed to use the amplification program indicated in Annex B.

Table 10. The PCR program for CFX96 thermal cyclers for packages S, U

Step	Temperature, °C	Time min: sec	Number of cycles (repeats)
1	80	01:00	1
2	94	01:30	1
3	94	00:15	50
4	64 √	00:20	
√ – optical measurements (Plate Read), set the fluorescence measurement on detection channels (Fam, Hex, Rox, Cy5, Cy5.5) at 64 °C			

[5] - Please, apply to Operation Manual for DTprime, DTprime II 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.

8.3 PCR, package U, manual dosing

WARNING!

1. For amplification use 0.2 mL single-use amplification tubes or 96-well PCR plates[7], sealed hermetically with thermal film. It is not recommended to use strips due to postamplification contamination hazard.
2. The reagents and tubes should be kept away from direct sun light.

8.3.1 Mark the required number of 0.2 mL tubes or a 96-well PCR plate for each test sample, negative control ("C-") and positive control ("C+").

Note. It is recommended to test at least 5 samples per test (3 test samples, negative control and positive control).

Example: to test 4 samples, mark 4 tubes/reserve 4 wells for samples, 1 tube/well for "C-" and 1 tube/well for "C+". The resulting number of tubes/wells is 6.

8.3.2 Shake the tube with PCR mix on vortex for 3–5 seconds, then centrifuge on vortex for 1–3 seconds.

8.3.3 Add 6.0 µL of PCR mix to each tube/well (including "C-" and "C+").

8.3.4 Vortex the tube with PCR buffer and TechnoTaq MAX polymerase for 3–5 seconds, then centrifuge on vortex for 1–3 seconds.

WARNING! Take TechnoTaq MAX polymerase out from the freezer immediately prior to use.

8.3.5 Prepare master mix. Add into the one tube:

6.0 x (N+1) µL of PCR buffer,

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

where N is the quantity of samples to be tested taking to account "C-", "C+".

Example: to test 4 samples, "C-" and "C+" in one PCR run, mark 6 tubes/reserve 6 wells (4 tubes/wells for test samples, 1 tube/well for "C-" and 1 tube/well for "C+"). Prepare master mix for 7 (6+1) tubes/wells. Mix 42 µL of PCR buffer and 2.1 µL of TechnoTaq MAX polymerase.

8.3.6 Vortex the tube with master mix for 3–5 seconds, then centrifuge on vortex for 1–3 seconds.

WARNING! Master mix must be prepared immediately prior to use.

8.3.7 Add 6.0 µL of master mix into each tube/well with PCR mix. Cover the tubes loosely.

WARNING! Follow the steps listed in pp. 8.3.8 – 8.3.14 within two hours after adding master mix to PCR mix.

8.3.8 Shake the tube with positive control "C+" on vortex for 3–5 seconds and centrifuge on vortex for 1–3 seconds.

WARNING!

1. Before introducing DNA preparation and negative control into tubes/wells with PCR mix, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using **PREP-NA**, **PREP-NA PLUS** extraction kits, shake the tubes with DNA preparation and negative control for 3–5 seconds on vortex mixer and centrifuge on vortex for 1–3 seconds.
3. To prevent contamination, only open the caps of the tubes into which the sample is to be added and close them before adding the next sample. Close the tubes tightly. Use filter tips.

[7] - 96-well plates are not used with DTlite detecting thermal cycler

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

8.3.10 Add 6.0 µL of negative control (“C-”) which passed whole DNA extraction procedure into the corresponding tube/well.

8.3.11 Add 6.0 µL of positive control (“C+”) into the corresponding tube/well.

8.3.12 **In case of using 96-well PCR plates:**

8.2.12.1. Place the plate carefully, without shaking into the DTpack sealing device.

8.2.12.2. Seal the PCR plate with polymer thermal film according to the DTpack operation manual.

8.2.12.3. Centrifuge the plate at RCF(g) 100 for 30 seconds.

8.3.13 **In case of using tubes:**

Centrifuge the tubes on vortex for 3–5 seconds.

8.3.14 Set the tubes into the real-time thermal cycler.

8.3.15 Launch the operating software for DT instrument[8]. Add corresponding test[9], specify the number and IDs of the samples, positive and negative controls. Specify position of the samples in thermal unit (see 8.3.14) and run PCR. See Table 11.

8.3.16 For CFX96 thermal cyclers perform PCR considering the volume of reaction mixture of 18 µL. See Table 10.

Table 11. The PCR program for DTlite, DTprime and DTprime II thermal cycler (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	25[1]			Holding		Holding
√ – optical measurements [1] – holding at 10 °C is allowed						

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

8.4 PCR, package U, using DTstream[10]

WARNING!

1. For amplification use 96-well or 384-well[11] PCR plates hermetically sealed with thermal film.
2. The reagents and tubes should be kept away from direct sun light.

Note. It is recommended to test at least 5 samples per run (3 test samples, negative control and positive control).

8.4.1. Shake the tube with PCR mix on vortex for 3–5 seconds, then centrifuge on vortex for 1–3 seconds.

8.4.2. Shake the tubes with PCR buffer and TechnoTaq MAX polymerase on vortex for 3–5 seconds, then centrifuge on vortex for 1–3 seconds.

WARNING! Take TechnoTaq MAX polymerase out of the freezer immediately before use.

8.4.3. Prepare master mix according to the software for DTstream.

8.4.4. Vortex the tube with master mix for 3–5 seconds, then centrifuge on vortex for 1–3 seconds.

8.4.5. Vortex the tubes with positive control for 3–5 seconds and centrifuge on vortex for 1–3 seconds.

WARNING!

1. Before introducing DNA preparation and negative control into tubes with PCR mix, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using **PREP-NA**, **PREP-NA PLUS** extraction kits, shake the tubes with DNA preparation and negative control for 3–5 seconds on vortex mixer and centrifuge on vortex for 1–3 seconds.

8.4.6. Set the tubes with PCR mix, master mix, tubes or deep-well plate with DNA samples, positive and negative controls, as well as PCR plate on the DTstream worktable and conduct dosage of the components according to DTstream user manual.

8.4.7. After the end of dosing program on DTstream put the PCR plate without shaking on the worktable of DTpack sealing device.

8.4.8. Run the process of sealing of PCR plate according to the user manual of DTpack sealing device.

8.4.9. Centrifuge the PCR plate at RCF(g) 100 for 30 seconds.

8.4.10. Set the PCR plate into the real-time thermal cycler.

8.4.11. Launch the operating software for DT instrument[12]. Add corresponding test[13], specify the number and IDs of the samples, positive and negative controls. Specify position of the samples in thermal unit (see 8.4.10) and run PCR. See Table 11.

8.4.12. For CFX96 thermal cyclers perform PCR considering the volume of reaction mixture of 18 µL. See Table 10.

[10] - only for DTprime, DTprime II and CFX96 thermal cyclers
[11] - only for DTprime 5X* and DTprime II 5X* thermal cyclers
[12] - Please, apply to Operation Manual for DTprime, DTprime II and DTlite Real-Time PCR instruments PART II.
[13] - Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

9. CONTROLS

The **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit** contains positive control “C+”.

Positive control is a cloned part of the microorganism’s genome. It is produced with genetic engineering techniques and characterized by automatic sequencing.

The PCR mix from the kit includes the internal control (IC). IC is intended to assess the quality of PCR performance.

To reveal possible contamination a negative control is required.

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

If positive control (“C+”) does **not** express growing fluorescence of the specific product or positive result, it is required to repeat the whole test. It may be caused operation error or violation of storage and handling.

If negative control (“C-”) expresses growing fluorescence of the specific product or positive result, all tests of the current batch are considered false. Decontamination is required.

10. DATA ANALYSIS

10.1. Calculation of amplification results is performed automatically by the software delivered together with the thermal cycler.

10.2. If using CFX96 detection thermal cyclers, use the regression type of analysis (Cq Determination Mode: Regression). In the “Baseline Setting” tab select “Baseline Subtracted Curve Fit”.

10.3. Result interpretation is carried out according to Table 12. The results are valid if the conditions for the interpretation of results obtained for control samples are met.

WARNING! In case of mixed infection, DNA extraction for two and more microorganisms on the corresponding detection channels is possible.

10.4. Invalid result may be due to the presence of inhibitors in the DNA preparation obtained from biological material; incorrect execution of the analysis protocol; noncompliance with the amplification temperature regime, etc. In this case it is necessary to repeat PCR with the available DNA preparation, or to re-extract DNA and perform PCR for this sample, or to re-collect biological material from the patient (performed sequentially).

10.5. If a positive result is obtained for a negative control, the results of the entire run batch are considered invalid. In this case it is necessary to carry out special measures to identify and eliminate possible contamination.

10.6. If a negative result is obtained for a positive control, the results of the entire run batch are considered invalid. In this case it is necessary to repeat amplification of the whole batch of samples.

Table 12. Results interpretation

Detection channel									**Result interpretation**
Fam, Cp/Cq		Hex, Cp/Cq	Rox, Cp/Cq		Cy5, Cp/Cq		Cy5.5, Cp/Cq		
Package S	Package U		Package S	Package U	Package S	Package U	Package S	Package U	
Test sample									
< 36	**< 35**	Not considered	Not considered		Not considered		Not considered		***Streptococcus pneumoniae* DNA is detected**
≥ 36, but **< 40**	**≥ 35,** but **< 40**	Not considered	Not considered		Not considered		Not considered		Low amount of *Streptococcus pneumoniae* DNA, unreliable result*
Not considered		Not considered	**< 36**	**< 35**	Not considered		Not considered		***Haemophilus influenzae* DNA is detected**
Not considered		Not considered	**≥ 36,** but **< 40**	**≥ 35,** but **< 40**	Not considered		Not considered		Low amount of *Haemophilus influenzae* DNA, unreliable result*
Not considered		Not considered	Not considered		**< 36**	**< 35**	Not considered		***Chlamydophila pneumoniae* DNA is detected**
Not considered		Not considered	Not considered		**≥ 36,** but **< 40**	**≥ 35,** but **< 40**	Not considered		Low amount of *Chlamydophila pneumoniae* DNA, unreliable result*
Not considered		Not considered	Not considered		Not considered		**< 36**	**< 35**	***Mycoplasma pneumoniae* DNA is detected**
Not considered		Not considered	Not considered		Not considered		**≥ 36,** but **< 40**	**≥ 35,** but **< 40**	Low amount of *Mycoplasma pneumoniae* DNA, unreliable result*
Not specified or ≥ 40		**Specified**	Not specified or ≥ 40		Not specified or ≥ 40		Not specified or ≥ 40		Target microorganisms' DNA is not detected
Not specified or ≥ 40		Not specified	Not specified or ≥ 40		Not specified or ≥ 40		Not specified or ≥ 40		Invalid result
Negative control									
Not specified or ≥ 39		**Specified**	Not specified or ≥ 39		Not specified or ≥ 39		Not specified or ≥ 39		**Negative result** Run results are valid
Positive control									
Specified		Not considered	**Specified**		**Specified**		**Specified**		**Positive result** Run results are valid

*** -** The obtained result indicates that DNA of the target microorganism is at the limit of detection level, which may be due to low microorganism load in the sample, cross-contamination with high-copy samples or PCR inhibition. The result may be not proved at repeated test. Please conduct repeated biomaterial collection and/or repeated DNA extraction and run PCR. In case the result reappears, please make a conclusion "…DNA is detected".

11. SPECIFICATIONS

a. Analytical specificity

In samples of human biological material containing DNA of *Streptococcus pneumoniae, Chlamydophila pneumoniae, Mycoplasma pneumoniae* or *Haemophilus influenzae*, the software of the detecting thermal cycler must register positive results of amplification of specific products (*Streptococcus pneumoniae, Chlamydophila pneumoniae, Mycoplasma pneumoniae* or *Haemophilus influenzae* genome fragments) in the corresponding tubes on the corresponding detection channels.

In samples of human biological material not containing DNA of *Streptococcus pneumoniae, Chlamydophila pneumoniae, Mycoplasma pneumoniae* or *Haemophilus influenzae*, during amplification the software of the detecting thermal cycler shall register negative results of amplification of specific products (*Streptococcus pneumoniae, Chlamydophila pneumoniae, Mycoplasma pneumoniae* or *Haemophilus influenzae* genome fragments) in the corresponding tubes on the corresponding detection channels and positive results of amplification of internal control on the detection channel Hex.

There is an absence of nonspecific positive amplification results in the presence of RNA of Influenzae virus A(H3N2), Influenzae virus A(H1N1pdm09), Influenzae B virus, Human Coronavirus 229E, Human Coronavirus HKU-1, Human Coronavirus NL-63, Human Coronavirus OC-43, Human Metapneumovirus, Human Parainfluenzae virus type 2, Human Parainfluenzae virus type 3, Human Parainfluenzae virus type 4, Human Parainfluenzae virus type 1, Human Rhinovirus, Respiratory syncytial virus, SARS-CoV-2, DNA of Human Adenovirus, Human Bocavirus, *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus* (methicillin-resistant), *Escherichia coli*, *Bordetella pertussis, Bordetella parapertussis, Bordetella bronchiseptica, Bordetella holmesii* in the sample, as well as human DNA in a concentration up to 6.0×10^6 copies per mL of sample.

b. Analytical sensitivity (limit of detection)

Limit of detection (LOD) is 5 copies of each microorganism's DNA per amplification tube.

LOD had been established by analysis of serial laboratory controls' dilutions.

Limit of detection corresponds to the following DNA concentration values when using the specified NA extraction reagent kits and the final elution (dilution) volume of the extracted DNA:

Biomaterial	Name of NA extraction kit	Volume of obtained preparation, µL	Limit of detection, copies per sample
Nasopharyngeal, oropharyngeal swab in transport medium	**PREP-NA**	50	50
	PREP-NA PLUS	100	100
	PREP-RAPID	500	500
	PREP-OPTIMA[14]	500	500
	PREP-MB-RAPID II	100	100
Bronchoalveolar lavage, phlegm, pleural fluid (extraction from 500 µL of sample) Endotracheal, nasopharyngeal aspirate (extraction from 1.0 mL of sample)	**PREP-NA**	50	50
	PREP-NA PLUS	100	100
	PREP-MB-RAPID II	100	100

[14] - STOR-F (DNA-Technology, Russia) was used as a transport medium

c. Reproducibility and repeatability

Reproducibility is 100 %.

Repeatability is 100 %.

Note. The claimed specifications are guaranteed when DNA extraction is performed with **PREP-NA, PREP-NA PLUS, PREP-RAPID, PREP-OPTIMA or PREP-MB-RAPID II** nucleic acid extraction kits.

d. Diagnostic characteristics

Biomaterial	Pathogen	Diagnostic sensitivity	Diagnostic specificity
Nasopharyngeal swabs	*Streptococcus pneumoniae*	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	Chlamydophila pneumoniae	100% (95% CI: 86.28% – 100%)	
	Mycoplasma pneumoniae	100% (95% CI: 86.28% – 100%)	
	Haemophilus influenzae	100% (95% CI: 86.28% – 100%)	
Oropharyngeal swabs	*Streptococcus pneumoniae*	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	Chlamydophila pneumoniae	100% (95% CI: 86.28% – 100%)	
	Mycoplasma pneumoniae	100% (95% CI: 86.28% – 100%)	
	Haemophilus influenzae	100% (95% CI: 86.28% – 100%)	
Bronchoalveolar lavage	*Streptococcus pneumoniae*	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	Chlamydophila pneumoniae	100% (95% CI: 86.28% – 100%)	
	Mycoplasma pneumoniae	100% (95% CI: 86.28% – 100%)	
	Haemophilus influenzae	100% (95% CI: 86.28% – 100%)	
Endotracheal aspirate	*Streptococcus pneumoniae*	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	Chlamydophila pneumoniae	100% (95% CI: 86.28% – 100%)	
	Mycoplasma pneumoniae	100% (95% CI: 86.28% – 100%)	
	Haemophilus influenzae	100% (95% CI: 86.28% – 100%)	
Nasopharyngeal aspirate	*Streptococcus pneumoniae*	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	Chlamydophila pneumoniae	100% (95% CI: 86.28% – 100%)	
	Mycoplasma pneumoniae	100% (95% CI: 86.28% – 100%)	
	Haemophilus influenzae	100% (95% CI: 86.28% – 100%)	
Phlegm	*Streptococcus pneumoniae*	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)

Biomaterial	Pathogen	Diagnostic sensitivity	Diagnostic specificity
	Chlamydophila pneumoniae	100% (95% CI: 86.28% – 100%)	
	Mycoplasma pneumoniae	100% (95% CI: 86.28% – 100%)	
	Haemophilus influenzae	100% (95% CI: 86.28% – 100%)	
Pleural fluid	*Streptococcus pneumoniae*	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	Chlamydophila pneumoniae	100% (95% CI: 86.28% – 100%)	
	Mycoplasma pneumoniae	100% (95% CI: 86.28% – 100%)	
	Haemophilus influenzae	100% (95% CI: 86.28% – 100%)	

12. TROUBLESHOOTING

Table 13. Troubleshooting

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

If you face any undescribed issues contact our customer service department:

Phone: +7(495)640.16.93

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com/support

13. QUALITY CONTROL

The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

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

Contact our customer service department with quality issues of **BacScreen Pneumo 2 REAL-TIME PCR Detection Kit.**

Technical support:

E-mail: hotline@dna-technology.ru

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14. KEY SYMBOLS

	For research use only		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for<n>tests		Catalogue number
	Use-by date		Manufacturer
	Batch code		Keep away from sunlight
	Caution		Non-sterile

Annex A

Shortened procedure for DNA extraction from test samples (nasopharyngeal, oropharyngeal smears) using PREP-NA, PREP-NA PLUS reagent kit

WARNING!

1. Before starting work:
   - heat thermostat to 65°C;
   - take out the NA extraction reagent kit from the refrigerator and check that there is no precipitate in the lysis solution. In case of precipitation heat the vial with lysis solution at 65°C to dissolve the precipitate completely. Then mix the solution by turning the vial upside down 5–10 times, avoiding foaming. Before use, cool the solution to room temperature (18°C–25°C). The precipitate can also be dissolved at room temperature (18°C–25°C) for approximately 12 hours.
2. Caps may open during heating! Use tubes with self-lock caps (e.g. Eppendorf Safe-Lock Tubes) or programmable thermostats with a clamp cover (e.g. solid-state programmable small-size thermostat TT-1-DNA-Technology).

Procedure:

1. Mark a 1.5 mL plastic tube for negative control ("C-").
2. Add 300 µL of lysis solution into each marked tube with 100 µL of biomaterial and into the "C-" tube. Do not touch the edges of the tube.
3. Add 100 µL of negative control into the "C-" tube.
4. Close the tubes tightly and shake on vortex for 3–5 seconds.
5. Incubate the tubes on thermostat at 65°C for 5 minutes.
6. Centrifuge the tubes on vortex for 3–5 seconds.
7. Add 400 µL of precipitation buffer into each tube without touching the edges of the tube, close the tubes and shake on vortex for 3–5 seconds.
8. Centrifuge the tubes at RCF(g) 12,000–16,000 at room temperature (18 °C–25 °C) for 10 minutes.
9. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.
10. Add 500 µL of wash solution No. 1 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3–5 times.
11. Centrifuge the tubes at RCF(g) 12,000–16,000 at room temperature (18 °C–25 °C) for 1 minute.
12. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.
13. Add 300 µL of wash solution No. 2 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3–5 times.
14. Centrifuge the tubes at RCF(g) 12,000–16,000 at room temperature (18 °C–25 °C) for 1 minute.
15. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate. It is allowed to leave up to 20–30 µL of liquid above the precipitate.
16. Open the tubes and dry the precipitate at 65°C for 5 minutes.
17. Add the amount of dilution buffer corresponding to the PREP-NA/PREP-NA PLUS extraction kit instruction to the precipitate, shake the tubes on vortex for 3–5 seconds and centrifuge on vortex for 3–5 seconds.
18. Heat the tubes on thermostat at 65°C for 5 minutes. Shake the tubes on vortex for 3–5 seconds.
19. Centrifuge the tubes at RCF(g) 12,000–16,000 for 30 seconds at room temperature (18 °C–25 °C) to spin down the condensate.

DNA preparation is ready to be introduced into PCR mix.

DNA preparation can be stored at temperature from minus 22°C to minus 18°C for up to 1 month or at temperature from minus 72°C to minus 68°C for up to 1 year.

Before using DNA preparation for PCR, thaw DNA preparation and negative control at room temperature (18 °C–25 °C) or at temperature from 2°C to 8°C, then shake the tubes with DNA preparation and negative control on vortex for 3–5 seconds and centrifuge on vortex for 1–3 seconds.

WARNING! It is only allowed to thaw DNA preparation once!

DNA preparation is ready to be introduced into PCR mix.

Annex B

Table B.1. Amplification program for DTprime, DTprime II and DTlite detecting thermal cyclers (allowed for joint runs with acute respiratory human viral infections RT-PCR DNA detection kits manufactured by DNA-Technology, Russia).

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	35	15	0	1		Cycle
2	92	0	30	1		Cycle
3	92	0	10	8		Cycle
	64	0	15		√	
4	90	0	5	40		Cycle
	64	0	15		√	
5	64	0	5	1		Cycle
6	25[1]	…	…	Holding		Holding
√ – optical measurements [1] – holding at 10 °C is allowed						