DNA-TECHNOLOGY

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RUO

For research use only

Coxiella burnetii

REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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REF

R1-P013-S3/4ER
R1-P013-23/4ER
R1-P013-UA/9ER

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1. INTENDED USE

The **Coxiella burnetii REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Coxiella burnetii REAL-TIME PCR Detection Kit** is designed to detect DNA of *Coxiella burnetii* in human biological material (nasopharyngeal, oropharyngeal swabs; bronchoalveolar lavage; endotracheal, nasopharyngeal aspirate; phlegm; pleural fluid; cerebrospinal fluid; blood; biopsy material; autopsy material) 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 **Coxiella burnetii REAL-TIME PCR Detection Kit.**

The **Coxiella burnetii REAL-TIME PCR Detection Kit** can be used in research practice.

Potential users: qualified personnel trained in research methods and rules of work in the laboratory.

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

2. METHOD

Method: polymerase chain reaction (PCR) with real time results detection; qualitative 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 from these primers with Taq polymerase.

To increase sensitivity and specificity of the amplification reaction, the use of a “hot” start is provided. For package S, “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. This eliminates the nonspecific anchoring of primers with the DNA target at lower temperatures. “Hot” start for package U is provided by using polymerase which activity is blocked by antibodies. The activation of the enzyme occurs only after preheating the reaction mixture at 94 °C. This eliminates the nonspecific anchoring of primers with the DNA target at lower temperatures.

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 destroyed probes (and hence the fluorescence level) increases in proportion to the number of specific amplification products formed. The fluorescence level is measured at each amplification cycle in real time.

PCR mix includes internal control (IC) designed to control the quality of polymerase chain reaction.

The DNA probe used to detect the *Coxiella burnetii* amplification product includes fluorescent dye Fam. The DNA probe used to detect the IC amplification product includes fluorescent dye Hex.

Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam	Hex/Vic	Rox	Cy5	Cy5.5
Coxiella burnetii	IC	-	-	-

The automatic analysis available on “DNA-Technology” made instruments: DTlite, DTprime or DTprime II real time thermal cyclers for **Coxiella burnetii 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 **Coxiella burnetii REAL-TIME PCR Detection Kit** is also approved for use with CFX96 (Bio-Rad) real-time thermal cyclers and Applied Biosystems QuantStudio 5 (Life Technologies Holdings Pte. Ltd).

3. CONTENT

The **Coxiella burnetii REAL-TIME PCR Detection Kit** contents is represented in Tables 2 – 4.

Table 2. The **Coxiella burnetii REAL-TIME PCR Detection Kit** content, package S, strips, for R1-P013-S3/4ER

Reagent	Description	Total volume	Amount
Paraffin-sealed PCR mix	Colorless or pink transparent liquid under waxy white fraction	20 µL in each	tubes, 6 strips of 8
Taq polymerase solution	Colorless transparent liquid	500 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	1 tube
Positive control[1]	Colorless transparent liquid	130 µL	1 tube
Strip caps	6 strips of 8		

Table 3. The **Coxiella burnetii REAL-TIME PCR Detection Kit** content, package S, tubes, for R1-P013-23/4ER

Reagent	Description	Total volume	Amount
Paraffin-sealed PCR mix	Colorless or pink transparent liquid under waxy white fraction	20 µL in each	48 individual 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[1]	Colorless transparent liquid	130 µL	1 tube

Table 4. The **Coxiella burnetii REAL-TIME PCR Detection Kit** content, package U, for R1-P013-UA/9ER

Reagent	Description	Total volume	Amount
PCR mix	Colorless or pink 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[1]	Colorless transparent liquid	130 µL	1 tube

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

The **Coxiella burnetii REAL-TIME PCR Detection Kit** (package S) is intended for single use and designed for 48 tests (no more than 12 runs), including analysis of test samples, negative controls and positive controls.

The **Coxiella burnetii REAL-TIME PCR Detection Kit** (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. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

The following equipment, reagents and consumables are required:

Equipment, reagents and consumables	Package S		Package U, dosing	
	strips	tubes	manual	automated
UV PCR cabinet	yes	yes	yes	yes
Real-time detecting thermal cycler[1]	yes	yes	yes	yes[2]
Vortex mixer[3]	yes	yes	yes	yes
Vortex rotor for 0.2 mL strips	yes	no	no	no
Refrigerator with freezer	yes	yes	yes	yes
Tube rack for 1.5 mL tubes	yes	yes	yes	yes
Tube rack for 0.2 mL tubes	no	yes	yes[4]	no
Tube rack for 0.2 mL strip tubes	yes	no	no	no
Single channel pipettes (dispensers covering 2.0-20; 20-200; 200-1,000 µL volume range)	yes	yes	yes	yes
RNase and DNase free filtered pipette tips (volume 20 µL; 200 µL; 1,000 µL)	yes	yes	yes	yes
Pipette rack	yes	yes	yes	yes
RNase and DNase free 1.5 mL microfuge tubes with caps	yes	yes	yes	yes
RNase and DNase free 0.2 mL PCR tubes or 96-well PCR plate[5]	no	no	yes	no
Powder-free surgical gloves	yes	yes	yes	yes
Container for used pipette tips, tubes and other consumables	yes	yes	yes	yes
DTstream dosing instrument, version 12M1 or 15M1	no	no	no	yes
RNase and DNase free filter pipette tips (volume 200 µL) for DTstream, or similar	no	no	no	yes
DTpack plate sealing device	no	no	yes[6]	yes
Centrifuge for microplates (RCF(g) at least 100)	no	no	yes[6]	yes
Polymer thermal film for plate sealing	no	no	yes[6]	yes
384-well PCR plate	no	no	no	yes
Transport medium (if necessary), the following are recommended: - **STOR-F** transport medium for biomaterial samples or transport medium recommended in the instruction to DNA extraction kit				
Physiological saline solution 0.9% NaCl (sterile)				
NA extraction reagent kits[7], the following are recommended: – **PREP-NA;** – **PREP-NA PLUS**; – **PREP-RAPID**; – **PREP-MB MAX**; – **PREP-OPTIMA[8]**; – **PREP-MB-RAPID II**.				
Notes: [1] – hereinafter – detecting thermal cycler; the required parameters are indicated below [2] – for DTprime *X*, DTprime II *X* detecting thermal cyclers (DNA-Technology, Russia) [3] – DTspin laboratory shaker (DNA-Technology, Russia) is recommended [4] – only if using tubes [5] – not used for DTlite detecting thermal cycler [6] – only PCR plates [7] – the choice of DNA extraction kit is determined by biomaterial type [8] – only in **PREP-OPTIMA MAX** version for DNA extraction from blood				

The following detecting thermal cyclers are validated for work with the **Coxiella burnetii REAL-TIME PCR Detection Kit**:

- DTprime in DTprime *M* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime;
- DTprime II in DTprime II *M* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime II;
- DTprime in DTprime *X* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime *X* (only for package U, automated dosing);
- DTprime II in DTprime II *X* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime II *X* (only for package U, automated dosing);
- DTlite in DTlite *S* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTlite (only for package S, and package U, manual dosing if using tubes);
- CFX96 (Optical Reaction Module CFX96) (manufactured by Bio-Rad Laboratories, USA), hereinafter – CFX96;
- Applied Biosystems QuantStudio 5 (manufactured by Life Technologies Holdings Pte. Ltd., Singapore), hereinafter - Applied Biosystems QuantStudio 5.

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 production.

5.1. Storage conditions

5.1.1. **Package S**

- All components of the **Coxiella burnetii REAL-TIME PCR Detection 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 out of light over the storage period.

5.1.2. **Package U**

- All components of the **Coxiella burnetii REAL-TIME PCR Detection 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 out of light over the storage period.
- TechnoTaq MAX polymerase must be stored in a freezer at the temperatures from minus 22°C to minus 18°C over the storage period.

WARNING! The excessive temperature and light can be 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 **Coxiella burnetii REAL-TIME PCR Detection Kit** must not be used.

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

Conformity of **Coxiella burnetii REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

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.

[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.

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.

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

The **Coxiella burnetii REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from nasopharyngeal, oropharyngeal swabs; bronchoalveolar lavage; endotracheal, nasopharyngeal aspirate; phlegm; pleural fluid; cerebrospinal fluid; blood; biopsy material; autopsy material.

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 quality of sampling, sample storage, transport and pretreatment are of great importance for obtaining correct results. If biomaterial from several biotopes is required, please repeat the procedure using new probe and new tube for each sample. Incorrect sampling may lead to unreliable results and, therefore, to the necessity for repeated sampling.

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 and close it before proceeding to the next tube.

7.2. 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 include the following endogenous and exogenous interfering substances: hemoglobin and medications found in the biomaterial sample due to incomplete removal during DNA extraction, as well as isopropyl alcohol and methyl acetate left in DNA sample due to incomplete removal of wash solutions during sample preparation.

Maximum interfering substance concentrations not affecting amplification of laboratory control and internal control: hemoglobin – 0.35 mg/mL in DNA sample, isopropyl alcohol – 100 µL/mL in DNA sample, methyl acetate – 100 µL/mL in DNA sample.

In order to assess possible drug interference, medications that could potentially be present in residues in human biological samples taken from the respective biotopes (chlorhexidine bigluconate, Lasolvan® RINO, RINOFLUIMUCIL®, Tizin® Classic, Tantum® Verde Spray, Hexoral® Solution, Berodual®, Salbutamol-Teva, Grippferon® Nasal Drops) were selected.

For all investigated drugs it was shown that there was no effect at concentrations up to 10% in the biomaterial sample.

7.3. Sample collection

WARNING! Sample preparation may be required before DNA extraction.

Method limitations[4]: local application of medicines (sprays, drops, creams and ointments) less than 24 hours before the test. If using aerosols or other inhalation medications for bronchial asthma treatment, collect the material no earlier than 3 hours after inhalation.

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.

After taking the material transfer the probe into the tube with "PREP-RAPID" reagent, lysis solution or transport medium for transport and storage of samples and rinse it thoroughly in the liquid for 10-15 seconds, avoiding splashing.

Remove the probe from the solution and press the probe against the inner wall of the tube above the solution level with a rotating motion to squeeze out excess liquid. Remove the probe completely from the tube and discard.

Close the tube tightly and mark it.

7.3.2 Bronchoalveolar lavage, pleural fluid

Biomaterial is collected according to NA extraction kit instruction for use.

If using PREP-NA and PREP-NA PLUS extraction kits:

Collect the material into empty plastic tightly screwed tubes up to 50 mL in volume.

Collect at least 500 µL of biomaterial. Close the tube tightly.

7.3.3 Endotracheal, nasopharyngeal aspirate

Biomaterial is collected according to NA extraction kit instruction for use.

If using PREP-NA and PREP-NA PLUS extraction kits:

Collect the material into empty plastic tightly screwed tubes up to 50 mL in volume.

Collect at least 1.0 mL of biomaterial. Close the tube tightly.

7.3.4 Phlegm, cerebrospinal fluid

Biomaterial is collected according to NA extraction kit instruction for use.

7.3.5 Peripheral blood

Biomaterial is collected according to NA extraction kit instruction for use.

Method limitations[4]: intravenous heparin injections, parenteral nutrition infusions less than 6 hours before the test.

7.3.6 Biopsy material, autopsy material

Biomaterial is collected according to NA extraction kit instruction for use.

If using PREP-NA extraction kit:

Collect biomaterial into 1.5 mL tubes with transport medium for transport and storage of samples.

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

7.4. Transport and storage of the samples

Transport and storage conditions of biomaterial samples are stated in the instructions for use of the NA extraction reagent kits or the transport media used for transport and storage of samples.

It is allowed to store biomaterial according to the conditions indicated in Table 5 (if it does not contradict the requirements stated in the instructions for use of the NA extraction reagent kits or the transport media used for transport and storage of samples):

Table 5. Biomaterial transport and storage conditions prior to DNA extraction

Biomaterial	Transport and storage temperature	Time before DNA extraction
Biopsy material Autopsy material	from 2°C to 8°C	up to 24 hours
	from minus 22°C to minus 18°C	up to 7 days
	minus 70°C	prolonged period
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
Blood Cerebrospinal fluid	from 18°C to 25°C	up to 2 hours
	from 2°C to 8°C	up to 6 hours
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

WARNING! Only one freezing-thawing of the material is allowed.

7.5. Sample preparation to DNA extraction

7.5.1. Nasopharyngeal, oropharyngeal swabs; phlegm; cerebrospinal fluid; blood

Sample preparation (if necessary) is performed in accordance with the instructions for use for the NA extraction reagent kits.

7.5.2. Bronchoalveolar lavage, pleural fluid

7.5.2.1. Transfer 500 µL of biomaterial into 1.5 mL plastic tube.

7.5.2.2. Centrifuge the tube at RCF(g) 12,000 – 16,000 for 10 minutes.

7.5.2.3. Remove supernatant leaving approximately 50 µL (precipitate + liquid fraction) in the tube.

7.5.2.4. Centrifuge the tube at RCF(g) 12,000 – 16,000 for 10 minutes.

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

7.5.2.6. Close the tubes tightly.

The sample is ready for DNA extraction.

7.5.3. Nasopharyngeal, endotracheal aspirate

7.5.3.1. Transfer 1.0 mL of biomaterial into 1.5 mL plastic tube.

7.5.3.2. Centrifuge the tube at RCF(g) 12,000 – 16,000 for 10 minutes.

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

7.5.3.4. Close the tubes tightly.
The sample is ready for DNA extraction.

7.5.4. Biopsy material, autopsy material

Sample preparation (if necessary) is performed in accordance with the instructions for use for the NA extraction reagent kits.

If using PREP-NA extraction kit:

7.5.4.1 Shake the tubes with biomaterial on vortex for 3-5 seconds and centrifuge on vortex for 3-5 seconds.

7.5.4.2 Remove supernatant.

The sample is ready for DNA extraction.

8. PROCEDURE

8.1 DNA extraction from biological material

We recommend the authorized DNA extraction kits for the corresponding biomaterial types: **PREP-NA**, **PREP-NA PLUS**, **PREP-RAPID**, **PREP-OPTIMA**, **PREP-MB MAX**, **PREP-MB RAPID II** (see Table 6).

Table 6. Kits recommended for DNA extraction (manufactured by DNA-Technology):

Reagent kit	Biomaterial	Minimal eluate volume, µL
PREP-NA	Nasopharyngeal, oropharyngeal swabs; bronchoalveolar lavage; endotracheal, nasopharyngeal aspirate; phlegm; pleural fluid; cerebrospinal fluid; biopsy material; autopsy material	50
	Nasopharyngeal, oropharyngeal swabs **(according to shortened method in Annex A)**	
PREP-NA PLUS	Nasopharyngeal, oropharyngeal swabs; bronchoalveolar lavage; endotracheal, nasopharyngeal aspirate; phlegm; pleural fluid; cerebrospinal fluid	300
	Nasopharyngeal, oropharyngeal swabs **(according to shortened method in Annex A)**	
PREP-RAPID	Nasopharyngeal, oropharyngeal swabs; cerebrospinal fluid	500
PREP-OPTIMA	Whole peripheral blood[5]	100
	Nasopharyngeal, oropharyngeal swabs; cerebrospinal fluid; biopsy material; autopsy material	400
PREP-MB MAX	Whole peripheral blood	50
PREP-MB RAPID II	Nasopharyngeal, oropharyngeal swabs	100

DNA is extracted in accordance with the instruction to the NA extraction reagent kit.

WARNING!

1. Simultaneously with DNA extraction from biological material a negative control must be prepared and carried through all the stages of sample preparation. Physiological saline solution can be used as a negative control sample in volumes as indicated in the instructions for use of extraction kits or negative control sample that is include in the corresponding extraction kit.
2. DNA extraction from biopsy material and autopsy material using **PREP-NA** extraction kit is carried out **in accordance with this instruction for use**.

[5] - only for **PREP-OPTIMA MAX** extraction kit

8.2 DNA extraction from biopsy material and autopsy material using PREP-NA extraction kit

8.2.1 General requirements

8.2.2.1. Use RNase and DNase filter tips. Change the tips after each solution removal from the tube. If using aspirator, use RNase and DNase free tips without filter.

8.2.2.2. When adding solution to the tube with biomaterial, be careful and do not touch the walls of the tube. If touching occurred, change the tip.

8.2.2.3. To prevent contamination only open the cap of the tube you are working with and close it before proceeding to the next one. It is not allowed to work with several open tubes simultaneously.

8.2.2.4. Test samples and negative control ("C-") must be treated in the same way simultaneously according to this instruction for use.

8.2.2 DNA extraction

WARNING!

1. Before starting work it is necessary to:
   - Heat the thermostat to 65°C;
   - Take out of the refrigerator the NA extraction kit and visually control the precipitate in lysis solution. In case there is precipitate, heat the vial with lysis solution on thermostat preheated to 65°C until the precipitate dissolves completely. Then turn the vial upside down 5-10 times, avoiding foaming. Before use, cool down the solution to room temperature (18°C – 25°C). You can also dissolve precipitate at room temperature (18°C – 25°C) for approximately 12 hours.
2. Tube caps may open during heating! Use self-lock tubes (e.g. Eppendorf Safe-Lock Tubes) or programmable thermostats with clamp cover (e.g. solid-state programmable thermostat TT-1-DNA-Tech by DNA-Technology, Russia).

8.2.2.1. Mark one 1.5 mL plastic tube for each test sample and negative control ("C-").

8.2.2.2. Add 300 µL of lysis solution to the tubes with prepared biopsy samples and autopsy samples (see **7.5.4**) and to the "C-" tube. Do not touch the walls of the tube.

8.2.2.3. Add 100 µL of negative control to the "C-" tube. Close the tubes tightly and shake on vortex for 3-5 seconds.

8.2.2.4. Heat the tubes at 65°C on thermostat for 30 minutes and centrifuge on vortex for 3-5 seconds.

8.2.2.5. Transfer supernatant to the corresponding marked tubes for test samples. Do not transfer supernatant into the "C-" tube.

8.2.2.6. Add 400 µL of precipitation reagent into each tube. Do not touch the walls of the tubes. Close the tubes tightly and shake on vortex for 3-5 seconds.

8.2.2.7. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 15 minutes.

8.2.2.8. Remove supernatant without touching precipitate. Use separate tip for each tube.

8.2.2.9. Add 500 µL of wash solution No. 1 to the precipitate. Do not touch the walls of the tubes. Close the tubes tightly and turn upside down 3-5 times.

8.2.2.10. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 5 minutes.

8.2.2.11. Remove supernatant completely without touching precipitate. Use separate tip for each tube.

8.2.2.12. Add 300 µL of wash solution No. 2 to the precipitate. Do not touch the walls of the tubes. Close the tubes tightly and turn upside down 3-5 times.

8.2.2.13. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 5 minutes.

8.2.2.14. Remove supernatant completely without touching precipitate. Use separate tip for each tube.

8.2.2.15. Open the tubes and dry the precipitate at 65°C for 5 minutes.

8.2.2.16. Add 50 µL of dilution buffer to the precipitate. Close the tubes tightly.

8.2.2.17. Shake the tubes on vortex for 3-5 seconds and centrifuge the tubes on vortex for 1-3 seconds to spin down the drops.

8.2.2.18. Heat the tubes at 65°C on thermostat for 10 minutes. Shake the tubes on vortex for 3-5 seconds.

8.2.2.19. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 30 seconds.

DNA preparation is ready for PCR.

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

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

WARNING! Only one thawing is allowed for DNA preparation!

DNA preparation is ready for PCR.

8.3 PCR, package S

WARNING!

1. The reagents and tubes should be kept away from direct sun light.
2. When using package S, strips, strictly observe the completeness of the strips and caps. Do not use the caps for the strips of the other kits!

8.3.1. Mark one tube/stripped tube with the paraffin-sealed PCR mix for each test sample, negative control (C-), positive control (C+).

WARNING! The volume of reagents is calculated for no more than 12 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 samples, one negative control tube “C-” and one positive control tube “C+”. Total number of tubes is 6.

8.3.2. Shake the tubes with Taq polymerase solution on vortex for 3-5 seconds and centrifuge on vortex for 1-3 seconds.

8.3.3. Add 10 µL of Taq polymerase solution to each tube. Avoid paraffin layer break.

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

8.3.5. Shake the tube with positive control on vortex mixer 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.
3. If using **PREP-MB MAX** extraction kit, centrifuge the tubes with DNA preparation and negative control on vortex for 1-3 seconds without shaking, then place the tubes into magnetic rack. If after extraction supernatant containing the extracted DNA was transferred into new tubes, shake the tubes with DNA preparation and negative control on vortex for 3-5 seconds and centrifuge on vortex for 1-3 seconds.

4. 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. If strips are used, close the strip caps after adding the sample before proceeding with the next sample. Close the tubes/strips tightly. Use filter tips.

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

8.3.7. Add 5.0 µL of negative control (“C-”) which passed whole DNA extraction procedure into “C-” tube (see 8.1). Avoid paraffin layer break.

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

8.3.9. Centrifuge the tubes/strips on vortex for 3-5 seconds.

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

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

8.3.12. For CFX96 and Applied Biosystems QuantStudio 5 detecting thermal cyclers: perform PCR considering reaction mixture volume of 35 µL according to amplification programs shown in Tables 8, 9.

Table 7. The PCR program for DTlite, DTprime and DTprime II thermal cyclers (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

[6] - Please, apply to Operation Manual for DTprime, DTprime II and DTlite Real-Time PCR instruments PART II.

[7] - Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

Table 8. The PCR program for CFX96 thermal cyclers (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 the Fam, Hex detection channels at 64 °C			

Table 9. The PCR program for Applied Biosystems QuantStudio 5 (packages S, U)

Stage	Step	Temperature, °C	Time, min:sec	Number of cycles (repeats)
Holding	1	80	01:00	1
	2	94	01:30	1
PCR	1	94	00:20	50
	2	64 **√**	00:20	
√ - optical measurements (Plate Read), set the fluorescence measurement on the Fam, Hex detection channels at 64 °C				

8.4 PCR, package U, manual dosing

WARNING!

1. For amplification use 0.2 mL single-use amplification tubes or 96-well PCR plates[8], 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.

8.4.1. 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.

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

8.4.3. Add 6.0 µL of PCR mix to each tube/well (including “C-” and “C+”).

8.4.4. 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.

8.4.5. 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,

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

[8] - 96-well PCR plates are not used with DTlite detecting thermal cycler

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 the mixture of PCR buffer and TechnoTaq MAX polymerase for 7 (6+1) tubes/wells. Mix 42 µL of PCR-buffer and 2.1 µL of TechnoTaq MAX polymerase.

8.4.6. Shake the tube with the mixture of PCR buffer and TechnoTaq MAX polymerase on vortex for 3-5 seconds, then centrifuge on vortex for 1-3 seconds.

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

8.4.7. Add 6.0 µL of mixture of PCR buffer and TechnoTaq MAX polymerase into each tube/well with PCR mix. Cover the tubes loosely.

WARNING! Follow the steps listed in pp. 8.4.8 – 8.4.14 within two hours after adding mixture of PCR buffer and TechnoTaq MAX polymerase to PCR mix.

8.4.8. Vortex the tube with positive control "C+" for 3-5 seconds, then 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.
3. If using **PREP-MB MAX** extraction kit, centrifuge the tubes with DNA preparation and negative control on vortex for 1-3 seconds without shaking, then place the tubes into magnetic rack. If after extraction supernatant containing the extracted DNA was transferred into new tubes, shake the tubes with DNA preparation and negative control on vortex for 3-5 seconds and centrifuge on vortex for 1-3 seconds.
4. 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.

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

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

8.4.11. Add 6.0 µL of positive control sample (C+) into the corresponding tube/well.

8.4.12. **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.

8.4.13. **In case of using tubes:**

Centrifuge the tubes on vortex for 3-5 seconds.

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

8.4.15. Launch the operating software for DT instrument[9]. Add corresponding test[10], specify the number and IDs of the samples, positive and negative controls. Specify position of the samples in thermal unit (see 8.4.14) and run PCR. See Table 10.

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

8.4.16. For CFX96 and Applied Biosystems QuantStudio 5 thermal cyclers perform PCR considering the volume of reaction mixture of 18 µL. See Tables 8, 9.

Table 10. 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

8.5 PCR, package U, using DTStream[11]

WARNING!

1. For amplification use 384-well 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 in 1 run (3 test samples, negative control and positive control).

8.5.1 Vortex the tube with PCR mix for 3-5 seconds, then centrifuge on vortex for 1-3 seconds.

8.5.2 Vortex the tubes with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then centrifuge on vortex for 1-3 seconds.

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

8.5.3 Prepare mixture of PCR buffer and TechnoTaq MAX polymerase according to the software for DTstream.

8.5.4 Vortex the tube with mixture of PCR buffer and TechnoTaq MAX polymerase for 3-5 seconds, then centrifuge on vortex for 1-3 seconds.

8.5.5 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.
3. If using **PREP-MB MAX** extraction kit, centrifuge the tubes with DNA preparation and negative control on vortex for 1-3 seconds without shaking, then place the tubes into magnetic rack. If after extraction

[11] - only for DTprime X* and DTprime II X* thermal cyclers

supernatant containing the extracted DNA was transferred into new tubes, shake the tubes with DNA preparation and negative control on vortex for 3-5 seconds and centrifuge on vortex for 1-3 seconds.

8.5.6 Set the tubes with PCR mix, the mixture of PCR buffer and TechnoTaq MAX polymerase, tubes or deep-well plate with DNA samples, positive and negative controls and PCR plate on the DTstream worktable and conduct dosage of the components according to DTstream user manual.

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

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

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

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

8.5.11 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 8.5.10) and run PCR. See Table 10.

9. CONTROLS

The **Coxiella burnetii REAL-TIME PCR Detection Kit** contains positive control "C+".

Positive control is a cloned part of the bacterial 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.

[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.

10. DATA ANALYSIS

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

10.2 When using CFX96 detection thermal cyclers, use regression type analysis (Cq Determination Mode: Regression). In the "Baseline Setting" tab select "Baseline Subtraction Curve Fit".

10.3 Result interpretation is carried out according to Table 11. The results are valid if the conditions for the interpretation of results obtained for control samples are met.

Table 11. PCR results interpretation

Detection channel		**Result interpretation**
Fam (target DNA), Cp/Cq/Ct	**Hex/Vic** (internal control), Cp/Cq/Ct	
Test samples		
Specified	Not considered	***Coxiella burnetii* DNA is detected**
Not specified	**Specified**	*Coxiella burnetii* DNA is not detected
Not specified	Not specified	Invalid result
Negative control		
Not specified	**Specified**	**Negative result** Run results are valid
Positive control		
Specified	Not considered	**Positive result** Run results are valid

10.4 Unreliable result may be due to the presence of inhibitors in the NA 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 negative control, the results of the whole batch should be deemed invalid. In this case special measures are required for detection and elimination of possible contamination.

10.6 If a negative result is obtained for positive control, the results of the whole batch should be deemed invalid. In this case it is required to repeat PCR for the whole batch of samples.

11. SPECIFICATIONS

a. Analytical specificity

For biomaterial samples containing DNA of *Coxiella burnetii*, thermal cycler software must register positive amplification result for specific product (*Coxiella burnetii* genome fragment) on Fam detection channel.

For biomaterial samples not containing DNA of *Coxiella burnetii*, thermal cycler software must register negative amplification result for specific product (*Coxiella burnetii* genome fragment) on Fam detection channel and positive amplification result for IC on Hex/Vic detection channel.

There were no nonspecific positive amplification results in the presence of RNA of Influenza virus A(H3N2), Influenza virus A(H1N1pdm09), Influenza B virus, Human Coronavirus 229E, Human Coronavirus HKU-1, Human Coronavirus NL-63, Human Coronavirus OC-43, Human Metapneumovirus, Human Parainfluenza virus type 2, Human Parainfluenza virus type 3, Human Parainfluenza virus type 4, Human Parainfluenza virus type 1, Human Rhinovirus, MERS-CoV, Respiratory syncytial virus, SARS-CoV-2, DNA of Human Adenovirus, Human Bocavirus, *Bordetella parapertussis, Bordetella pertussis, Chlamydophila pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae, Legionella pneumophila, Moraxella catarrhalis, Mycoplasma pneumoniae, Staphylococcus aureus* (methicillin-resistant), *Streptococcus pneumoniae,* as well as human DNA in concentration 1.0 x 10^8 copies/mL of sample.

b. Analytical sensitivity (limit of detection)

Limit of detection is 5 copies of *Coxiella burnetii* DNA per amplification tube.

Limit of detection was established via analysis of laboratory control's serial dilutions.

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

Biomaterial	**DNA extraction kit**	**Preparation volume, µL**	**Limit of detection, copies per sample**
Nasopharyngeal, oropharyngeal swabs in 500 µL of transport medium[14]	**PREP-NA**	50	50
	PREP-NA PLUS	300	300
	PREP-RAPID	500	500
	PREP-OPTIMA	400	400
	PREP-MB-RAPID II	100	100
Bronchoalveolar lavage, phlegm, pleural fluid (extraction from 500 µL of sample) Nasopharyngeal and endotracheal aspirate (extraction from 1.0 mL of sample)	**PREP-NA**	50	50
	PREP-NA PLUS	300	300
Cerebrospinal fluid (extraction from 500 µL of sample)	**PREP-NA**	50	50
	PREP-NA PLUS	300	300
	PREP-RAPID	500	500
Cerebrospinal fluid (extraction from 1.0 mL of sample)	**PREP-OPTIMA**	400	400
Biopsy material	**PREP-NA**	50	50
	PREP-OPTIMA	400	400
Biopsy and autopsy material	**PREP-NA**	50	50
Whole peripheral blood (500 µL[15])	**PREP-OPTIMA MAX**	100	100
Whole peripheral blood (100 µL)	**PREP-MB MAX**	50	50

[14] - **STOR-F** transport medium was used

[15] - when adding 100 µL of lysis solution

c. Diagnostic characteristics

Type of biomaterial	Diagnostic sensitivity	Diagnostic specificity
Nasopharyngeal swab	100 % (95 % CI: 86.28 % – 100 %)	100 % (95 % CI: 86.28 % – 100 %)
Oropharyngeal swab	100 % (95 % CI: 86.28 % – 100 %)	100 % (95 % CI: 86.28 % – 100 %)
Bronchoalveolar lavage	100 % (95 % CI: 86.28 % – 100 %)	100 % (95 % CI: 86.28 % – 100 %)
Endotracheal aspirate	100 % (95 % CI: 86.28 % – 100 %)	100 % (95 % CI: 86.28 % – 100 %)
Nasopharyngeal aspirate	100 % (95 % CI: 86.28 % – 100 %)	100 % (95 % CI: 86.28 % – 100 %)
Phlegm	100 % (95 % CI: 86.28 % – 100 %)	100 % (95 % CI: 86.28 % – 100 %)
Pleural fluid	100 % (95 % CI: 86.28 % – 100 %)	100 % (95 % CI: 86.28 % – 100 %)
Cerebrospinal fluid (liquor)	100 % (95 % CI: 86.28 % – 100 %)	100 % (95 % CI: 86.28 % – 100 %)
Whole peripheral blood	100 % (95 % CI: 86.28 % – 100 %)	100 % (95 % CI: 86.28 % – 100 %)
Biopsy material	100 % (95 % CI: 86.28 % – 100 %)	100 % (95 % CI: 86.28 % – 100 %)
Autopsy material	100 % (95 % CI: 86.28 % – 100 %)	100 % (95 % CI: 86.28 % – 100 %)
Total	100 % (95 % CI: 98.67 % – 100 %)	100 % (95 % CI: 98.67 % – 100 %)

d. 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**, **PREP-OPTIMA MAX**, **PREP-MB-RAPID II** and **PREP-MB MAX** extraction kits.

12. TROUBLESHOOTING

Table 11. Troubleshooting

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

If you encounter any undescribed issues, contact our customer service department:

Phone: +7(495) 640.16.93

E-mail: hotline@dna-technology.ru

https://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 with quality issues of **Coxiella burnetii REAL-TIME PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

Manufacturer:

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14. KEY TO 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 method of DNA extraction from biomaterial (nasopharyngeal, oropharyngeal swabs) using PREP-NA, PREP-NA PLUS reagent kits

WARNING!

1. Before starting work it is necessary to:
   - Heat the thermostat to 65°C;
   - Take out of the refrigerator the NA extraction kit and visually control the precipitate in lysis solution. In case there is precipitate, heat the vial with lysis solution on thermostat preheated to 65°C until the precipitate dissolves completely. Then turn the vial upside down 5-10 times, avoiding foaming. Before use, cool down the solution to room temperature (18°C – 25°C). You can also dissolve precipitate at room temperature (18°C – 25°C) for approximately 12 hours.
2. Tube caps may open during heating! Use self-lock tubes (e.g. Eppendorf Safe-Lock Tubes) or programmable thermostats with clamp cover (e.g. solid-state programmable thermostat TT-1-DNA-Tech by DNA-Technology, Russia).

Procedure

1. Mark a 1.5 mL plastic tube for negative control (C-).
2. Enter 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. Heat 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 walls 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 corresponding amount of dilution buffer to the precipitate according to the extraction kit instruction. Shake the tubes on vortex for 3-5 seconds and centrifuge to spin down the drops 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 for PCR.

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

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

WARNING! Only one thawing is allowed for DNA preparation!

DNA preparation is ready for PCR.