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For Professional Use Only

# CC-EPh

# PCR kit

## Instruction Manual

**AmpliSens®**

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## 1. INTENDED USE

**CC-EPh PCR kit** is an *in vitro* nucleic acid amplification test for qualitative detection of *Chlamydiaceae* microorganisms DNA in the biological material of birds and animals (conjunctival swabs, urethral swabs, avian excrement, parenchymatous organs, sperm, urine) by using electrophoretic detection of the amplified products in agarose gel.

## 2. PRINCIPLE OF PCR DETECTION

*Chlamydiaceae* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen DNA specific region using special *Chlamydiaceae* primers. After PCR the amplified product is detected in agarose gel. **CC-EPh** PCR kit is a qualitative test, which uses the principle of endogenous control – amplification of β-globin gene. DNA-target selected as endogenous internal control is the fragment of animal or avian genome and must be present in a sample in sufficient quantity equivalent to that of cells in the sample. **CC-EPh** PCR kit uses "hot-start", which greatly reduces frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by separation of nucleotides and Taq-polymerase by using wax layer. Wax melting and reaction mix components occur only at 95 ºC.

## 3. CONTENT

**CC-EPh** PCR kit is produced in 2 forms:

CC-EPh PCR kit variant 50 R (tubes 0.2 ml) **REF** B70-50-R0,2-CE;

CC-EPh PCR kit variant 50 R (tubes 0.5 ml) **REF** B70-50-R0,5-CE.

**CC-EPh PCR kit** variant 50 R includes:

| ***Reagent*** | ***Description*** | ***Volume (ml)*** | ***Quantity*** |
|---|---|---|---|
| **PCR-mix-1-R CHLA-COM** ready-to-use single-dose test tubes (*under wax)* | colorless, clear liquid | 0.005 | 55 tubes of 0.5 or 0.2 ml |
| **PCR-mix-2 blue** | blue, clear liquid | 0.6 | 1 tube |
| **Mineral oil for PCR** | colorless, viscous liquid | 2.0 | 1 dropper bottle |
| **Positive Control DNA *Chlamydophila psittaci* (C+ *C. psittaci*)** | colorless, clear liquid | 0.1 | 1 tube |
| **DNA-buffer** | colorless, clear liquid | 0.5 | 1 tube |
| **Negative Control (C-)*** | colorless, clear liquid | 1.2 | 1 tube |

* must be used in the isolation procedure as Negative Control of Extraction.

**CC-EPh** PCR kit variant 50 R is intended for 55 reactions, including controls.

## 4. ADDITIONAL REQUIREMENTS

- DNA isolation kit.
- Agarose gel detection kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- PCR box.
- Tube racks.
- Personal thermocyclers (for example, Terzik (DNA-Technology, Russia), GeneAmp PCR System 2700 (Applied Biosystems, USA), Maxygene (Axygen Scientific, USA) or equivalent).
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).
- Refrigerator with temperature between 2 and 8 °C.
- Deep-freezer with temperature no more than minus 16 °C.
- Waste bin for used tips.

## 5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact immediately flush with water, seek medical attention.

- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one directional, it should begin in the Extraction Area move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.
- Observe the rules and instructions to prevent semination of the environment objects.

Some components of this kit contain Sodium Azide as a preservative. Do not use metal tubing for reagent transfer.

## 6. SAMPLING AND HANDLING

**CC-EPh** PCR kit is intended for analysis of DNA extracted with DNA isolation kits from the biological material of birds and animals:

- mucous scrapes (conjuctival swabs, urethral or cloaca swabs),
- avian excrement,
- parenchymatous organs of dead birds and animals (carrion or coercive killed), parts of foetus membrane, parenchymatous organs with foetus abomasum),
- frozen sperm (ejaculate samples),
- urine.

### 6.1. Material sampling

The liquids – 20 ml.

The parts of tissues and organs – about 1x1x1 (sm)

Materials are to be analyzed the next day if they stored at the temperature 2–8 °C. Urine must be analyzed in the day of the sampling.

Materials are to be stored at the temperature no more than minus 16 °C for one month.

### 6.2. Preparation of the samples

*Water samples* (10 ml) transfer into the tube. Centrifuge them for 10–15 min at 3000 rpm. If the sediment is absent, add 10 ml into the same tube one more time and repeat the centrifugation. Carefully remove and discard the supernatant using a tip with aerosol barrier and leaving about 200 µl of the liquid on the sediment. Resuspend the sediment in solution and use 100 µl for DNA extraction.

*Parenchymatous organs, fetus membrane samples* pestle in sterile porcelain mortars or glass homogenizer, add equal volume of saline and thoroughly homogenize. Transfer 100 µl of the upper part of prepared suspension in a sterile tube for DNA extraction.

*Sperm* (0.5 ml). Add the equal volume of the saline. Centrifuge for 10–15 min at 11000-12000 rpm. The sediment resuspended in 100 µl saline use for DNA extraction.

*Mucous scrapes*. Add 0.5–1.0 (ml) of the saline. Centrifuge for 10–15 min at 11000-12000 rpm. Carefully remove the supernatant. The sediment resuspended in 100 µl saline use for DNA extraction. If mucus is very thick, add 1–2 ml of β-mercaptoethanol (0.1 M solution), mix on vortex. Keep it in 15–20 min at the room temperature mixing periodically. Centrifuge again in the same regime. Resuspend the sediment in saline and use 100 µl for DNA extraction.

*Avian excrement samples* (0.3–0.8 g). Slurry thoroughly in 5 ml of saline (preparing 5-10 % suspension). Centrifuge suspension for 5 min at 1500 rpm. Use 100 µl of supernatant for DNA extraction.

## 7. PROTOCOL

### 7.1. DNA Isolation

It's recommended to use the following nucleic acid extraction kits:

- "DNA-sorb-B", **REF** K1-2-50-CE

Carry out the amplification according to the manufacturer instruction. The volume of clinical sample is 100 µl.

Add 100 µl of Negative Control (C-) into tube used as Negative Control of Extraction.

Add 90 µl of Negative Control (C-) and 10 µl of Positive Control DNA *Chlamydophila psittaci* (C+ $_{C.\ psittaci}$) into tube used as Positive Control of Extraction.

### 7.2. Preparing the PCR

Total reaction volume – **25 µl**, volume of cDNA sample – **10 µl**.

#### 7.2.1 Detection of *Chlamydiaceae* DNA

1. Prepare the required number of PCR tubes with **PCR-mix-1-R CHLA-COM** and wax for amplification of DNA from clinical and control samples.
2. Add **10 µl** of **PCR-mix-2 blue** to the surface of the wax layer of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-R CHLA-COM**.
3. Add above **1 drop** of **mineral oil for PCR** (about **25 µl**).
4. Using tips with aerosol barrier add **10 µl** of **DNA samples** obtained from clinical or control samples.
5. Carry out the control amplification reactions:

NCA -Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+ -Add **10 µl** of **Positive Control DNA *Chlamydophila psittaci* (C+ *C. psittaci*)** to the tube labeled C+.

### 7.2.2 Amplification of *Chlamydiaceae* DNA

Run the following program on the thermocycler (see Table 1, Table 2). When the temperature reaches 95 ºC (pause regimen), insert tubes into the cells of amplifier and press the button to continue.

It is recommended to precipitate drops from walls of tubes by short vortex (1–3 sec) before their insertion in a thermocycler.

Table 1

**Programming thermocyclers for *Chlamydiaceae* DNA amplification**

| | Thermocyclers with active temperature adjustment: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **"Terzik" (DNA-Technology), "Omn-E" (Hybaid)** | | | **"GeneAmp PCR System 2700" (Applied Biosystems), "Palm Cycler" (Corbett Research)** | | | **"Maxygene" (Axygen)** | | |
| Step | Tempera-ture, °C | Time | Cycles | Tempera-ture, °C | Time | Cycles | Tempera-ture, °C | Time | Cycles |
| 0 | **95** | pause | | **95** | pause | | **95** | pause | |
| 1 | **95** | 5 min | 1 | **95** | 5 min | 1 | **95** | 5 min | 1 |
| 2 | **95** | 10 sec | 42 | **95** | 10 sec | 42 | **95** | 30 sec | 42 |
| | **63** | 10 sec | | **63** | 25 sec | | **63** | 45 sec | |
| | **72** | 10 sec | | **72** | 25 sec | | **72** | 45 sec | |
| 3 | **72** | 1 min | 1 | **72** | 1 min | 1 | **72** | 1 min | 1 |
| 4 | **10** | storage | | **4** | storage | | **10** | storage | |

Table 2

**Programming thermocyclers for *Chlamydiaceae* DNA amplification**

| Thermocyclers with block temperature adjustment: **"Ampli-3" (Biocom), "Biometra", "MiniCycler", "PTC-100" (MJ Research)** | | | |
|---|---|---|---|
| Step | Temperature, °C | Time | Cycles |
| 0 | **95** | pause | |
| 1 | **95** | 5 min | 1 |
| 2 | **95** | 10 sec | 42 |
| | **63** | 10 sec | |
| | **72** | 10 sec | |
| 3 | **72** | 1 min | 1 |
| 4 | **10** | storage | |

After the reaction is finished PCR tubes must be collected and sent to the room for PCR products analysis.

Analysis of amplification products is performed by separation of DNA fragments in agarose

gel.

The amplified samples can be stored for 16 h at room temperature, for 1 week at 2–8 °C (be sure to warm the samples to room temperature before running electrophoresis).

## 8. DATA ANALYSIS

It's recommended to use the following detection agarose kit:

- "EPh" variant 200, **REF** K5-200-CE.

Analysis of results is based on the presence or absence of specific bands of amplified DNA in agarose gel (1.7%). The lengths of specific amplified DNA fragments are:

- *Chlamydiaceae* – 300 bp (basic pairs)

Put the protective mask or use the glass barrier while watching and photographing the gel.

Start analysis from results for controls (see Table 3).

Table 3

**Results for controls**

| Control | Controlled step | Specific bands in the agarose gel 300 bp | Interpretation |
|---|---|---|---|
| C- | DNA isolation | No | OK |
| PCE | DNA isolation | Yes | OK |
| NCA | Amplification | No | OK |
| C+ | Amplification | Yes | OK |

- The sample is considered to be positive if the band of 300 bp is present in agarose gel.
- The sample is considered to be negative if the band of 300 bp is absent.
- Besides specific bands the indistinct washed-out bands of primer-dimers may be seen in lanes, they are situated lower than level of 100 bp.

## 9. TROUBLESHOOTING

Analysis results are not obtained as per the following examples:

1. If the results of control samples do not correspond to the listed above (Table 3), then the tests should be repeated.
2. If in lanes corresponding to positive control (PCE, C+) specific band of 300 bp is absent, it may be mistake of preparation of reagents, amplification or program error of thermocycler.
3. If in lanes nonspecific bands appear at different levels, it may be caused by lack of "hot start" or false temperature regime in thermocycler.
4. If in lanes corresponding to negative control (NCA, C–) specific band of 300 bp appears, it means that reagents or samples contamination has taken place. In such

cases analysis results must be considered as irrelevant. Test analysis should be repeated and measures for detecting contamination source must be undertaken.

## 10. STABILITY AND STORAGE

All components of **CC-EPh** PCR kit are to be stored at the temperature 2–8 ºC when not in use. All components of the PCR kit are to be stable until labeled expiration date.

## 11. SPECIFICATIONS

### 11.1. Sensitivity

Analytical Sensitivity of **CC-EPh** PCR kit is no less than $1x10^3$ copies per 1 ml of a sample (cop/ml).

The claimed analytical features of CC-EPh PCR kit are guaranteed only when additional kits of reagents, “DNA-sorb-B” and “EPh” (manufactured by Federal State Institute of Science Central Research Institute of Epidemiology) are used.

### 11.2. Specificity

Specificity of **CC-EPh** PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.

## 12. REFERENCES

1. “Development of test-system for diagnostics of cats’ chlamidiosis by using PCR” by Russian State Centre of Quality and Standardization of Medicaments for Animals and Feed, symposium “Genodiagnostics of infectious diseases”, vol.2, Moscow, 2007.

## 13. QUALITY CONTROL

In compliance with Federal State Institute of Science Central Research Institute of Epidemiology ISO 13485 – certified Total Quality Management System, each lot of **CC-EPh** PCR kit is tested against predetermined specifications to ensure consistent product quality.

## 14. KEY TO SYMBOLS USED

| | | | |
|---|---|---|---|
|  | Manufacturer |  | Temperature limitation |
|  | Expiration Date | LOT | Batch code |
| RUO | Research Use Only | VER | Version |
| REF | Catalogue number | **NCA** | Negative control of Extraction |
|  | Sufficient for | **PCE** | Positive control of Extraction |
|  | Consult instructions for use |  | Caution, consult accompanying documents |
| **C+** | Positive control of Amplification | **C-** | Negative control of Extraction |

## List of Changes Made in the Instruction Manual

| VER | Location of changes | Essence of changes |
|---|---|---|
| 24.05.13 FN | Cover page, text | Federal Budget Institution of Science “Central Research Institute for Epidemiology” was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology” |
| | Content, text | Positive Control DNA *Chlamydophila psittaci* was changed to Positive Control DNA *Chlamydophila psittaci* (C+ $_{C.\ psittaci}$) |
| | Key to Symbols Used | The “Explanation of Symbols” section was renamed to “Key to Symbols Used” |
| | | The explanation of symbols was corrected |
| 11.06.13 FN | Cover page | Symbol IVD was changed to RUO |
| | Key to Symbols Used | |