RUO

For Professional Use Only

MS-EPh

PCR kit

Instruction Manual

AmpliSens®

Federal Budget Institute of
Science “Central Research
Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia

TABLE OF CONTENTS

1. INTENDED USE

MS-EPh PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Mycoplasma synoviae* DNA in the biological material of sick bird by using electrophoretic detection of the amplified products in agarose gel.

2. PRINCIPLE OF PCR DETECTION

Mycoplasma detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen DNA specific region using special *Mycoplasma synoviae* primers. After PCR the amplified product is detected in agarose gel. **MS-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. **MS-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 °C.

3. CONTENT

MS-EPh PCR kit is produced in 2 forms:

MS-EPh PCR kit variant 50 R (0.2-ml tubes) **REF** B73-50-R0,2-CE;

MS-EPh PCR kit variant 50 R (0.5-ml tubes) **REF** B73-50-R0,5-CE.

MS-EPh PCR kit variant 50 R includes:

Reagent	***Description***	***Volume (ml)***	***Quantity***
PCR-mix-1-R MYC-SYN 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 *Mycoplasma synoviae* (C+)	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.

MS-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

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

- Nasal swabs, conjunctive swabs, outflows.
- Joint synovial fluid.
- Parenchymatous organs (lungs, spleen).
- Whole blood.

6.1. Material sampling

Blood with 6 % EDTA (20:1)

The parts of tissues and organs – about 1x1x1 (cm) or less.

Materials are to be analyzed the next day after sampling if they stored at the temperature 2-8 °C.

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

6.2. Preparation of the samples

Whole blood probes with EDTA, medicinal serum and cell cultures are used for DNA extraction without treatment.

Other water probes (1.5 ml). Centrifuge for 5 min at 10000 rpm. Carefully remove and discard the supernatant using a tip with aerosol barrier and leaving about 200 µl of the liquid on the sediment. If the sediment is hidden, transfer material again (1.5 ml) and repeat centrifugation. Suspend the sediment in supernatant and use 100 µl for DNA extraction.

Parenchymatous organs pestle in sterile porcelain mortars or glass homogenizer, add equal volume of saline and homogenize thoroughly. Keep 30 min. Transfer 100 µl of the upper

part of prepared suspension in a sterile tube for DNA extraction.

7. WORKING CONDITIONS

MS-EPh PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. 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 *Mycoplasma synoviae* into tube used as Positive Control of Extraction.

8.2. Preparing the PCR

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

8.2.1. Detection of *Mycoplasma synoviae* DNA

1. Prepare the required number of PCR tubes with **PCR-mix-1-R MYC-SYN** 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 MYC-SYN**.
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 *Mycoplasma synoviae*** to the tube labeled C+.

8.2.2. Amplification of *Mycoplasma synoviae* DNA

Run the following program on the thermocycler (see Table 1). 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 *Mycoplasma synoviae* DNA amplification

	Thermocyclers with active temperature adjustment						Thermocyclers with block temperature adjustment		
	Terzik (DNA-Technology), Omn-E (Hybaid)			**GeneAmp PCR System 2700 (Applied Biosystems), Palm Cycler (Corbett Research)**			**Ampli-3 (Biocom), Biometra, MiniCycler, PTC-100 (MJ Research))**		
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 s	41	**95**	10 s	41	**95**	1 min	41
	61	10 s		**61**	25 s		**61**	1 min	
	72	10 s		**72**	25 s		**72**	1 min	
3	**72**	1 min	1	**72**	1 min	1	**72**	1 min	1
4	**10**	storage		**4**	storage		**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).

9. 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:

- *Mycoplasma synoviae* – 710 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 2).

Table 2

Results for controls

Control	Controlled step	Specific bands in the agarose gel 710 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 710 bp is present in agarose gel.
- The sample is considered to be negative if the band of 710 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.

10. 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 2), then the tests should be repeated.
2. If in lanes corresponding to positive control (PCE, C+) specific band of 710 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 509 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.

11. TRANSPORTATION

MS-EPh PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of **MS-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. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

13. SPECIFICATIONS

13.1. Sensitivity

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

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

13.2. Specificity

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

14. REFERENCES

1. Detection of *Mycoplasma synoviae* in poultry environment samples by culture and polymerase chain reaction. Corinne Marois, Fabienne Oufour-Gesbert and Isabelle Kempf**.** Veterinary Microbiology, Volume 73, Issue 4, 11 May 2000, Pages 311-318**.**

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Total Quality Management System, each lot of **MS-EPh** PCR kit is tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
RUO	Research use only	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**C–**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
	Expiration Date	**IC**	Internal Control
CRIE	Central Research Institute of Epidemiology, Moscow, Russia	**PCE**	Positive control of Extraction

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
17.04.11 LA	Text	The name of the PCR kit was changed from MYC-SYN-EPh to MS-EPh
	Page footer	List Numbers of the PCR kit were changed from VET-5-R0,5-K2-E and VET-5-R0,2-K2-E to B73-50-R0,5-CE and B73-50-R0,2-CE
	Cover page	Phrase “For Professional Use Only” was added
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used””
	Stability and Storage	The information about the shelf life of open reagents was added
	Key to Symbols Used	The explanation of symbols was corrected
12.02.14 ChA	Front page, Key to symbols used	Symbol IVD was changed to RUO
	Front page, text	The name of “Federal State Institution of Science Central Research Institute of Epidemiology” was changed to “Federal Budget Institute of Science “Central Research Institute for Epidemiology”