Reference and informational publication

Based on handbook

«Sampling, transportation and storage of clinical material for PCR-diagnostics»

Developed by Federal State Institute of Science Central Research Institute of Epidemiology of Federal service for surveillance on consumers' rights protection and human well-being, based on methodical recommendations "Organization of work in laboratories which use nucleic acids amplification methods while working with clinical material containing microorganisms I-IV groups of pathogenicity " МУ1.3.2569-09, Moscow, 2009

Moscow 2012

Guidelines of sampling and hadling of material for PCR-diagnostics

1. Sampling of clinical material should be performed according to instruction by sterile disposal instruments, in sterile disposal vials, tubes, containers, using disposal gloves.
2. Sampling of clinical material should be performed in tubes with transport medium, supplied by manufacturer of PCR-kits (in case of necessity of using transport medium). Do not use transport medium of others manufacturers!
3. Immediately after sampling close tubes and vials with clinical material. Do not touch tubes and vials internal surface and internal surfaces of their caps!
4. While transferring clinical material from tubes and vials to new ones use only new sterile disposal tips with aerosol barrier for each sample.
5. While working with clinical material do not make flicks to prevent spattering and spilling to avoid contamination of sample and work surface.
6. Keep the rules of clinical samples storage and transportation. Before transportation of clinical material the cooling agent should be refrigerated to required temperature.
7. For RNA extraction from clinical material use plastic (pipette tips and tubes) free from DNAses and RNAses.

Materials and equipment required for sampling and pretreatment of clinical materials for PCR analysis

Materials:

1. Disposable polypropylene micro tubes with tightly closed or screw caps, 1.5 ml of volume (Axygen (USA)).
2. Disposable polypropylene tubes with screw caps, 50 ml of volume (Axygen (USA)).
3. Plastic container, 60 ml of volume.
4. Vacuum tubes with EDTA-K3, 6,0 ml 13x100 mm (for example, Green Cross (South Korea).
5. Gynecological probe for sampling of biological material (polymeric, villous, for sampling of biological material from urethra and cervical canal) (Sweden).
6. Cervical cytological brush.
7. Disposal pipette tips with aerosol barrier up to 200 and 1000 μl (Axygen (USA)).
8. Microtubes (1,5 ml volume) racks, pipette tips racks.
9. Container with disinfectant.
10. Disposable gloves and laboratory coat.

Equipment:

1. Desktop laboratory centrifuge (for example “Elecon”).
2. Refrigerator for 2–8 °C and deep-freezer for –20 °C and –70 °C.

Clinical sample	HIV DNA	HIV RNA	HCV, HBV, HDV, HGV	HAV, HEV	*Treponema pallidum*	*Neisseria gonorrhoeae* / *Chlamydia trachomatis* / *Trichomonas vaginalis* / *Mycoplasma genitalium* / *Mycoplasma hominis*	*Candida* sp.	*Gardnerella vaginalis*	*Lactobacillus* spp.	HPV	CMV	HSV 1 и 2	HHV 6	EBV	VZV	*Toxoplasma gondii*
Cervical canal scraping						.				.	.	.				
Urethral scraping						.					.	.				
Vaginal discharge						.	.	.	.							
Seminal fluid, prostate secretion						.										
Erosive-ulcer lesions scraping					.							.			.	
Urine											.	.				
Amniotic fluid											.	.	.	.	.	.
Biopsy material of lungs											.	.	.			.
Lymph gland biopsy material					.								.			.
Hepatic biopsy material			.	.												.
GIT biopsy material											.					
Bronchoalveolar lavage (BAL)							.				.		.			.
Whole blood	.										.	.	.	.	.	
Peripheral blood (cells)	.										.	.	.	.	.	
Peripheral blood (plasma)		.	.	.							.			.	.	
Umbilical cord blood											.	.	.	.	.	.
Nasopharyngeal swab																
Oropharyngeal swab							.					.	.	.		
Sputum							.				.					
Conjunctiva secretion						.					.					
Placenta											.		.	.	.	.
Pleural fluid													.			.
Synovial fluid						.										
Saliva											.	.	.	.	.	
Cerebrospinal fluid					.						.	.	.	.	.	.
Feces				.												
Autopsy material											.	.	.	.	.	.
Ticks																
Mosquitoes																
Lice																
Fleas																
Concentrated washes sample				.												

Clinical sample	*Rubella virus*	*Parvovirus B19*	*Chlamydophila pneumoniae* *Mycoplasma pneumoniae*	*Pneumocystis carinii*	*Mycobacterium tuberculosis complex*	*Streptococcus spp.*	*Heamophilus spp.*	*Legionella sp.*	*Neisseria meningitidis A,B,C*	*Enterovirus*	*Streptococcus pyogenes* *Corinebacterium diphteriae*	*Adenovirus*	*Adenovirus F group*
Cervical canal scraping													
Urethral scraping													
Vaginal discharge						•							
Seminal fluid, prostate secretion													
Erosive-ulcer lesions scraping													
Urine					•								
Amniotic fluid	•	•								•		•	
Biopsy material of lungs			•	•	•			•					
Lymph gland biopsy material					•								
Hepatic biopsy material													
GIT biopsy material													
Bronchoalveolar lavage (BAL)			•	•	•			•					
Whole blood		•								•			
Peripheral blood (cells)		•								•			
Peripheral blood (plasma)	•	•								•			
Umbilical cord blood	•	•								•			
Nasopharyngeal swab						•	•					•	
Oropharyngeal swab	•	•	•			•	•	•			•	•	
Sputum			•	•	•			•					
Conjunctiva secretion												•	
Placenta	•	•											
Pleural fluid			•	•	•			•					
Synovial fluid						•	•						
Saliva	•	•								•		•	
Cerebrospinal fluid	•	•			•	•	•		•	•			
Feces													•
Autopsy material	•	•						•					
Ticks													
Mosquitoes													
Lice													
Fleas													
Concentrated washes sample								•		•			•

						•		•						•																	SARS
													•	•	•																RSV Influenza virus A/B Parainfluenza virus
					•	•																		•							Influenza virus A H5N1
•						•																									Astrovirus Norovirus 1,2 Rotavirus
•						•															•										Helicobacter pylori Yersinia pseudotuberculosis
•						•																									Clostridium defficile Yersinia enterocolitica Salmonella spp., Shigella spp.,
					•				•										•				•								Brucella spp.
					•								•						•							•					Bacillus anthracis
•					•		•											•					•			•					Leptospira spp.
																	•					•	•	•						Hantavirus
				•	•		•											•												Tick-borne encephalitis virus
			•	•			•											•												West Nile virus
	•	•		•														•												Rickettsiae spp.
				•		•												•				•	•	•						Flavivirus
				•	•												•													CCHF virus Borrelia burgdorferi s.l.
			•															•												Bortanella spp.
							•		•		•					•	•	•							•					Listeria monocytogenes

MATERIAL SAMPLING AND STORAGE

Blood (plasma), blood serum

Blood should be collected to a tube with 6% EDTA solution:

Cat. №:

GV0312 - GV0439

Blood (plasma) samples are used for quantitative and qualitative analysis, blood serum samples are used only for qualitative analysis by means of Nucleic acids amplification method.

Material sampling

To obtain plasma blood sampling should be preformed after overnight fasting or after 3 hours after food ingestion from cubital vein using disposable needle (d 0,8-1,1 mm) to vacuum tube (lilac lid – 6% EDTA) or using disposable syringe to plastic tube with sodium citrate (3,8% sodium citrate solution at a ratio 1:9). The closed tube should be inverted carefully several times to ensure proper mixing with anticoagulant (otherwise the blood coagulates and DNA/RNA extraction will be impossible).

Heparin should not be used as anticoagulant!

To obtain blood serum blood sampling should be performed after overnight fasting from cubital vein using disposable needle (d 0,8-1,1 mm) to disposable tubes without anticoagulant.

The additional sample pretreatment is required.

The conditions of material and pretreatment samples storage and transportation

Whole blood samples:

- at temperature 20–25 °C — for 2 hours;
- at temperature 2–8 °C — for 6 hours from the time of material sampling for quantitative nucleic acids detection; for qualitative nucleic acids detection - for 12 hours;

Do not freeze the whole blood samples!

Plasma and serum blood samples:

- at temperature 2–8 °C — for 5 days;
- at temperature -20 °C — for one year;
- at temperature -70 °C — for a long time.

Only one freeze-thaw cycle of clinical material is allowed, that's why the plasma or blood serum samples for long time storage should be divided to small portion (0,1-0,2 ml) and be placed in separate sterile tubes 1,5 or 2 ml of volume.

CLINICAL MATERIAL FROM THE WOMEN UROGENITAL TRACT

For detection of urogenital system infection, including STI,
the different clinical material is used - mucosal tunic scraping discharge or swab, urine, prostate secretion, seminal fluid. The selection of clinical material is depended on diagnostics task and gender.

The condition of scrapings, swabs and mucosal tunic discharge sampling

The special disposal probes, sponge probes, cytological brushes are used for scraping discharge and swabs sampling. **The using of instruments recommended by the test systems manufacturer is required!** Scraping and swabs should be placed into tubes with a transport medium recommended by the test systems manufacturer.

The conditions of material storage and transportation

Are determined by manual for transport medium and the nucleic acids extraction kit.

In case of using

Transport medium with mucolytic cat.№ 952-CE

- at room temperature 18–25 °C — for 28 days;
- at temperature 2–8 °C — for 3 months;
- at temperature <- 20 °C— for a long time.

In case of using

Transport medium for swabs cat. № 956 и 987

- at room temperature 18–25 °C — for 48 hours;
- at temperature 2–8 °C — for 7 days;
- at temperature <- 20 °C— for a long time.

In case of using

Transport medium TM EDEM from the EDEM nucleic acid extraction kit (cat. № K2-17-100-CE)

- at room temperature 18–25 °C — for 48 hours;
- at temperature 2–8 °C — for 14 days;
- at temperature <- 20 °C— for a long time.

The type of clinical material is determined by diagnostics task

Clinical material	Diagnostic task	Detected microorganisms
Cervical canal scraping	Cervical screening with using HPV test	HPV high carcinogenic risk
	Etiological diagnostics of cervicitis. Monitoring of antibiotic therapy of cervicitis.	STI: *Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Mycoplasma genitalium, Treponema pallidum, HSV II*, and also opportunistic microorganisms: *Ureaplasma spp, Streptococcus spp, Staphylococcus spp* and others.
Erosive-ulcer lesions scraping	Differential diagnostics of infections which cause erosive-ulcer lesions	*Treponema pallidum, HSV I/II*
Epithelium scraping from condylomatous formations	Differential diagnostics of infections, which cause condylomatous formations	HPV low carcinogenic risk
Vaginal discharge or swab	Screening for HPV high carcinogenic risk (for women older than 25-30 years)	HPV high carcinogenic risk
	Screening for STI	STI: *Chlamydia trachomatis, Neisseria*

	Diagnostics of bacterial vaginosis, candidiasis, vaginitis	*gonorrhoeae, Trichomonas vaginalis, Mycoplasma genitalium* Opportunistic microorganisms concerning with bacterial vaginosis (*Lactobacillus spp., Gardnerella vaginalis, Atopobium vaginae* and others.), vaginal candidiasis (*Candida albicans/glabrata/krusei*) or nonspecific vaginitis (*Streptococcus spp., Staphilococcus spp., E.coli* and others)
Urine	Differential diagnostics of urethritis, cystitis	STI: *Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Mycoplasma genitalium* Opportunistic microorganisms: *E.coli, Streptococcus spp, Staphylococcus spp, Klebsiella spp, Proteus, Pseudomonas, Ureaplasma spp,* and others

SCRAPING DISCHARGE OF CERVICAL CANAL

Cytological brush **Cat. №: ЦЩ**	
Material should be collected to a tube:   **Information for order:** **Transport medium with mucolytic agent (TMM): cat. № 952-СЕ** **Transport medium for swabs: cat. № 987** **Transport medium TM -EDEM** from **the EDEM nucleic acid**	**Material sampling** The disposable or sterile reusable gynecological speculum is used for cervical canal approach. The material sampling should be performed to a tube with special transport medium with mucolytic agent «TMM» using cervical cytological brush. As the virus is the intracellular agent for HPV analysis it is required the sufficient amount of epithelial cells. The minor presence of cervical mucus or blood additive is allowed. In some case the material sampling can be performed by using universal gynecological probe, in this case the volume of scraping discharge will be less and the cells amount can be insufficient. Mucus and vaginal discharge should be removed from uterine cervix surface by using sterile gauze sponge. Insert the working part of cytological brush into cervical canal and rotate clockwise 2-3 turns. Take out cytological brush and place its working part, containing taken material, into the tube with transport medium. Working part of the probe is to be broken off (no more than 1 cm of plastic part of cytological brush) and left in the tube with transport media. In some case for material sampling from cervical canal universal probe can be used (pregnant women, nullipara, when the screening diagnostic of HPV is not required). The material should be collected as described above. The universal probe is to be broken off in place of special cut. For this purpose place working part of probe into the tube with transport medium. When the probe touches tube bottom fold fine part of probe with additional effort, placing into the tube its wide part until the cut. Brake off the probe and left it in the tube. **It should be noted that not enough cells quantity from mucosal surface can be collected due to small surface area of universal probe.** In case of difficulties with probe or cytological brush broking the clinical material **should be washed thoroughly** from their working part into the tube with transport medium. Work part should be pressed to tube inside and be rotated 5-10 times clockwise and contra clockwise. **The using of reusable scissors for cutting of cytological brush or universal probe working part is not allowed. It may cause cross contamination of clinical material and false positive results.** Thoroughly vortex the content of the tube for mucus solution before nucleic acid extraction procedure. Centrifuge the tube with clinical material at 1500-3000 rpm for 5s, and then carefully mix by pipette. ***The pretreatment of samples is not required.*** Only one freeze-thaw cycle is allowed.

extraction kit **cat. № K2-17-100-CE**	

SCRAPING DISCHARGE OR VAGINAL SWAB

Probe **Cat. №: ЗГУ_ЦМ** **Sponge-probe** **Cat. №: 300202**	 
Material should be collected to a tube: 	

Information for order: **Transport medium with mucolytic agent (TMM):** **cat. № 952-CE** **Transport medium for swabs:** **cat. № 987** **Transport medium TM EDEM** from **the EDEM nucleic acid extraction kit** **cat. № K2-17-100-CE**	**Material sampling** The discharge should be sampling from surface of vaginal sides. Using of gynecological speculum can restrict its approach. The material sampling should be performed in sufficient amount to a tube with transport medium using sponge-probe or universal probe. The minor presence of cervical mucus or blood additive is allowed. Collect discharge thoroughly from vaginal side walls surface using rotary motion of working part of sponge-probe. Transfer sponge-probe into the tube with transport medium. The working part of sponge-probe which contains sampling material is to be broken off and left in the tube with transport media. The using of scissors for cutting of sponge-probe working part is not allowed! In case of transport medium with mucolytic agent using the medium color can be changed due to changing of pH (at acid pH of vaginal discharge –see the picture). Close the tube tightly excluding gap and deformation of inside part of lid. Mark the tube. Before nucleic acid extraction procedure centrifuge the tube with clinical material at 1500-3000 rpm for 5 sec and carefully mix the content of tube on vortex excluding sparging and material transferring on inside part of lid. ***The pretreatment of samples is not required.***

URINE

Material should be collected to container: **Cat. №: ИЛС-КП-60-С** 	**Material sampling** The first pass urine is used for analysis. Collect 15-25 ml of urine into special dry sterile container 50-60 ml of volume. Urine sampling is performed after thorough genitals washing for excluding presence of genital secretions in urine. If possible put tampon into vagina before material collecting for excluding urine contamination of vagina discharge. You shouldn't collect material during a period. ***The pretreatment of samples is required.*** **The conditions of storage and transportation of material and pretreatment samples** Native and pretreatment samples of urine: at temperature 2–8 °C — for 1 day; - at temperature -20 °C — for one week; - at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed. Material should be collected into container: Cat. №: ИЛС-КП-60-С

CLINICAL MATERIAL FROM THE MEN UROGENITAL TRACT

The type of clinical material is determined by diagnostic task

Clinical material	Diagnostic task	Detected microorganisms
Urethral scraping	Screening for STI, etiological diagnostics of urethritis, balanoposthitis Monitoring of antibiotic therapy of urethritis, balanoposthitis	STI: *Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Mycoplasma genitalium,* Opportunistic microorganisms: *Ureaplasma spp, Streptococcus spp, Staphylococcus spp* and others
Scraping discharge of prepuce of penis		
Urine	Screening for STI, etiological diagnostics of urethritis	
Erosive-ulcer lesions scraping	Differential diagnostics of infections which causes	*Treponema pallidum, HSV II*

	erosive-ulcer lesions	
Scraping of epithelium from neoplasms of balanus, perianal region	Differential diagnostics of infections, which causes condylomatous formations	HPV low carcinogenic risk
Seminal fluid, prostate secretion	Etiological diagnostics of bacterial prostatitis, diagnostics of male infertility	Opportunistic microorganisms: *E.coli, Serratia, Klebsiella, Enterobacter spp, Acinetobacter spp., Pseudomonas aeruginosa, Ureaplasma spp, Streptococcus spp, Staphylococcus spp* and others STI: *Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Mycoplasma genitalium.*

SCRAPING DISCHARGE OF MEN URETHRA

Probe **Cat. №: ЗГУ_ЦМ**	
Material should be collected to a tube:  **Information for order:** **Transport medium with mucolytic agent (TMM): cat. № 952-CE** **Transport medium for swabs: cat. № 987** **Transport medium TM EDEM** from **the EDEM nucleic acid extraction kit cat. № К2-17-100-CE**	**Material sampling** Before sampling of urethral scraping treat balanus in region of external urethral orifice with sterile physiological solution. Perform urethra massage. In case of presence of free-draining urethral discharge delete them with dry sponge. Enter probe into urethra for 1-2 cm. Perform scraping of epithelial cells with several rotate movements and transfer probe into a tube with transport medium. The probe is to be broken off and left in the tube. **In case of cut absent** immerse working part of probe into medium. Press it to tube inside and rotate during 5-10 sec. Remove probe, close the tube tightly. It should be kept in mind that in this case the considerable amount of clinical material can be lost and material will be inadequate for analysis. **The using of scissors for cutting of probe working part is not allowed.** Close the tube tightly excluding gap and deformation of inside part of lid. Mark the tube. Before nucleic acid extraction procedure centrifuge the tube with clinical material at 1500-3000 rpm for 5 seconds and carefully mix the content of tube on vortex excluding sparging and material transferring on inside part of lid. Collect discharge in sufficient amount. The minor presence of mucus, blood and purulence additives is allowed. ***The pretreatment of samples is not required.***

URINE

Material should be collected to container: **Cat. №: ИЛС_КП_60_С** 	**Material sampling** Before urination open external urethral orifice totally abducting skin fold. The first void urine is used for analysis. Collect 15-25 ml of urine into special dry sterile container 50-60 ml of volume. ***The pretreatment of samples is required.*** **The conditions of storage and transportation of material and pretreatment samples** Native and pretreatment samples of urine: at temperature 2–8 °C — for 1 day; - at temperature -20 °C — for one week; - at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed.

PROSTATE SECRETION

Material should be collected to a tube: **Cat. №: МСТ-200-С**  **To a container:** **Cat. №: ИЛС_КП_60_С** 	**Material sampling** Before obtaining of prostate secretion treat balanus with sterile cotton tampon. Prostate secret should be collected after prostate massage per rectum. The doctor performs prostate massage by energetic pressing from foundation to top. After prostate massage collect 0.5-1 ml prostate secretion into sterile dry disposable plastic tube 2ml volume or sterile dry container 50-60 ml volume. Close the tube tightly excluding gap and deformation of inside part of lid, mark it. ***The pretreatment of samples is not required.*** If prostate secretion obtaining is impossible collect 15-25 ml of first pass urine (which contains prostate secretion) just after prostate massage (see rules of urine sampling). **The conditions of material storage:** at room temperature — for 6 hours; at temperature 2–8 °C — for 1 day; at temperature -20 °C — for one week; at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed.

SEMINAL FLUID

Material should be collected to a container: **Cat. №: ИЛС-КП-60-С** 	**Material sampling** Seminal fluid should be collected into special dry sterile container, 50-60 ml volume. ***The pretreatment of samples is required.*** **The conditions of material storage:** at room temperature — for 6 hours; at temperature 2–8 °C — for 1 day; at temperature -20 °C — for one week; at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed.

FECES

Material should be collected to a container: **Cat. №: ИЛС-КПЛ-60-С** 	**Material sampling** Use 1–3 g (1–3 ml) fecal samples. Analysis of swabs is not informative due to low concentration of pathogens. The sample 1 g is transferred to a sterile container using disposable pipette tips with aerosol barrier or disposable spatula. ***The pretreatment of samples is required.*** **The conditions of material and pretreatment samples storage and transportation:** Native feces samples: at room temperature — for 6 hours; at temperature 2–8 °C — for 3 days; Fecal suspension with glycerol, bacterial fraction and clarified fecal extract: at temperature -20 °C — for one week; at temperature -70 °C — for a long time.

CEREBROSPINAL FLUID

Material should be collected to a tube: **Cat. №: МСТ-200-С** 	**Material sampling** Cerebrospinal fluid not less than 1 ml should be collected into disposable plastic tubes 1.5 and 2 ml volume using disposable needle. ***The pretreatment of samples is not required.*** ***Perform samples concentration in case of necessity (please see page 27).*** **The conditions of material storage and transportation:** - at temperature 2–8 °C — for 1 day; - at temperature -20 °C — for one week; at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed.

LACHRYMAL FLUID

Material should be collected to a tube: **Cat. №: MCT-200-C** 	**Material sampling** Lachrymal fluid (not less than 0.5 ml) should be collected in disposable sterile plastic tubes 1.5 or 2.0 ml using disposable plastic pipettes. To increase lacrimation perform provocation by lachrymatory agent (usually liquid ammonia is used). ***The pretreatment of samples is not required.*** **The conditions of material storage and transportation:** - at temperature 2–8 °C — for 1 day; - at temperature -20 °C — for one week; at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed.

BIOPSY OR AUTOPSY MATERIAL

Material should be collected to a tube:  **Information for order:** **Tubes:** **Cat. №: MCT-200-C** **Transport medium:** **Cat. № 956** **Tubes with pre-dispensed transport medium: Cat. № 987**	**Material sampling** Material was collected from zone of proposed infectious agent location, from affected tissue or from region boundary with affected part. The tissue pieces (diameter not more than 5 mm) were collected into disposable sterile tubes, 2 ml of volume, with appropriate transport medium. Close tube thoroughly. Macro autopsy material should be collected into container with physiological solution or special transport medium. ***The pretreatment of samples is required.*** **The conditions of material storage and transportation** **Sample of autopsy and biopsy material, intended for DNA or RNA extraction:** at room temperature — for 6 hours; at temperature 2–8 °C — for 3 days; - at temperature -20 °C — for one week; at temperature -70 °C — for a long time.

CONJUNCTIVAL SECRETION

Sponge probe **Cat. №: 300202**	

Material should be collected to a tube:

Information for order:

Tubes:

Cat. №: MCT-200-C

Transport medium:

Cat. № 956

Tubes with pre-dispensed transport medium: Cat. № 987

Material sampling

Material is collected using dry sterile cotton sponge with plastic basis with local anesthetic (2 drops of dicainum). Abduct inferior eyelid. Perform sampling from conjunctiva with 4-5 rotate movements cover lateral and medial angles of eye.

After material sampling sponge (work part of probe with cotton sponge) is transferred in disposable sterile tube with flip cover of 2 ml volume, containing relevant transport medium. Work part of probe is entered in transport medium, the probe is to be broken off no more than 0.5 cm above work part, and work part with material is left in the tube. Close the tube tightly.

The pretreatment of samples is not required.

The conditions of material storage and transportation

at room temperature — for 6 hours;

at temperature 2–8 °C — for 3 days;

- at temperature -20 °C — for one week;

at temperature -70 °C — for a long time.

CLINICAL MATERIAL OF RESPIRATORY TRACT

NASOPHARYNGEAL SWABS

Sponge probe **Cat. №: 300202**	
Material should be collected to a tube:   **Information for order:** **Tubes:** **Cat. №: MCT-200-C** **Transport medium for respiratory swabs storage and transportation:** **cat. № 957 or 958** **Tubes with pre-dispensed transport medium: Cat. № 959**	**Material sampling** Swabs (mucus) are collected using dry sterile cotton sponge with plastic basis. Sponge is entered by light moving along outer dorsum of the nose for 2-3 cm before lower scroll bone. Then sponge is moved down and is entered in lower nasal passages under lower scroll bone. Make rotation moving and remove tampon along outer dorsum of the nose. After material sampling sponge (work part of probe with cotton sponge) is transferred in disposable sterile tube with flip cover, containing relevant transport medium. The probe is to be broken off no more than 0.5 cm above work part, and work part with material is left in the tube. Close the tube tightly. ***The pretreatment of samples is not required.*** **The conditions of material storage and transportation** at room temperature — for 6 hours; at temperature 2–8 °C — for 3 days; - at temperature -20 °C — for one month; at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed.

WASHING FROM NASAL CAVITY

Material should be collected to a tube: **Cat. №: SCT-10ML-S** 	**Material sampling** Material sampling is performed from patient in sitting position with reflexed head by entering warm sterile isotonic solution of sodium chloride (3-5 ml) in each nasal passages using disposal syringe (probe). Washing fluid is collected in one sterile tube using sterile funnel. Re-using funnel without disinfection by steam under pressure is not allowed. *The pretreatment of samples is not required.* **The conditions of material storage:** at temperature 2–8 °C — for 6 hours; at temperature -20 °C — for one week; at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed.

OROPHARYNGEAL SWAB

Sponge probe **Cat. №: 300202**	
Material should be collected to a tube:   **Information for order:** **Tubes:** **Cat. №: МСТ-200-С** **Transport medium for respiratory swabs storage and transportation:** **cat. № 957 or 958** **Tubes with pre-dispensed transport medium: Cat. № 959**	**Material sampling** Swabs are collected using dry sterile cotton sponge with plastic basis by rotate moving from tonsils surface, faucial pillars and back oropharyngeal wall. After material sampling sponge (work part of probe with cotton sponge) is transferred in disposable sterile tube, containing relevant transport medium. The probe is to be broken off no more than 0.5 cm above work part, and work part with material is left in the tube. Close the tube tightly. *The pretreatment of samples is not required.* **The conditions of material storage :** at room temperature — for 6 hours; at temperature 2–8 °C — for 3 days; at temperature -20 °C — for one month; at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed.

OROPHARYNGEAL WASHING

Material should be collected to a container: **Cat. №: ИЛС-КП-60-С** 	**Material sampling** Preliminary mouth rinsing with water is performed before washing sampling from oropharyngeal cavity. After that the thorough mouth rinsing with 25-40 ml of isotonic solution of sodium chloride is performed during 10-15 s. Washing fluid is collected in sterile container 50-60 ml of volume using sterile funnel. *The pretreatment of samples is not required.* **The conditions of material storage:** at room temperature — for 6 hours; at temperature 2–8 °C — for 3 days; at temperature -20 °C — for one week; at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed.

SPUTUM

Material should be collected to a container: **Cat. №: ПЛС-КП-60-С** 	**Material sampling** Material sampling (not less than 1 ml) is performed in disposable graduated sterile containers with wide screw cap, 50 ml of volume. *The pretreatment of samples is required.* **The conditions of material and pretreatment sample storage and transportation:** at room temperature — for 6 hours; at temperature 2–8 °C — for 3 days; at temperature -20 °C — for one week; at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed.

SALIVA

Material should be collected to a tube: **Cat. №: МСТ-200-С** 	**Material sampling** Three-time mouth rinsing with physiological solution is performed before saliva sampling. Saliva samples (not less than 1 ml) are collected in disposable sterile plastic tubes, 2 ml of volume. Close the tube tightly. *The pretreatment of samples is not required.* **The conditions of material storage:** at room temperature — for 6 hours; at temperature 2–8 °C — for 1 day; at temperature -20 °C — for one week; at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed.

BRONCHOALVEOLAR LAVAGE OR EPITHELIAL LINING FLUID

Material should be collected to a tube: **Cat. №: SCT-50ML-25-S** 	**Material sampling** Material sampling is performed in disposable tubes with tight-screw lid, 50 ml of volume. *The pretreatment of samples is required.* **The conditions of material and pretreatment sample storage and transportation:** at temperature 2–8 °C — for 1 day; at temperature -20 °C — for one week; at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed.

BUBONIC PUNCTATE

Material should be collected to a tube:  **Information for order:** **Tubes:** **cat. №: MCT-200-C** **Transport medium:** **cat. № 956** **Tubes with pre-dispensed transport medium: cat. № 987**	**Material sampling** Sampling material is performed using sterile syringe. If bubo has unbroken skin clean the skin with spirit. Bubo puncturing is performed in the center and in the periphery. Material from broken bubo should be collected in places with saved tissue, also bubonic discharge is collected. Material (0.1-0.3 ml) is transferred in tube with relevant transport medium. *The pretreatment of samples is not required.* **The conditions of material storage:** at temperature 2–8 °C — for 1 day; at temperature -20 °C — for one month; at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed.

MATERIAL FROM VESICLES AND PUSTULES

Material should be collected to a tube:  **Information for order:** **Tubes:** **cat. №: MCT-200-C** **Transport medium:** **cat. № 956** **Tubes with pre-dispensed transport medium: cat. № 987**	**Material sampling** Clean skin elements with ethanol or ester cotton tampon before sampling. Puncture it at their bottom using sterile needle or thin capillary of Pasteur pipette. For activating of material egress elements is pressed by pincers. Crust or upper part of vesicle is separated from skin using needle, lancet. Material is collected in a tube with transport medium. *The pretreatment of samples is not required.* **The conditions of material storage:** at room temperature — for 6 hours; at temperature 2–8 °C — for 3 days; at temperature -20 °C — for one week; at temperature -70 °C — for a long time.

SAMPLES FROM ENVIROMENTAL OBJECTS

TICKS, MOSQUITOES AND ECTOPARASITES (LICE AND FLEAS)

Material should be collected to a tube: **Cat. №: MCT-150-C**	**Material sampling** After sampling and transportation material to laboratory, ticks, mosquitoes, lice and fleas are treated with ester until immobilization using cotton wad with drop of ester. After species and gender determination material can be combined to pools according to species, gender, place and time of collecting and put into dry clean tubes 1,5 ml of volume.

	Samples grouping are performed according to МУ 3.1.1027-01 «Collecting, recording and pretreatment to laboratory analysis of bloodsucking arthropods— vector of natural focal infections agent». In case of analysis for plague 20-30 species (lice, small ticks or fleas) are included in one sample (not more than 50). Ixodic ticks are analyzed separately according to evolution stage in case of analysis for plague and other natural focal infections. One probe can contain no more than 3 sucked females, to 30 foodless species, to 15 sucked nymphs, to 50 foodless nymphs, to 30 sucked larvae. In case of analysis for tularemia one probe should contain up to 50 imago of ixodic ticks, 50-100 nymphs and 100-200 larvae. Lice, fleas and gamasid mites are analyzed up to 100 species in one probe. Bloodsucking dipterous are grouped in samples: to 100 mosquitoes, to 250 blackfly and 20-25 greenheads. In case of analysis for abrovirus infections mosquitoes is grouped in pool for 50-100 species. In case of necessity particular species are analyzed. *The pretreatment of samples is required.* **The conditions of material and pretreatment sample storage and transportation:** Material after samples dividing and formation at temperature -20 °C — for one month; at temperature -70 °C or in Dewar flask with liquid nitrogen — for a long time. Treated material (after homogenization and clarification) is stored for a long time at temperature -70 °C or in Dewar flask with liquid nitrogen. Only one material freeze-thaw process is allowed.

FOOD PRODUCTS

Material should be collected to a container: **Cat. №: ИЛС_КП$_{60}$-С** 	**Material sampling** Sampling is performed in sterile wide-mouthed container using sterile spoon, pincers or knife in consideration of aseptic rules. Containers brim is burned by flame of alcohol lamp. After sample collecting the containers should be covered by sterile paper and be banded. *The pretreatment of samples is required.* **The conditions of material storage and transportation:** at temperature 2–8 °C — for 1 day; at temperature -20 °C — for one month; at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed.

SOILS, GRASS, FORAGE, PLANT LITTER

Material should be collected to a container: **Cat. №: ИЛС_КП$_{60}$-С**	**Material sampling** Soils sample from place of probable pathogenic microorganisms contamination (place of forced slaughter, cattle camp and stock watering) are collected in quantity of 20–30 g at a depth of 15 cm, on the territory of animal burial sites – at a depth of up to 2 m using soil tube. In this case the upper soil layer (2-3 cm) is deleted. The forage sample is collected from surface layer – not less than 400 g on 4 square meters of surface in case of bulk type of storage. The upper layer of brick is cut from cubed feed. Sample collecting is performed using sterile trial probe. Samples of rough feed (velour, haulm) are collected from different place of haystack using scissors or pincers – one sample (40 g) for each 4 square meters of haystack surface. Collected hinges of velour or haulm are chaffed on paper

	using scissors or pincers, then are placed it into containers. Grass cuttings are placed in a tube or container using pincers. *The pretreatment of samples is required.* **The conditions of material storage and transportation:** at temperature 2–8 °C — for 1 day; at temperature -20 °C — for one month; at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed.

WATER, SEWAGE, WASHOUT

Material should be collected to a container: **Cat. №: ИЛС_КП_60_С** 	**Material sampling** Faucet water and water from surface water reservoir are collected for analysis in quantity of 1 liter for one sample in two volumes of 500 ml in sterile vessel with waterproof plug. From water taps sampling is performed after preliminary burning by flame and water drain during 10 minutes at total tap opening. Domestic sewage is collected for analysis by two ways: 1 liter divided to 2 volume of 500 ml or using sponge from gauze pads 10x15 cm put in 10–layers. These sponges are left in the place of water collection for 1 day and then are placed in sterile container with physiological solution. Washouts from surfaces are collected using sterile cotton sponge or gauze pads. Before sampling sponges and pads are irrigated with sterile physiological solution. After sampling sponge (pad) is placed in a vessel with physiological solution. *The pretreatment of samples is required.* **The conditions of material storage and transportation:** at temperature 2–8 °C — for 1 day; at temperature -20 °C — for one month; at temperature -70 °C — for a long time. Only one material freeze-thaw process is allowed.

MATERIAL PRETREATMENT

Type of clinical material	Pretreatment
Blood	**Blood plasma** is obtained by centrifugal separation of tubes with whole blood at 800–1600 g (3000 rpm on centrifuge «MiniSpin», Eppendorf, Germany) during 20 minutes at room temperature. Than collect plasma (not less than 1 ml) in sterile tubes 1.5 or 2.0 ml of volume using separate tips with filters (Pasteur pipette). In case of detection of leucocytal viruses **blood cells** (white cells fraction of whole blood, buffy coat layer) should be collected after centrifugal separation of whole blood and removing of blood plasma. Collect white cells thoroughly from surface of cell sediment in volume of 0.2 ml and transfer it in sterile tube 1.5-2.0 ml of volume using tip with filter. For **serum blood** sampling the tubes with blood (without anticoagulant) are left to settle during 30 minutes at room temperature until total clot formation or placed in thermostat for 15 minutes at 37°C. Centrifuge the tubes during 10 minutes at room temperature at 800–1600 g (3000 rpm on «MiniSpin», Eppendorf, Germany). Transfer blood serum in sterile tubes 1.5 or 2.0 ml of volume using separate tips with filters (Pasteur pipette). Blood serum shouldn't be hemolyzed. Blood serum is allowed to use only for qualitative detection of Hepatitis viruses in case of impossibility to obtain blood plasma. It is mportant to remember that in case of blood coagulation part of virus (HIV,HCV, HBV) remains in blood clot.

Urogenital tract	Thoroughly vortex content of the tubes for mucus lysing and obtaining of homogeneous cells suspension before working with samples. In case of mucus presence thoroughly vortex epithelium scraping sampling from endo- and exo- parts of cervical canal collecting with combined probe into tube of 5 ml volume with transport medium for more full mucus lysing and obtaining of homogeneous suspension. Transfer 0.5 ml of sample in plastic tube 1.5 ml of volume using tip with aerosol filter. Centrifuge during 5 minutes at 10000 g (12000 rpm on «MiniSpin», Eppendorf, Germany). Remove 0.4 ml of supernatant, mix cells sediment with 0.1 ml of remaining fluid.
Seminal fluid	Transfer 0.05 ml of seminal fluid in disposal sterile tube 1.5 ml of volume using tip with filter before nucleic acids extraction. Add 0.15 ml of transport medium and thoroughly vortex sample.
Urine	Shake container with urine. Transfer 1 ml of urine in sterile disposable tube 1.5 ml of volume using tip with aerosol barrier. Centrifuge during 5 minutes at 10000 g (12000 rpm on «MiniSpin», Eppendorf, Germany). In case of presence of big amount of salts suspend only upper layer of salts sediment in 1 ml of volume and then concentrate again. Totally remove supernatant using vacuum suction pump with catcher's retort and individual tip without aerosol barriers for each sample. Don't pick up sediment. Add transport medium to sediment to finish volume 0.2 ml and thoroughly vortex content of the tube. In case of analysis for Leptospira spp. it is possible to use consistent concentration: first, centrifuge 20 ml of urine during 10 minutes at 9000 g (11000 rpm on «MiniSpin», Eppendorf, Germany), then centrifuge sediment with 1 ml of supernatant urine during 10 minutes at 11000 g (13000 rpm on «MiniSpin», Eppendorf, Germany).

Type of clinical material	Pretreatment
Feces	In case of native feces analysis without preliminary freezing prepares feces suspension (in case of feces watery consistency the suspension preparing is not required). **Preparing of feces suspension** Add 0.8 ml of phosphate buffer (or sterile isotonic sodium chloride solution) in microcentrifuge tubes 1.5 ml volume. The quantity of tubes corresponds with quantity of samples. Add 0.1 g (0.1 ml) of feces in each tube using individual tip with filter (or disposable scapula) and thoroughly vortex until homogeneous suspension formation. If you haven't possibility to perform analysis of material during 24 hours or in case of long time storage add glycerol in end concentration 10-15 % to 10-20% feces suspension in phosphate buffer (or isotonic sodium chloride solution). Freeze thus prepared samples only after thorough homogenization and exposition with glycerol during 30-40 minutes. **Preparing of bacterial feces fraction for detection of bacterial agents** For preparing of bacterial feces fraction use watery consistency of feces, just prepared feces suspension or suspension freeze with glycerol. Centrifuge tubes with suspension (watery consistency feces) during 5 minutes at 7000-12000 g (10000-13000 rpm on «MiniSpin», Eppendorf, Germany). Take 0.05 ml of bacterial fraction (upper white-yellow part of obtained sediment) using individual tip with filter for each tube. In case of absence of sediment or white-yellow bounder layer between sediment and supernatant take 0.1 ml from tube bottom or from border between sediment and supernatant, accordingly. Transfer separated part of sample which contains bacteria in high concentration, in new micro centrifuge tube 1.5 ml volume with 0.8 ml of phosphate buffer (or isotonic sodium chloride solution). Thoroughly vortex sediment and centrifuge during 15 minutes at 7000-12000 g (10000-13000 rpm on «MiniSpin», Eppendorf, Germany). Remove supernatant, vortex sediment in 0.3 ml of phosphate buffer (or isotonic sodium chloride solution). **Preparing of defecate feces extraction for viruses agents detection** For preparing of defecate feces extraction use watery consistency of feces, just prepared feces suspension or suspension freeze with glycerol. Vortex feces indiscernible substance intensively. Defecate obtained suspension by centrifuge during 5 minutes at 10000 g (12000 rpm on «MiniSpin», Eppendorf, Germany). Mix supernatant (0.1 ml) with negative control samples (50% blood serum of cattle diluted with phosphate solid buffer) (0.1 ml) in the ratio 1:1 and use for DNA or RNA extraction. In case of storage necessity transfer supernatant in separate disposable tube.

Type of clinical material	Pretreatment
Cerebrospinal fluid	To concentrate virus containing cells and bacteria in case of some infection diseases (west-Nile fever, tick-borne encephalitis, leptospirosis, borreliosis, and toxoplasmosis) centrifuge 1 ml of cerebrospinal fluid at 10000–11000 rpm and analyze sediment with 100 μl of supernatant.
Lachrymal fluid	Not required

Biopsy and Autopsy material	The pretreatment of **Micro biopsy specimen (punctate)** or **Micro autopsy specimen** of liver, lien, prostatic gland, gastrointestinal tract, cervix uteri and others is not required. Place specimens in micro tubes with screw caps or in tubes 1.5 ml of volume with lock which contain 0.1 ml transport medium. Than perform DNA or RNA extraction according to kit manual. **Macro biopsy specimen** or **Macro autopsy specimen** In case of virus agents detection place pieces of tissue 0.1-1 g in cooled porcelain mortar and add cooled isotonic sodium chloride solution 0.5-1 ml. Mill it using sterile scissors and pestle. Take supernatant (0,1–0,2 ml) through cotton tampon using sterile tip with filter and place it in sterile tubes. In case of **bacterial agents** detection the process of macro biopsy specimen preparing (macro punctuate) is similar but without porcelain mortar and isotonic solution cooling. Another way place biopsy specimen in liquid nitrogen just before nucleic acid extraction. Pestle careful in porcelain mortar preliminary cooled by liquid nitrogen. Weight 100 mg of tissue pieces and pestle it in porcelain mortar in liquid nitrogen until powder obtaining. Then for RNA extraction transfer powder in tissue grinder and follow to manual for RNA extraction. For DNA extraction add equal volume of sterile physiological solution (0.1 ml) to obtained powder, mix thoroughly and take required quantity of material according to manual for DNA extraction. Porcelain ware and tissue grinders should be treated with bichrome and be sterilized. In case of several sample homogenization it is required to treat table surface with 0.2% solution of disinfect tablets ДП$_{2Т}$ (active material is trichloroisocyanuric acid) after each sample, then with water and 70% of ethanol. Change gloves before treatment of next sample.
Conjunctival secretion	Not required
Nasopharyngeal swabs	Not required
Washing from nasal cavity	Not required
Oropharyngeal swabs	Not required
Oropharyngeal washing	Not required

Type of clinical material	**Pretreatment**
Sputum	Before nucleic acids extraction it is required to perform sputum deliquating using “Mucolysin” solution (Na2HPO4 77,4 mM, NaH2PO4 22,6 mM, β-mercaptoethanol 99,4 mM, 5% sodium aside in the final concentration 0,05%). Add “Mucolysin” in container with sputum in ratio 5:1 (5 parts of “Mucolysin” to 1 part of sputum), follow to container calibration and add sterile glass beads. Shake periodically container during deliquating of sputum (20-30 minutes). Take 1 ml of diluted sputum using automatic pipette and tip with filter, place it in tube with screw cap or in micro centrifuge tube with lock 1.5 ml of volume and centrifuge during 10 minutes at 5000-7000 g (8000-10000 rpm on «MiniSpin», Eppendorf, Germany). Remove 0.8 ml supernatant, mix cells sediment with 0.2 ml of remaining liquid. The DNA/RNA extraction from 0.1 ml diluted sputum without centrifuge stage is allowed.
Saliva	Not required
Bronchoalveolar lavage or epithelial lining fluid	Mix bronchoalveolar lavage or epithelial lining fluid by tube turning over. Take 1 ml of clinical material using automatic pipette and tip with filter, place it in tube with screw cap or in micro centrifuge tube with lock 1.5 ml of volume and centrifuge during 10 minutes at 7000 g (10000 rpm on «MiniSpin», Eppendorf, Germany). Remove 0.9 ml supernatant, mix cells sediment with 0.1 ml of remaining liquid.
Bubonic punctate	Not required
Vesicles and pustules	Not required

Ticks, mosquitoes and ectoparasite (lice and fleas)	- place ticks in micro centrifuge tubes 1.5 ml of volume, add 1 ml of 96% ethanol, vortex and centrifuge during 3-5 sec. at 2000 g (5000 rpm on «MiniSpin», Eppendorf, Germany) for removing drops from tube lid; - remove ethanol from tube using vacuum suction pump and individual tip for each sample; - add 1 ml of 0.15M sodium chloride solution in tube, shake the tube and centrifuge during 3-5 sec. at 2000 g (5000 rpm on «MiniSpin», Eppendorf, Germany) for removing drops from tube lid - remove sodium chloride solution from the tube using vacuum suction pump and individual tips for each sample; - transfer ticks in sterile porcelain cup, add 0,7–1,0 ml 0,15 M sodium chloride solution and homogenize the sample; - transfer sample in micro centrifuge tube 1.5 ml of volume using tip with filter and centrifuge during 2 minutes at 1200 g (3000 rpm on «MiniSpin», Eppendorf, Germany) for sample defecating. RNA and DNA extraction is performed from 0.1 ml of supernatant. In case of RNA and DNA extraction from mosquitoes, lice and fleas use the same method excluding the step of elution by 96% ethanol and 0,15M sodium chloride solution. Directly homogenize insects in sterile porcelain mortar in 0,15M sodium chloride solution.

Type of clinical material	**Pretreatment**
Food products	Place 1-10 g solid food product in sterile porcelain mortar, add 0.9% sodium chloride solution in ratio 1:10 and grind for obtaining homogeneous condition. Defecate and take supernatant through cotton tampon. Perform DNA extraction from supernatant. Liquid food products (0.2 ml) are transferred in micro centrifuge tube 1.5 or 2.0 ml of volume for DNA extraction.
Soils, grass, forage, plant litter	Add 0.9% sodium chloride solution to clinical material in ratio 1:10, thoroughly mix during 15 minutes, defecate during 10 minutes for sedimentation of large particles. Centrifuge supernatant separately: first during 2-3 minutes at 2000 g (5000 rpm on «MiniSpin», Eppendorf, Germany), then during 15 minutes at 10000 g (12000 rpm on «MiniSpin», Eppendorf, Germany). Suspend sediment in 0.2-0.5 ml of distillated water.

Water, sewage, washouts	In case of bacterial agents and mycosis agent detection sample treatment is performed by divided centrifugation or vacuum filtration on filters with a pore size 0,45 and 0,65, 0,8, 1,2 microns accordingly. In case of virus agents detection for sample treatment only vacuum filtration on filters with a pore size 0,2 is used. **Divided centrifugation** Transfer 125 ml of collected samples into 4 centrifuge glass 250 ml of volume with screw caps (or 80 ml into 6 centrifuge tubes, or 50 ml into 10 centrifuge tubes) and centrifuge during 15 minutes at 10000 g (12000 rpm on «MiniSpin», Eppendorf, Germany). Suspend the sediment in each tube in 0,2 ml of 0,9% sodium chloride solution. Transfer obtained suspensions into micro centrifuge tubes 1,5 ml or 2,0 ml of volume and centrifuge during 1 minute at 10000 g (12000 rpm on «MiniSpin», Eppendorf, Germany). Transfer supernatant into micro tube 1,5 ml of volume using tip with filter. For DNA extraction 0,1-0,2 ml of supernatant is used. It is possible to centrifuge one sample in one centrifuge glass (tube). For this transfer 50-125 ml of sample in centrifuge glass (tube) and centrifuge during 15 minutes at 10000 g (12000 rpm on «MiniSpin», Eppendorf, Germany). Remove supernatant and add again corresponded volume of analyzed sample. Centrifuge whole volume of the sample by the same way. After the last centrifugation suspend the sediment in 1 ml of 0,9% of sodium chloride solution and centrifuge during 1 minute at 10000 g (12000 rpm on «MiniSpin», Eppendorf, Germany). Transfer supernatant into micro tube 1,5 ml of volume using tip with filter. For DNA extraction 0,1-0,2 ml of supernatant is used. **Vacuum filtration** For analysis sterile filters with corresponded pore size are used. In case of heavily polluted analyzed water sample by mechanic or oil impurities, which can be detected visually, preliminary filter the sample using glass cone and sterile cotton or paper filter. Prepared by this way water sample is infiltrated through membrane filter. After filtration transfer membrane filters into sterile vial (Petri dish) or sterile plastic bag (100 ml volume) with 0.9% of sodium chloride solution using burned surgical forceps. Sterilize water after filtration by adding dry calcium hypochlorite in an amount of 200 g for 1 litter. For bacteria and viruses sorption from filter shake the vial during 10 minutes. The filter inside the plastic bag is ground by hand during 1 minute. Scissor up the filter from Petri dish. Incubate during 10 minutes with shaking. Transfer the washout from filter surface into sterile tube. For PCR analysis transfer 1 ml into micro centrifuge tubes (1,5 ml of volume) and centrifuge during 10 minutes at 10000 g (12000 rpm on «MiniSpin», Eppendorf, Germany). In case of bacterial agents detection leave 0,1 ml of supernatant and suspend the sediment. Perform DNA extraction from obtained suspension. In case of virus agent detection the 0,1-0,2 ml of supernatant is used for DNA extraction.