

LN069-99

State System for Sanitation-Epidemiological  
Standardization of the Russian Federation

Federal Sanitation Regulations, Norms and  
Hygiene Standards

---

## 1.2 EPIDEMIOLOGY

# SAFETY IN WORKING WITH GROUP I AND II PATHOGENICITY MICROORGANISMS

Sanitation Regulations  
SP 1.2.011-94

Official Publication

Russian State Committee for Sanitation and Epidemiological Oversight  
(Goskomsanepidnadzor **Rossii**)

Moscow

1994

SP 1.2.011-94

BBK 51.9 ya 82  
B 39

Safety in Working with Group I and I Pathogenicity Microorganisms: Sanitation Regulations, Moscow, Information Publication Center, Russian State Committee for Sanitation and Epidemiological Oversight, 152 pages

1. Developed by:

- The Russian State Committee for Sanitation and Epidemiological Oversight (Federov, Yu. M.)
- Anti-Plague Center, Russian State Committee for Sanitation and Epidemiological Oversight (Ponomareva T.N., Korolev Yu.S., Kyuregyan A.A., Golovchenko N.N., Bezsmertnyy V. Ye.)
- "Mikrob" Russian Anti-Plague Scientific Research Institute (Kokushkin A.M., Popov Yu.A., Vasenin A.S.)
- Scientific Research Institute for Preventive Toxicology and Disinfection (Panteleyev L.G., Arefyeva L.I., Sokolova N.F., Potemkin A.S., Ramkova N.V.)
- Irkutsk Scientific Research Anti-Plague Institute of Siberia and the Far East (Zakhlebnaya O.D.)
- Stavropol Scientific-Research Antiplague Institute of Caucasia and Transcaucasia (Yundin Ye.V.)
- Volgograd Scientific Research Anti-Plague Institute (Anisimov B.I.)
- D.I. Ivanovskiy Virology Institute (Lvov D.K., Gaydamovich S.Ya., Fadeyeva L.L., Deryabin P.G., Budenko A.M., Terskikh I.I., Slepshkin A.N.)
- N.F. Gamaleya Scientific Research Institute of Epidemiology and Microbiology (Vertiyev Yu.V., Tarasevich I.V.)

2. Approved and entered into force by Decree of the Russian State Committee for Sanitation and Epidemiological Oversight of 5/4/94.

LN069-99

3. Introduced for the first time to replace "Instruction on Anti-epidemiological Regime for Working with Material Contaminated or Suspected of Being Contaminated by Pathogens of Infectious Diseases Groups I and II," approved the USSR Health Ministry on June 29, 1978

ISBN 5-7508-0007-5

©: Russian State Committee for Sanitation and Epidemiological Oversight

## Table of Contents

1. Scope .....	7
2. Normative References .....	8
3. Requirements on the Organization of Work with Biological Material of Group I and II Pathogenicity .....	9
3.1. General .....	9
3.2. Premises and Equipment of Microbiological Laboratories .....	22
3.3. Work Clothing .....	28
3.4. Supplemental, for Work with Viruses and Rickettsia .....	31
3.5. Supplemental for Working with Deep Mycoses Pathogens .....	32
3.6. Supplemental, for Working in Laboratories of Industrial Departments .....	34
3.7. Supplemental, for Working with Botulism Toxin and Biological Poisons .....	34
3.8. Zoological and Parasitological Operations .....	35
3.9. Procedures for Trapping, Transporting and Keeping Wild Vertebrates and Arthropods During Experimental Work .....	37
3.10. Procedures for Controlling Accidents During Work with Biological Material .....	40
3.11. Work in Hospitals, Isolation Wards, and Observation Wards .....	44
3.12. Medical Observation of the Populace. Disinfection and Pathological/Anatomical Work at the Foci of Especially Dangerous Infections .....	51
3.13. Procedures for Exiting from Institutions Working with Biological Material .....	53
4. Organization of Monitoring .....	55
Attachment 5.1 (Mandatory) Classification of Microorganisms Pathogenic to Man ..	57

Attachment 5.2 (Mandatory) Statute on a Board to Monitor Compliance with the Biological Safety Requirements at an Institution (Enterprise) . . . . . 69

Attachment 5.3 (Reference) Means and Methods of Disinfection Used in Work with Pathogenic Microorganism . . . . . 72

Attachment 5.4 (mandatory) Modes of decontamination of various objects which have been infected by pathogenic organisms . . . . . 82

Attachment 5.5 (Reference) Chemical tests for monitoring temperature parameters of steam sterilizer operating mode . . . . . 124

Attachment 5.6 (Mandatory) Bacteriological method of testing the effectiveness of steam sterilizer operation . . . . . 128

Attachment 5.7 (Mandatory) Procedure for replacing fine filters of the ventilation exhaust system and determining their efficacy . . . . . 132

Attachment 5.8 (Mandatory) Requirements for disinfection of laboratory vessels with infected nutritive media in portable disinfection chambers . . . . . 135

Attachment 5.9 (Mandatory) Requirements for examination of waste water for pathogenic microflora . . . . . 137

RSFSR Law "On Public Sanitation-Epidemiological Welfare"

"Sanitation regulations, norms and hygiene standards (henceforth sanitation regulations) are normative documents establishing the criteria for safety and/or absence of harm to humans from environmental factors, and the requirements for assurance of safe conditions in daily activities.

sanitation regulations must be complied with by all state organs and public associations, enterprises and other administrative entities, organizations and institutions, regardless of their subordination or forms of ownership, and by officials and citizens." (Article 3).

"Illegal, culpable (deliberate or careless) acts (action or inaction) associated with failure to comply with sanitation legislation of the RSFSR, including sanitation regulations in force, are deemed to be sanitation violations . . .

"RSFSR officials and citizens committing sanitation violations may suffer disciplinary, administrative, or criminal prosecution." (Article 27)

Approved  
by Decree of the Russian State Committee for Sanitation  
and Epidemiological Oversight dated 5/4/94 No. 011

## 1.2. EPIDEMIOLOGY

### SAFETY IN WORKING WITH GROUP I AND II PATHOGENICITY MICROORGANISMS

#### **Sanitation Regulations and Standards SP 1.2.011-94**

---

#### Regulations

##### 1. Scope

These regulations were prepared in accordance with the "Statute on Procedures for Development, Approval, Publication, and Entry into Force of Federal, Republic and Local Sanitation Regulations, as well as Operation Procedures of Union sanitation regulations on RSFSR Territory," approved by decree of the RSFSR Council of Ministers of 7/1/91 No. 375 and establishes the requirements for organization of work with group I and II pathogenicity microorganisms.

---

**Official Publication:** These sanitation regulations cannot be reproduced, copied, or distributed, fully or in part, without the permission of the Russian State Committee for Sanitation and Epidemiological Oversight

The requirements are mandatory and must be complied with by all institutions on the territory of the Russian Federation regardless of their departmental affiliation and forms of ownership, and well as by officials and citizens involved in working with Group I and II pathogenicity microorganisms (attachment 5.1) or material suspected of containing them.<sup>1</sup>

---

<sup>1</sup>Henceforth, group I and II pathogenicity biological material

The regulations are aimed at assuring personal and public safety in work with group I and II pathogenicity biological material and calls for the following measures:

- organizational, including rational planning of premises equipped with the appropriate technical and engineering systems; supplying laboratories with the necessary systems for individual and group protection; manning with appropriately trained personnel; use in operations of only tested and safe work procedures, as well as reliable decontamination systems and methods; effective plans for localization and control of accident consequences; strict monitoring of performance of these regulations by associates on the part of the institution administration and a board to monitor compliance with the requirements of biological safety<sup>2</sup>; provision of a reliable electric power source, water supply etc. (degree of reliability is determined depending on the type of biological material, and the nature of the investigations being carried out);

- regulatory, restricting the circle of individuals cleared for working with group I and II biological material; [regulating] the departure of associates working with this material or participating in localization and elimination of epidemic foci; departure of populace during epidemic manifestations of plague, cholera, and highly contagious viral hemorrhagic fevers;

- special, governing the admission to work of persons who have no contraindications to vaccine prophylactics; medical observation of personnel; use of specific immuno-prophylaxis for personnel; isolation and prophylactic treatment of persons who have come in contact with a pathogen in an accident and performance of measures for thorough disinfection in order to prevent spread of the infection beyond the boundaries of the building where the accident occurred.

## 2. Normative References

These regulations make reference to the following normative documents:

- 2.1. RSFSR Law "On Public sanitation-Epidemiological Welfare."
- 2.2. Order of the USSR Health Ministry dated 9/29/89 No. 555 "On Improving the System of Medical Examination of Workers and Drivers of Individual Transportation Means."
- 2.3. "Statute on Procedure for Accounting, Storage, handling, Release and Transfer of Cultures of Bacteria, Viruses, Rickettsia, Fungi, Rudimentary Mycoplasmas, Bacterial Toxins, and Poisons of Biological Origin,." Approved by the USSR Ministry of Health on 8/18/79

---

<sup>2</sup>Henceforth, the Board



2.4. "Instruction on Freeze-Drying of Infectious Disease Pathogens Group I and II in the Collector Apparatus of the K. Ye. Dolinov System," Approved by the USSR Health Ministry, 3/12/79

2.5. sanitation-Epidemiological Regulations "Safety of Work with Recombinant DNA Molecules," Approved by the USSR Health Ministry, 1/12/89

2.6. "Instruction on Primary Processing of Material Contaminated or Suspected of Contamination by Pathogens of Plague, Cholera, Tularemia, Brucellosis and Anthrax During Histocytoenzymochemical Investigations." Approved by the USSR Health Ministry GUKI\* (1984)

2.7. "Methodological Instructions on Monitoring the Operation of Steam and Air Sterilizers," Approved by the USSR Health Ministry GEU (1991).

2.8. "Instruction of Work Regime with Aerosols of Pathogens of Especially dangerous and Other Bacterial Infections." Approved by the USSR Health Ministry 11/18/76.

2.9. "Regulations for Safety Procedures, Industrial Sanitation and the sanitation-Epidemiological Regime for Enterprises in the Production of Bacterial and Viral Preparations." Approved by the USSR Health Ministry 7/10/67.

### **3. Requirements on the Organization of Work with Biological Material of Group I and II Pathogenicity**

#### **3.1. General**

3.1.1. Working with group I and I pathogenicity biological materials is permitted for laboratories having the conditions to meet the requirements of work safety provided for by these regulations and for complete separation of diagnostic and experimental investigations (diagnostic includes investigations of of objects of a biotic and abiotic nature with identification of culture; experimental includes all types of work with culturing of group I and II pathogens).

3.1.2. The procedure for granting permission to work with group I and II pathogenicity biological material is regulated by "Sanitation Regulations for Work Safety with Microorganisms," Part I, "Procedure for Issuance of Permission to Work with Group I-IV Pathogenicity Microorganisms and Recombinant DNA Molecules," SP 1.2.006-93.

Investigations of group I pathogenicity material are carried out by anti-plague and other specialized institutions.

3.1.3. Scheduled diagnostic investigations of cholera and botulism toxins performed for the purpose of prophylaxis of these infections are carried out by bacteriological laboratories having permission to work with group III-IV microorganism.

3.1.4. Permission to work with group I and II biological materials is considered to have lapsed if the premises are redesigned or the work techniques are changed.

3.1.5. Projects for the construction of new and reconstruction of existing laboratories must be concurred with the services of the State sanitation-Epidemiological Oversight Committee and approved by the institution director.

3.1.6. Work with group I-II biological material is carried out by specialists with higher and intermediate medical, biological, and veterinarian education (for veterinary institutions), who have graduated from the appropriate courses of specialization and have no contraindications against vaccine prophylaxis or treatment with specific medications.

3.1.7. Access to work with experimental and diagnostic material is authorized by order of the institution director once every two years after a test of knowledge on work safety. The engineering and technical personnel, disinfection specialists and laboratory assistants are trained in the structural subunit and are cleared for work in accordance with their official duties by institution order.

3.1.8. Permission to visit the laboratory is issued to engineering and technical personnel not permanently assigned there by the institution director. The visit is made after the work is stopped and disinfection performed, under escort by an associate of the structural subunit and recorded in the log.

3.1.9. Specialists (medical doctors and veterinarian, biologists etc) who are not permanently assigned to the institution are given access to the building where work is being done with biological material per the written authorization of the institution director. The purpose of the visit and its duration are recorded in the log.

3.1.10. Associates who by reason of their work come into contact with group I and II biological material (except for cholera) are vaccinated. Those with contraindications (to vaccination) are allowed to work per a separate institutional order. Persons having contraindications against vaccinations are not authorized to work in aerosol laboratories or with material that is contaminated or suspected of contamination by the Q fever pathogen.

3.1.11. All associates working with group I and II biological material (excluding cholera and toxins of a biological origin) receive a body temperature check daily, and the results are recorded in the log and certified by signature of the physician in charge (or science

associate). For persons working with the cholera pathogen, mandatory examination is organized to check for vibrio infection in the event of disjunction of the gastro-intestinal tract.

3.1.12. All associates working with group I and II biological material are under clinical observation. Periodic medical examinations are carried out in accordance with the order of the USSR Health Ministry dated 9/29/89 No. 555. Those working with deep mycoses receive allergy tests.

3.1.13. If an associate shows symptoms typical of an infectious illness caused by a pathogen with which he has worked, the associate is obliged to notify the director of the section or the institution duty officer. Subsequent decisions are made by the institution director.

3.1.14. In the event of sickness of an associate working with infectious material or cultures of plague, cholera, or group I virus pathogens, an institute physician will be sent to the apartment of the patient in order to clarify the epidemiological history and decide the question of whether it needs to be isolated. The results of the visit are recorded in the log and brought to the attention of the institution directory. A physician from the general medical network is called in only after the patient is visited by the institution physician, the exception being treatment per vital signs. Here the patient or his relatives must inform the arriving physician on the nature of the work he does and inform the structural subunit director of what has occurred.

3.1.15. Associates who for specific reasons cannot go to work are obliged within two hours to notify the section supervisor of this. If an associate does not show up at the institution for two hours after the start of work and there is no information about his location, the section supervisor will take steps to establish his location and reason for absence.

3.1.16. The transfer of equipment, laboratory or other vessels, reagents, tools and so forth inside the institution will occur with the consent of the supervisors of the sections in question. Removal of these materials from the institution will occur only after they are disinfected, with the written consent of the institution supervisor.

3.1.17. All types of work with group I and II pathogenicity biological material will be carried out in compliance with the principle of paired team operations (no fewer than two persons, one of whom is a physician or scientific associate). The time of continuous work with such material is restricted to 4 hours, after which there must be a 30-60 minute break. Work in the evening and night, as well as on days off and holidays is possible only with the written permission of the institution director, provided there is compliance the break regimen and two persons are present.

3.1.18. For individual protection of personnel, protective clothing must be worn. It is described in section 3.3. The protective suits must be disinfected before being turned in for laundering.

3.1.19. Various objects used in working with group I and II biological material are disinfected in accordance with these regulations (attachment 5.4), as well as the instructions and manuals in force.

3.1.20. It is forbidden to call associates when they are doing any sort of work with biological materials.

3.1.21. Reception of visitors, public work, and eating may be done only in specially designated premises.

3.1.22. The grounds and buildings of the institution must be under twenty-four-hour security.

3.1.23. When working with group I and II pathogenicity biological material, demanding special procedures, it is necessary to develop supplements to these regulations, allowing for the requirements which they put forward. The supplements, put forward in the form of procedural recommendations, are examined by a board and approved by the institution director.

3.1.24. Fire and addition safety regulations in the laboratory (institution0 must be compiled with allowance for the requirements of these regulations.

3.1.25. The director is responsible for organization of biological safety for the institution as a whole, and the supervisors are responsible in the sections. Compliance with the requirements of these regulations is monitored by the deputy director for science work, and in other institutions by the deputy director for epidemiological work.

3.1.26. The premises of the microbiological laboratories, and in a number of cases, the grounds of the institution, are divided into three zones in terms of degree of hazard to personnel:

I. "Contamination" zone--areas in which work with group I and II pathogenicity biological material is carried out, and personnel are clothed in the appropriate type of protective clothing.

II. "Possible contamination" zone--areas of the laboratories (in a number of cases also the institution grounds) where work with biological material is not conducted, and the

personnel wear their work clothing (jumpsuits, socks, slippers). Within this zone no biological material is transported between blocks of the "contamination" zone.

III. "Clean" zone--areas where no work is done with biological material, and personnel wear their personal clothing.

3.1.27. Associates proceed from the "clean" zone to the "possible contamination" zone through the sanitary check point. The outer clothing is left in individual lockers intended for is storage, exchange their footwear for slippers and proceed to the area to don their work clothing.

It is permitted to carry only writing implements through the sanitary check point.

Drinking of water and smoking may be done only in areas set aside for that purpose. Areas in the "possible contamination" zone are cleaned daily with detergents and disinfectants.

3.1.28. Transfer of materials and equipment within the limits of "possible contamination" zones is by personnel clothed in work clothing.

Transfer of pathogen cultures in containers (test tubes) from one section to another is by persons cleared to work with group I and II pathogenicity biological material, clothed in work clothes, with an escort.

Transfer of infective material in tanks for autoclaving, delivered in metal trays with high (20 cm) sides, is by junior and middle personnel clothed in a type III anti-plague suit and apron, accompanied by a laboratory assistant cleared to work with biological material. The movement is along specified routes. During the transfer of material to the autoclave, other movement will cease along that route.

3.1.29. The production operations regime in targeted rooms of a "possible contamination" zone (radioisotope, biochemical, electron microscopy, medicinal etc. ) must conform to the type of work done, in compliance with safety requirements.

3.1.30. Entrance of personnel into bacteriological rooms (wards) and departure from them are through vestibules (airlocks). The associates don and remove protective clothing in the vestibule (airlock).

3.1.31. Bacteriological rooms (wards) are cleaned daily before the start of the work day by junior personnel in work clothing, supplemented by rubber gloves, using disinfectants and supervised by a laboratory assistant. After wet mopping, the bactericidal lamps are turned on for 30-40 minutes. The laboratory tables and safety isolation wards are prepared by ~~the~~

laboratory assistants.

At the end of work with biological material, objects with sown cultures are transferred to storage (safes, refrigerators, autoclave tanks, incubators, and so forth), the working surfaces in the area are disinfected, hands are washed with 70° ethyl alcohol, and bactericidal lamps are turned on for 30-40 minutes.

Before leaving the premises, the associates make sure that gas, water, and unneeded instruments and so forth are turned off. Areas in the "contamination" zone of the laboratory are sealed and locked. Seals are opened and removed, and the entire laboratory is locked and sealed by associates (scientific associates, physicians, laboratory assistants) having the appropriate authorization by the institution (laboratory) director.

3.1.32. It is forbidden to perform diagnostic and experimental investigations in one and the same area, or simultaneous work with diagnostic material and live vaccines.

It is forbidden to perform any type of experimental work with virulent antibiotic-resistant strains, if there are no medicines in the institution (no fewer than two) to which the utilized strains are sensitive.

It is permitted to conduct simultaneous work with different types of pathogens in the same bacteriological room, if it is necessary. The biological safety is assured by the requirements imposed on working with the most dangerous type.

3.1.33. Inspection of agar cultures and conduct of bacteriological work with diagnostic material, as well as with low concentrations (less than  $10^{10}$  KOE/ml)<sup>3</sup> and low volumes (less than 500 ml) of virulent group I and II pathogen cultures (except for glanders, melioidosis and pathogens of deep mycoses) are done at the bacteriological table in a type IV antiplague suit. When working with pathogens of glanders, melioidosis and deep mycoses, the type IV suit is supplemented with a respirator and gloves.

Work with high concentrations (more than 10 KOE/ml), high volumes (more than 500 ml), in the absence of isolation boxes is performed in a type I antiplague suit (without shoes).

3.1.34. Contamination of ectoparasites with group I and II pathogens on a biomembrane (placed in a container with high sides), pulverization and sowing of contaminated ectoparasites on nourishment media are done at the bacteriological table in a type

---

<sup>3</sup>KOE is the number of colony-forming units or number of microbe cells in 1 milliliter.

IV suit, supplemented with cotton gauze bandaging and rubber gloves.

Contaminated ectoparasites are inspected in a type IV suit.

Contaminated ectoparasites are stored in test tubes and flasks placed in refrigerators, incubators and lockers.

3.1.35. During pipetting, it is necessary to use bulbs or automatic devices. The end of the pipette must always be below the level of the fluid in the vessel, or the fluid may drain from the pipette along the inside wall of the vessel. It is forbidden to pour live cultures, or to blow air through them from the pipette. Cultures are collected from the surface of an agar with loops or a metal, glass or plastic spatula.

Before a vessel, pipette, equipment, syringe, etc. is used, it must be checked for intactness and functionality.

A bacteriological <sup>loop</sup> closed must be closed in a continuous ring and have an arm no longer than 6 cm.

3.1.36. It is forbidden to fix smears by heating. For fixation, use 96° ethyl alcohol, a Nikiforov mixture (equal amount of alcohol and ether), or when investigating material containing anthrax pathogen or one of unknown etiology, 96° ethyl alcohol with an addition of 3% hydrogen peroxide. The fixing time is 30 minutes.

3.1.37. It is forbidden to leave unfixed smears, objects with sown cultures or other materials containing biological material in open places or in unsealed storage areas at the end of work.

It is permitted to leave an identified vessel, without sown cultures, on tables and in storage boxes.

When it is necessary to leave the bacteriological room for a short time (5-10 minutes), the associate can leave objects with biological material on the table (in the safety box) if there is another associate in the room, or if the room's door can be locked.

3.3.38. Work involving the risk of formation of an aerosol of virulent microorganisms (with high concentrations and large volumes of pathogens, centrifuging, homogenization, grinding of tissues, destruction of pathogens, transfer with replicators, shaking and so forth) are done in safety rooms by personnel clothed in a type IV suit.

Centrifuging, shaking, vibrating of cultures on apparatus without protection (not in safety rooms) is possible, as in safety rooms, only if the containers with the material are made of unbreakable material and equipped with screw-on sealed covers. The personnel must be clothed in a type I suit.

3.1.39. Before the start of work in a safety room, turn on the exhaust ventilation, and from the room manometer, make sure there is a negative pressure, check the functionality of the equipment in the room and load the material.

All work must be done close to the back wall of the room and be visible from the outside.

During the work, it is forbidden to open the door of the safety room, and after work is over, the door can be open only when the infective material is put away in containers and routine disinfection carried out (attachment 5.4).

After the containers of biological material are removed, the door of the safety room is closed, and the bacterial lamps are turned on inside the room.

Bear in mind that the safety rooms V/O "Isotope" are made of organic glass, are explosion and fire hazards, so that the burning of bacterial loops should be done with electric heater units or alcohol burners, rather than gas burners.

3.1.40. The storage of group I and II biomaterials, accounting, exchange with other institutions and destruction are carried out in accordance with the current "Statute on Procedures for Accounting, Storing, Handling, Releasing and Transferring Cultures of Bacteria, Viruses, Rickettsia, Fungi, Rudimentary Mycoplasmas, Bacterial Toxins, and Biological Poisons."

3.1.41. Group I and II pathogenicity biological material cultures from diagnostic investigations are sent to territorial anti-plague institutes or lead institutes for institution problems. These strains are transmitted with the permission of the directors of these institutions. Documents of transmission and destruction are filled out as required.

At anti-plague stations, it is permitted to store a minimal quantity (determined by the territorial anti-plague institute) of typical and atypical cultures of the given natural focus, needed for scientific or practical work.

Laboratories which do not have authorization for work with the cholera pathogen, after identification transfer the cultures to the territorial anti-plague institution and destroy them.



upon concurrence with the latter.

At institutions (institutes) working with group I and II microorganisms, it is permitted to have collections (a museum) of strains for the performance of scientific research and a collection of typical cultures for diagnostic purposes.

3.1.42. Labels on items with cultures and plantings must be legible with a detailed designation of the sown material and an indication of the date of sowing.

Items with pathogen cultures are stored in metal, water-proof containers, with tightly closing covers, which are placed in a refrigerator or iron locker. Temporary storage of cultures prepared for autoclaving in tanks made of heat-resistant material (metal, plastic) with openings in the top part is permitted. Tanks sealed with the seal of the scientific associate (physician) in storage are placed in trays with sides 20 cm high.

All storage facilities (thermostat boxes, refrigerators, and lockers) are locked and sealed. Keys to storage facilities and personal seals are kept by the associates or the section (laboratory) supervisor.

Vaccine strains are stored separately from pathogenic ones. Antibiotic-resistant strains are stored separately from sensitive. It is not permitted to store live cultures of microorganisms and diagnostic and medicinal preparations in the same refrigerator.

3.1.43. The work of freeze-drying cultures of group I and II infectious disease pathogens is done in accordance with the current "Instruction on Freeze-Drying Group I-IV Infectious Disease Pathogens in the K. Ye. Dokonov Collector Apparatus." It is permitted to use more up-to-date Apparatus.

3.1.44. Ampules with dried cultures are opened in the museum (collection) area for live cultures in a safety room with an exhaust ventilation system. The pulled-back end of the ampule is heated over a burner flame, then a moist end of a sterile cotton tampon is applied to the heated part, with the result that cracks appear. The end of the ampule is covered with three layers of gauze wet with a disinfectant solution, then wrung out and squeezed with pincers. After the ampule is opened, it remains covered with the same gauze for one or two minutes. Then the gauze is carefully removed and along with glass residue is submerged in the disinfectant solution. The open ampule is covered with a sterile gauze tampon for 1-2 minutes, then a solution is put in the ampule to prepare the suspension, which is then applied to solid and liquid nourishment media. The cultures in the nourishment media are then sent to the laboratory.

3.1.45. Laboratories keep an account of the biological material on authorized forms and in special logs.

The logs must be numbered, sewn and secured with a seal.

3.1.46. During serological investigations, first disinfect the material:

- serums and suspensions of blood are decontaminated by an addition of sodium Merthiolate, checked for bactericidal action, to a concentration of 1:10,000 with subsequent heating at 56 °C for 30 minutes. To collect the blood and smears from internal organs, it is permitted to use filtration paper soaked with sodium Merthiolate in a concentration of 1:1000. Decontamination occurs after an hour's exposure at room temperature.

- suspensions of internal organs or the marrow of animals, material from sick persons, as well as bacterial suspensions of plague, tularemia, and brucellosis are decontaminated by an addition of formalin tested for bactericidal action, to a 1-2% concentration, after which it is kept for no less than 12 hours, or to a concentration of 4% with exposure at room temperature for 1 hour. Cultures of cholera vibrio are decontaminated by boiling for 30 minutes. The effectiveness of decontamination is checked by testing for specific sterility.

If it is necessary to conduct an urgent analysis in the presence of group I and II pathogen antigens, and there is no time to decontaminate the material or check a sample for sterility, the serological reactions are placed in test tubes with a round bottom. The laboratory vessel and instruments are decontaminated.

3.1.47. All the tasks associated with the acceptance and primary treatment of biological material, from persons, rodents, ectoparasites, environmental samples etc., as well as contamination of animals and their investigation, may be done only in areas of the block for infected animals. Contamination of laboratory animals is done by the physician jointly with the laboratory assistant. Autopsies of wild and bio-sample animals and sowing of organs for bacteriological investigation are carried out by the physician or laboratory assistant. When field material is investigated, the disinfection specialist may be asked to perform combing and dissections of wild rodents, and the physician or laboratory assistant take the material for investigation. Cleanup in premises for infected animals, their care and feeding are done by the laboratory assistants and disinfection agents. In exceptional cases, when field material is investigated, laboratory employees may be recruited by order of the institutional director for cleanup.

Visits to the infected animal block are recorded in the log, with indication of the time of the visit and the nature of the work done.

Entry of personal in the unit to work with infected animals is through a room where protective clothing is donned, and the unit is exited through a room where the protective clothing is removed and decontaminated. It is forbidden to don protective clothing and remove, it after working with biological material, in the same room.

3.1.48. Small animals and ectoparasites are kept in areas of the block for infected animals in compliance with the following rules:

- small animals are placed in jars, boxes and cages which are first checked for integrity, and to which labels are affixed. Boxes and jars are covered with screen covers which prevent escape of the animals;

- ectoparasites are placed in jars and flasks which are tightly secured with fine screen material, as well as in test tubes covered with cotton gauze or a cork plug;

jars with animals are placed in metal (wooden) shelves painted with an oil paint, or in screened lockers, and vessels with ectoparasites in similar lockers, refrigerators or thermostat.

- Cans with animals infected with pathogens of anthrax or deep mycoses are placed on metal or wooden shelves lined with iron.

- When a large amount of bedding material (1/3 of the jar) is accumulated in the jars or cages, the animals are moved to clean jars, and the used ones are cleaned with disinfectant solution or autoclaved (attachment 4).

3.1.49. Depending on the nature of the work done in the unit for infected animals and the degree of the hazard to personnel, strictly specified types of protective clothing are used:

- In the investigation of material from patients suspected of having plague, deep mycoses, glanders, melioidosis or a disease of uncertain etiology, a type I protective suit is used; for anthrax, tularemia, brucellosis, cholera, and Legionella a type II suit.

- In the investigation of wild rodents and biotest animals, as well as their carcasses (weighing, measurement, combing, collection of ectoparasites, dissections, sowing of organs, preparation of suspensions, examination of nests), a type II protective suit.

- In infection of bio-test animals with material from wild rodents and other objects suspected of having plague, deep mycoses, glanders, and melioidosis, a type I suit is used; for material suspected of being contaminated with pathogens of anthrax, tularemia, brucellosis, cholera and Legionella, a type II suit.

- When laboratory animals are infected with virulent cultures of pathogens of plague, glanders, melioidosis, anthrax, tularemia, brucellosis, and deep mycoses, and during all handling of infected animals (blood sampling, temperature measurement, feeding of ectoparasites on rodents, combing, treatment and transfer of animals, feeding etc.), and dissections, a type I suit is used.

- When laboratory animals are infected with cultures of cholera and Legionella pathogens, and during investigation of such animals and their dissection, a type II suit is used.

3.1.50. Live rodents intended for dissection are killed with chloroform, ether or other means in conformity with the requirements of biological safety and the nature of the investigation.

During the dissection of rodents the physician (laboratory assistant) holds the carcass with long pincers (tongs), submerged for 10-15 seconds in a 3% aqueous soap solution, and after drainage (on a screen), moves it to the dissection board.

The dissected animal is disinfected after the material is removed for examination. Animals which are dead of melioidosis, for complete disinfection are kept in a 5% solution of Lysol for 7 days. After animals are dissected, the instruments, dissection boards, jars, barrels, and cages for the animals etc. are disinfected (attachment 4).

To get rid of solid decontaminated waste and carcasses of animals, a crematorium is used, or separate burial places concurred with the territorial Center for State Sanitation and Epidemiological Oversight. If there is no crematorium or burial places on the grounds of the institution, a pit is dug, located as far as possible from utilities and wells, in conformity with the following requirements:

- The pith 1.5-2 meters deep must not extend to the water table.
- The pit is covered with a dense roof, without cracks, with a hatch that can be locked.
- The pit area must be raised to prevent flooding by rain or runoff.
- After each deposit of material, freshly prepared disinfectant solutions are poured in (20% lime, carbolic acid, cresol and so forth).
- When the pit is filled (no more than 1 m from the surface), it is covered with earth and tamped down. The roof can be removed and used again.

When it is necessary to dissect rodents and examine them in sparsely populated or unpopulated areas, it is permitted to bury disinfected carcasses of dissected rodents in pits that are dug especially each time.

3.1.51. In maximally isolated laboratories, when working in units for infected animals, infection, dissection and all other handling of animals are done in the safety room system, and the animals are kept in ventilated low-pressure lockers. Personal clothed in type II anti-plague suits perform the work and cleanup.

3.1.52. Biological material is disinfected in accordance with current documents and attachments 5.4 and 5.8 of these regulations.

The introduction of new systems for disinfection and insect and rat extermination is permitted only after they are tested and with the permission of the Russian State Committee for Sanitation and Epidemiological Oversight. All series of disinfectants arriving in the warehouse must be tested for effectiveness and a finding made regarding their suitability.

The methods and systems for decontamination are determined in each specific case depending on the type of pathogen and the nature and volume of the material to be decontaminated.

Notes regarding the results of investigations and counts of items with biological material are kept on separate sheets (protocols) which are disinfected by submersion in disinfectant.

The transfer of decontaminate material between laboratories of one institution and outside of its boundaries is permitted after a check of specific sterility. The transfer of such material beyond the limits of the institution is done with the permission of the institution director.

3.1.53. There must be a sufficient quantity of disinfectant solutions and an untouchable reserve of them for accidents. Containers of disinfectants are marked. When working with ectoparasites, the same requirements apply to insecticides.

The disinfect solutions are prepared by a laboratory assistant or disinfection specialist; a physician monitors quality of preparation. Freshly prepared disinfectant solutions are used.

Responsibility for correct disinfection of material is borne by the director of the structural section or a physician assigned to that duty (or scientific associate). When there is a centralized autoclave, the responsibility for disinfection is borne by the autoclave manager.

3.1.54. Work with recombinant DNA molecules is regulated by the sanitation-epidemiological regulations "Safety of Work with Recombinant DNA Molecules."

Histocytoenzymatic investigations are conducted in accordance with the requirements of the current "Instruction on Primary Processing of Material Contaminated or Suspected of Contamination by Pathogens of Plague, Cholera, Tularemia, Brucellosis or Siberian Plague during Histocytoenzymatic Investigations."

3.1.55. New methods and procedures must be examined by the institution board for monitoring compliance with the requirements of biological safety and approved by the supervisor before they may be used in the laboratory.

3.1.56. Laboratories which work with biological material must have an emergency prevention medicine chest in case of accident, along with a reserve of working and protective clothing, a hand sprayer or automatic spray, which are stored in a specially allocated place in the "possible contamination" zone.

The emergency prevention medicine chest must contain 70° ethyl alcohol, a 1% solution of Protargol, iodine, dry potassium permanganate, sterile distilled water, a set of specific-action antibiotics, optical pipettes, a syringe for preparing solutions of antibiotics, scissors, and bandages.

The laboratory medicine chest must include a 1% solution of boric acid.

Laboratories working with botulism toxin must have homological antitoxin serums.

The shelf life of the preparations and the completeness of the medicine chest are checked by the physician in charge appointed by the section supervisor.

3.1.57. Institutions working with group I and II pathogenicity biological material regularly monitor the effectiveness of filters in the exhaust ventilation system (attachment 5.7), check for the presence of residual concentrations of disinfectant in waste water (attachment 5.9) and investigate them for pathogenic microflora, and when working with virulent cultures of anthrax, check for building contamination once a month.

### **3.2. Premises and Equipment of Microbiological Laboratories**

3.2.1. Fixed laboratories where work is done with group I and II pathogenicity biological material must have the following basic set of premises:

In the "contamination" zone:

- isolation cabinet(s) separated from the "possible contamination" zone by pre-cabinets or airlocks;

- block for working with infected animals, consisting of a reception and primary processing room for biological material, a room for working with this material (infection, dissection, sowing), a room for decontamination of equipment for maintaining the biotest animals (cages, boxes, etc) and a room for holding the infected animals. The block for work with infected animals is separated from the "possible contamination" zone by cabinets for donning and removing protective clothing (the schematics for planning the block rooms are shown in figure 1).

- an autoclave room for disinfecting material;

- a thermostat room (not mandatory).

In the "possible contamination" zone:

- room(s) for specific purposes—luminescent, biochemical, serological and other for working only with disinfected material;

- preparation room(s)—laboratory assistant's room, medicinal preparations, etc.;

- room(s) for making entries in logs;

- corridors.

The areas of the "possible contamination" zone are separated from the "clean" areas by sanitation checkpoints.

In the "clean" zone:

- a room (wardrobe) for outer clothing;

- room(s) for administrative work, work with the literature, eating, rest and so forth.

3.2.2. The block for working with infected animals, the autoclave area for decontamination, the preparation and sanitation checkpoints can be used by several sections of one institution.

3.2.3. Laboratories are as a rule accommodated in a separate building with two entrances or at the back end of one. The name (number) of the laboratory must be indicated on the entry door, along with the international "Biological Hazard" sign (fig. 2). The door must have a lock. All laboratory areas must be rodent-proof.

3.2.4. General requirements for areas in the "contamination" zone.

Areas must be isolated from other zones. Lighting is established depending on the type of work according to the requirements of SNiP. The floor, walls and ceiling must be smooth, easily washed, and resistant to disinfectants. Floors must not be slippery.

Projecting and penetrating pipes (radiators) must be at a sufficient distance from walls to be disinfected; areas of insertion of engineering utilities, window and door spaces must be sealed.

Air vents must be screened off against insects. Windows of the first and second floors should be closed by metal grates; window alarms do not preclude the need for them.

It is not permitted to set up a water supply system in the "contaminated" zone areas which is not protected by against inleakage and reverse flow by technical systems.

In areas of the block for working with infected animals, high thresholds (30 cm) are installed to prevent the penetration of rodents and ectoparasites.

A plenum exhaust ventilation system is set up in the laboratories. The ventilation system on the boundary of the "contamination" and "clean" zones are equipped with fine filters which are tested for their protection effectiveness. The procedure for replacing them and testing them is given in attachment 5.7.

In all areas there are bactericidal lamps installed, figuring  $2.5 \text{ W/m}^3$ .

In a hot climate it is possible to install air conditioners, provided that they are turned off when working with biological material. Air conditioners are not permitted in rooms where infected animals are kept.

The premises must be supplied with fire extinguishing systems.

3.2.5. In the vestibules (airlocks), as well as the rooms for removal of protective clothing, water taps (sinks) are installed, and tanks of disinfectant solutions in case of accident. There must be a rug on the floor, wetted with disinfectant solution.



Emergency acoustic or visual signals will be sent from the isolation cabinets to those areas of the "possible contamination" or "clean" zones where personnel are continuously present.

In the vestibules (airlocks) as well as the rooms for donning protective clothing, there must be mirror.

Both doors of the vestibule (airlock) must close tightly and have observation windows. The door giving on the "possible contamination" zone must have a lock.

3.2.6. Autoclave rooms for disinfection and thermostat rooms, where no work directly with biological material is carried out, need not have vestibules (airlocks).

3.2.7. Areas of the "possible contamination" zone must assure safe conditions for work with personnel. Rooms are equipped and fitted in accordance with the requirements of the normative documents, depending on their purpose (biochemical, serological, medicinal etc.).

3.2.8. The sanitation checkpoint must have separate rooms for personal and work clothing with individual lockers, as well as shower rooms located between these two areas.

3.2.9. Laboratory equipment and furniture (tables, shelves for keeping animals, chairs etc.) must be strong, without sharp edges or roughness, water proof and resistant to the action of disinfectants.

The refrigerators are periodically cleaned to remove ice and to disinfect them.

All vacuum lines are supplied with systems for air disinfection (filters etc.).

Containers for storage and transfer of infective material are made of strong, anti-corrosive material. The bottom must be lined with soft, adsorbent material in a quantity sufficient to absorb all liquid material in the event that it leaks. The cover must close tightly. Containers are equipped with a convenient handle (handles).

In containers for autoclaving, along the top edge of the side walls there are openings providing free circulation of steam. The container for autoclaving is transferred on a tray. The integrity of the containers and trays is checked periodically.

The centrifuge barrels must be unbreakable, hermetically sealable, and resistant to the action of disinfectants.

Steam sterilizers (autoclaves) can be of any design, if they provide effective disinfection of objects and condensate. The operation of the autoclaves is monitored in accordance with the "Procedural Instructions on Monitoring the Operation of Vapor and Air Sterilizers," approved by the USSR Ministry of Health GEU, by chemical, physical (maximal thermometers) and bacteriological methods (attachments No. 5.5, 5.6).

The effectiveness of disinfection is monitored every month by seeding (determining the cytopathic effect) of a microorganism culture used in the laboratory and killed by autoclaving.

3.2.10. To reduce the risk of work associated with possible formation of an aerosol (centrifuging, grinding, intensive shaking, ultrasound processing, opening of objects with infected material, large volumes and high concentration of infectious agents), it is necessary to use safety cabinets.

The safety cabinets utilized include V/O "Isotope" biological cabinets, imported class III biological safety cabinets, safety cabinets of flexible, transparent film, as well as other sealed structures which provide maximal protection to personnel. The safety cabinets are equipped with fine filters at the inlet and outlet of air flow, manometers, bactericidal lamps, and lighting, and are connected to the exhaust ventilation. The exhaust ventilation must assure a vacuum of 10 mm H<sub>2</sub>O (98.1 Pa) and lower, or a rate of air movement in the open spaces of 0.4-0.75 m/s. Recirculation of air from the cabinet into its surroundings is forbidden. After the safety cabinets are installed, the effectiveness of their filter operation is checked out (attachment 5.7), the manometer readings are read, and the rate of air motion in the space is measured. The results of the check are recorded in a certificate. The vacuum in the cabinet is checked during operation from the manometer readings.

Equipment installed inside the biological safety cabinets whose operation does not lead to the accumulation of gases and explosive materials in critical concentrations. If necessary, the safety cabinets are connected to one another, creating assembly lines. The locations of engineering utilities and points of cabinet connections with one another are carefully sealed.

3.2.11. It is forbidden to drain (release) non-disinfected fluids from the areas of the "contamination" zone into the sewage network, even if there is a centralized waste water treatment system.

3.2.12. Requirements for the planning of laboratories, the internal finishing, design of areas and equipment will vary depending on the specific tasks:

A. Temporary laboratories for epidemiological detachments and expeditions, intended for conduct of diagnostic investigations, can be accommodated in adapted buildings, wards,

yurts, or on truck beds. Here it is necessary to strive for the most complete isolation of the areas from the environment, and for use of improvised materials, dust-and water-proof and resistant to the action of disinfectants, in refitting operations (polyethylene, oilcloth, plastic, wood and plywood painted with resistant paints and so forth), and in the extreme case, fabric. In one common room, separated by light partitions or plastic sheet (fabric), the following work places can be organized:

- for storage of trapped rodents and other field material;
- for primary processing of parasitological material;
- for zoological processing of rodents;
- for dissection of rodents and infection of laboratory animals;
- for keeping biotest animals;
- for removing and disinfecting protective clothing.

The area for bacteriological operations need not be equipped with a pre-cabinet (airlock). The protective clothing is donned and removed in the bacteriology room.

The sanitation checkpoint can be in one area where there are separate places equipped for the storage of personal and work clothing.

B. In fixed laboratories, conducting only diagnostic work and having a minimal group of areas, there need not be a "possible contamination" zone. Here the areas belonging to that zone can be located in the "clean" half, and the sanitation checkpoint on the boundary of the "clean" and "contamination" zones.

C. In fixed laboratories conducting diagnostic and/or experimental work, not associated with the study of wild rodents and the use of biological methods, there need not be a block for working with infected animals.

Diagnostic material for bacteriological (serological) investigations go to the room for reception, registration and sorting of samples through an individual entry or window.

D. In fixed laboratories where only experimental work with group I and II pathogens is done, the block for working with infected animals need not have a room for reception and initial processing of material.

When diagnostic material arrives with epidemiological signs, work with experimental material is halted and final disinfection is conducted. The sorting and initial processing of arriving samples, depending on their nature, are carried out in one of the bacteriological rooms or in a room in the block for working with infected animals.

E. In fixed, maximally isolated laboratories, where continue experimental work is conducted using new methods potentially associated with a higher hazard of aerosol formation, the requirements on the areas and their equipment are more strict, supplemental to paragraphs 3.2.4-3.2.11.

A laboratory must have a backup electrical generator or other power source, independent of the main one, to support operation of the main equipment, the exhaust ventilation system, the thermostat boxes, and the refrigerators. The system of general water supply of the "possible contamination" zone must be equipped with technical systems to prevent the inleakage or reverse flow of water.

The windows of the "contamination" zone areas must be carefully sealed. The ventilation system is installed figuring a negative pressure of 2-5 mm H<sub>2</sub>O (19.62-19.05 Pa) in the work areas and 10-15 mm H<sub>2</sub>O lower in class III cabinets. Air must go through a fine filter from the "possible contamination" zone and be removed through a system of such filters (no fewer than two) from the work areas. Ventilation assemblies are installed outside the "contamination" zone and are backed up in the event that one of them malfunction.

Such laboratories must have a central autoclave for disinfection.

F. Work areas and equipment of aerosol laboratories must conform to the requirements of these regulations and the current "Instruction on Work Regime with Aerosols of Pathogens of Especially Hazardous and Other Bacterial Infections."

### **3.3. Work Clothing**

3.3.1. To work with biological material, each associate must be supplied with work clothing and footwear (jumpsuits, 3 sets, socks, 3 pair, slippers, 2 pair) and well as 6 anti-plague coveralls, 2 medical smocks, 6 kerchiefs, and other types of special clothing and footgear provided for by the standards.

To go out onto the institution grounds ("possible contamination" zone), the associates are also supplied with headgear, warm coveralls (quilted jackets), and appropriate footgear.

3.3.2. To work in the "contamination" zone, in order to protect personnel from

pathogenic microorganisms, they use protective clothing (antiplague suits, insulating type KZM-1 suits and other means of individual protection authorized for use).

The following types of antiplague suits exist:

Type I – a jumpsuit or overalls; socks, slippers, a large kerchief (120x120x150) or hood; antiplague smock (like a surgical smock, length to the lower third of the shin, flaps must extend far over one another, long ties at the collar, waist and sleeves; cotton gauze mask (of 125x50 cm gauze with a layer of cotton 25x17x1.5–2 cm, weight 20 g) or an anti-dust respirator, or a filtration gas mask, tightly seated safety glasses, or disposable cellophane film (15x39 cm, with 6 cm on each side for tying with laces of 30 cm each; rubber gloves; rubber shoes (rubbers), towel.

Type II – jumpsuit or overalls, socks, slippers, large kerchief (hood), anti-plague smock, cotton-gauze mask, rubber gloves, shoes, towel.

Type III – jumpsuit or overalls, socks, slippers, large kerchief, anti-plague smock, rubber gloves, protective shoes, towel.

Type IV – jumpsuit or overalls, socks, slippers, cap (small kerchief), anti-plague or surgical smock.

3.3.3. The anti-plague suit is donned before entering the area where the work with biological material is being done, in a strictly defined sequence. The order of donning the clothing is as follows: work clothing and footwear, hood (large kerchief), anti-plague smock and boots. Laces of the smock collar, as well as the waist are tied forward on the left side, in a slip not, after which the straps on the sleeves are tied. The respiratory (mask) is donned so that the mouth and nose are covered. To do this, the top edge of the mask must be at the level of the bottom part of the orbits, and the bottom under the chin. The top straps are tied in loops at the temple, and the bottom at the parietal bone (like a sling bandage). After the respirator (mask) is donned, along the sides of the nostrils dense cotton wadding is inserted so that air does not filter by the mask.

The glasses (cellophane film) must be pressed tightly, and the glass rubbed with a pencil (to prevent fogging) or a piece of dry soap. At the points of likely air filtration, cotton wadding is inserted. Then the gloves are donned after their integrity is checked. The towel is placed at the waist of the smock on the right side.

When the bodies of humans or camels are dissected, the following are also donned, in this order: an oil-cloth or polyethylene apron, sleeves of the same material, and a second pair

of rubber gloves. The towel placed at the waist of the apron on the right side.

If it is necessary to use a phone-endoscope, it is donned before the hood or large kerchief.

3.3.4. The protective clothing intended for work at foci of infectious diseases, hospitals, isolation wards, or blocks for working with infected animals are disinfected immediately after use. During disinfection of the suit, all of its parts are completely submerged in the disinfectant solution. In those cases when the disinfection is performed by autoclave, boiling or in a disinfection chamber, the suit is placed in a steam sterilizer, tank or chamber bag respectively.

During work in bacteriological rooms, the protective clothing is disinfected at least once a week, and then send for laundering. The list of disinfecting agents and the disinfection regime are described in attachments 5.3 and 5.4.

To disinfect the individual protection means, there must be individual tanks of disinfectant solution or steam sterilizers (chamber bags): to treat protective footwear; hands in gloves when removing protective clothing; glasses, cellophane, phonendoscope; cotton-gauze masks; smocks, kerchiefs and towels; gloves; gas max boxes.

3.3.5. The suit is removed slowly, in the required order, submerging gloved hands into the disinfectant solution after removing each of the parts. Shoes or rubbers are wiped from the top down with wipes liberally wetted with disinfectant. The towel is removed. The apron is wiped with a cotton wipe wetted with disinfectant, then removed, folding the outside down. The sleeves and second pair of gloves are removed. Phonendoscope removed. Glasses removed (cellophane film), pulling them forward up and back behind the head with both hands. The cotton gauze mask is untied and removed without touching the face with its outer side. The ties of the smock collar and waist are untied. The top edge of the gloves is pushed down and the laces of the sleeves are untied, then the smock is removed and rolled up with its outer side in. The kerchief is removed, carefully collecting all the ends into one hand at the temple. Remove the gloves (if loss of integrity is suspected, check then in disinfectant, not in air). Remove the boots.

After the protective suit is removed, clean the hands with 70° ethyl alcohol, then wash thoroughly with soap.

3.3.6. Depending on the nature of the work done, and the degree of hazard to personnel, use strictly defined types of protective clothing.

### **3.4. Supplemental, for Work with Viruses and Rickettsia**

3.4.1. Work with group I pathogenicity viruses is done only in laboratories especially designed for that purpose. The requirements on the laboratory work areas are basically the same as for fixed maximally isolated laboratories (para. 3.2.12.E of these Regulations).

3.4.2. All investigations with group I viruses, from the moment of their arrival until their destruction, are carried out in a system of interconnecting safety cabinets. A mandatory requirement for fitting the work areas of the "contamination" zone is that there is a central autoclave with automatic door interlock. Entry into the "contamination" zone is through a sanitation checkpoint with shower or airlock, where the work clothing is changed for the appropriate protective clothing. During work in the airlock, an ultraviolet light source (bactericidal lamp) should be turned on.

The entry doors into the airlocks must be self-closing and equipped with locks.

During work the doors of the "contamination" zone work areas must be closed. In this time it is forbidden to leave or enter the virology cabinet. For communication with other work areas, a telephone or intercom is used.

Biological material is stored in sealed, low-temperature resistant, non-breakable containers which are placed in low-temperature lockers or vessels with liquid nitrogen.

Transfer of biological material between the technology lines and to storage is in sealed closed moisture-proof containers. Before removal from the technological lines, the containers are disinfected by submersion in a tank of disinfectant solution.

All waste from the virology laboratory, including used shower water, is disinfected; routine and final disinfection of work areas and technological lines are done in accordance with attachment 5.4.

During work, personnel use a type II antiplague suit. At the end of work, personnel take a hygienic shower.

3.4.3. Work with group II viruses is conducted in specialized laboratories. The requirements on work areas and equipment conform to the requirements imposed on stationary laboratories and indicated in section 3.2. It is advisable to use safety cabinets.

Personnel work in all the work areas of the "contamination" zone in overalls or type III antiplague suits, supplemented with a cotton gauze mask or respiratory.

3.4.4. During serological investigations, antigens and serums are used which have been checked for absence of active virus.

3.4.5. The requirements on work areas where work with group II rickettsia is conducted, conform to those indicated in section 32, requirements for fixed laboratories.

3.4.6. All work with biological material is conducted in a work area consisting of individual cabinets with pre-cabinets equipped with thermostat boxes for culturing of rickettsia in chick embryos, cell cultures, ectoparasites etc.

3.4.7. Centrifuging and vacuum drying of infective material is also carried out in separate cabinet areas.

3.4.8. Infection of chick embryos, animals, ectoparasites, and centrifuging and vacuum drying of biological material are carried out in a type I protective suit.

Ampules with dried rickettsia culture, and homogenization of rickettsia biomass are done in a table cabinet with exhaust ventilation while wearing a type II protective suit.

Other types of work with biological material in a "contamination" block are also carried out in a type II protective suit. When animals are being cared for, an oilcloth apron and sleeves are also donned.

### **3.5. Supplemental for Working with Deep Mycoses Pathogens**

3.5.1. All handling of cultures in the mycelial phase, as well as the study of fungus viability in all phases, are conducted in a sealed cabinet, with a filter and ventilation system, including filtration of the air entering the cabinet and emerging from it through fabric Petryanov fabric or other highly effective sterilizing filters. Air emerging from the cabinet, in addition, passes through a filter of glass wool soaked in a Lysol solution or a 105 solution of Lysol (in a vessel), or goes to a common filtration system, at the outlet of which there are filters of Petryanov fabric. The work area housing the cabinet is maximally sealed.

3.5.2. Fifteen minutes before the start of work in the cabinet, the bactericidal lamps and the exhaust ventilation system are turned on. During loading of the cabinet, the exhaust system is turned off, and then turned on again after loading. The work of the vacuum pump is monitored from the volume rate of air motion using a rheometer or manometer, which determine the vacuum in the cabinet; the latter must be 2-5 mm of H<sub>2</sub>O (19.62-49.05 Pa).

3.5.3. In order to prevent the entry of fungal pathogens into the work area housing the



cabinet. its pressure integrity is checked at installation and then at least once a quarter. The bacteriological investigation of the air downstream from the filter is checked once a month. Lysol in a vessel or soaking the glass wool is changed once ever 7-10 days, and the glass wool as it hardens.

During work in the cabinet, if air exhaust from it ceases, the work must be stopped immediately.

3.5.4. Plantings of mycelial cultures in the cabinets are made after preliminary introduction of the physiological solution or bouillon in the test tubes or beds. During production smears of mycelial cultures, the fluid is introduced in the beds through plugs with a syringe with a long needle. The plantings are incubated in metal containers.

3.5.5. During work with mycelial phases of fungi, in order to avoid aerogenic infection, the agar plates with plantings are kept in a thermostat box for no more than 5 days (before the start of spore production). Beds and test tubes with plantings of the fungal mycelial phase are not opened outside the cabinet.

3.5.6. Examination of plantings with fungal mycelial phases in cabinet rooms are performed while in type IV suits with a cotton gauze mask.

Work with yeast phases of fungi is done in a cabinet room n a type III suit with mask. Serological investigations are performed in a type IV suit.

3.5.7. To count cellular elements in a Goryayev chamber, the fungus suspensions are either autoclaved or (to speed up the count) 10% formalin is added to it and it is kept in a thermostat box for 2 hours at 37°C.

3.5.8. In order to produce antigens and vaccines and to conduct other work, the grown mycelium is disinfected:

- by autoclaving at 0.5 atm for 30 min;
- by adding formalin to a final concentration of 0.5% (yeast forms of pathogens of histoplasmosis, blastomycosis, and mycelial phases of all pathogens).

3.5.9. To check the fungal mass or filtrations for sterility, they are sown in a test tube with 0.5 ml of sugar, beef-extract agar and bouillon, solid and liquid Saburo medium, liquid must and mut-agar (4 test tubes of each medium). The suspensions, killed with formaline, are first diluted by a factor of 20-100 with a physiological solution or bouillon. The plantings are

kept for 10 days at 28° and 37°. Until the results of the test are obtained, the suspensions and filters are stored at 4°C.

3.5.10. Investigation of soil samples, rodent nests, bird droppings and so forth are conducted in a cabinet with filter and ventilation unit.

3.5.11. During infection of laboratory animals, the area of introduction of the material is treated with a 1% tincture of iodine (iodonium)

3.5.12. During serological investigations, material is disinfected by autoclaving or by the addition of formalin to a final concentration of 0.5-1%, after which it is kept for at least 2 days at 37°C and checked for sterility. Before addition of serological reagents, serums are heated at 56°C for 30 minutes, and then thiomersal (Merthiolate) is added to it to a concentration of 1:10,000 and they are examined no sooner than 24 hours later.

### **3.6. Supplemental, for Working in Laboratories of Industrial Departments**

3.6.1. The regime in laboratories of industrial departments when working with group I and II pathogenicity microorganisms are established in accordance with the current "Regulations of Technical Safety, Industrial Sanitation and Sanitation-Epidemiological Regime for Enterprises Producing Bacterial and Viral Preparations," and these regulations.

3.6.2. In accordance with these regulations, industrial laboratories must develop supplements to them based on the specific features of the production technology, utilized equipment and other conditions. The supplements are approved by the enterprise (institution) director.

### **3.7. Supplemental, for Working with Botulism Toxin and Biological Poisons**

3.7.1. When working with botulism toxin not purified of spores, it is necessary to allow for the specific features of biological work safety with spore cultures.

3.7.2. Work with botulism toxin purified of spores (biological poisons) is done in a cabinet with a pre-cabinet, while wearing a medical (surgical) smock, medical cap, slippers, along with a cotton mask (respirator) for work with dry toxin.

3.7.3. Infection of laboratory animals with botulism toxin (poison) is done in a separate room from the general vivarium, while wearing a medical (surgical) smock and cotton gauze mask (respirator).

3.7.4. A 6% solution of hydrogen peroxide and autoclaving ( $132^{\circ} \pm 2^{\circ}$  for 90 minutes) is used for decontamination. See attachment 5.4.

### 3.8. Zoological and Parasitological Operations

3.8.1. The workers of anti-plague and other medical and biological institutions and departments of OOI Centers of the State Sanitation and Epidemiological Oversight conducting trapping of rodents, collection of ectoparasites (carriers and transmitters of natural focal diseases) as well as other field operations with while vertebrates and invertebrates must be supplied with protective clothing in keeping with the season:

a) In warm weather, a cotton work suit (pants and shirt, protective anti-encephalitis suit, combined suit for protection against flies and ticks), leather shoes and rubber marsh boots for working in wet lands, canvas mittens, and headgear. One worker should have 2 sets of suits and three pairs of cotton gloves.

b) In cold weather, a cotton suit, quilted jacket or heated jacket with waterproof top layer, cotton breeches, leather shoes or felt boots with galoshes, canvas and warm mittens, headgear (such as a cap with ear flaps or with ear muffs).

3.8.2. When working at natural foci of plague, overalls and shoes are impregnated with persistent repellants (DETA KyuZOL etc.,  $80 \text{ g/m}^2$ , see special instruction). This is repeated every 7 days and after every laundering. The clothing must be comfortable and fitted well.

During research work in mountain foci of the marmot type, the impregnation of the overalls and shoes with persistent repellants is not mandatory because there is no migration of marmot fleas.

3.8.3. For work of rodent extermination, all workers must be supplied with protective clothing: overalls, socks or stockings, foot gear (boots) and mittens. The clothing is impregnated with repellents or persistent insecticides of the pyrethrin type.

3.8.4. In the process of work to trap rodents and collect arthropod carriers and ectoparasites, as well as to destroy them, before breaks in the work, before smoking and at the conclusion of work it is necessary to disinfect the hands and instruments with the appropriate disinfectant solutions (attachment 5.4).

3.8.5. The stopping points in the field must be selected so as not to be close to rodent burrows. If this is impossible, exterminate the rodents. The location of tents must be treated with powder insecticides.

3.8.6. Field equipment which during the work comes into contact with rodents and ectoparasites (traps, presses, taps for trapping ectoparasites, test tubes, sacks etc) should be transported and carried in a closed container. The delivery of such equipment and field material to the laboratory is by transportation means of the field team, or transport allocated by the laboratory, escorted by a person familiar with the work regime. Store such equipment, as well as the field material obtained, in special areas inaccessible to outside persons.

3.8.7. If necessary trapped animals are killed right in the trap by crushing the neck with a pincers or tongs. The carcasses for transport safety are stowed in cloth sacks and the latter go into packs, boxes, or canvas (oilcloth) sacks. The fabric sacks are tied tightly (second time through the folded edge of the sack) in order to prevent scattering of ectoparasites.

3.8.8. Live rodents are placed in metal or galvanized iron containers or boxes. Ectoparasites for parasitological and microbiological investigation are delivered in test tubes closed by cotton gauze plugs and placed in metal cases, or in thick-walled glass flasks with tight plugs, placed in cloth sacks.

3.8.9. Rodents delivered dead, after they are removed from the sacks, are combed, and those delivered alive are dusted in the containers. Delivered ectoparasites are separated from sand and other substrates.

3.8.10. Insect extermination and disinfection of cloth sacks in which animals and other material has been delivered are done after each use by boiling for 30 minutes in a soap and soda solution with subsequent thorough rinsing in clean water. Flasks and test tubes for ectoparasites are disinfected by boiling in water.

3.8.11. Disinfection of traps and other instruments is done systematically at the end of daily operations by heating in the sun (in the summer), boiling, treatment with disinfectants (with subsequent airing and lubrication with vegetable oil). The boxes and containers are also disinfected (attachment 5.4).

3.8.12. Determination of the type of ectoparasites, laboratory investigation (preparation of suspensions and sowing) are done in a bacteriological room. The ectoparasites, before determination, are immobilized by ether vapors, placed on a broad slide and examined in dry form under a microscope.

For purposes of identification, if it is necessary to examine the ectoparasites alive in a droplet of water under a cover glass, the slide is placed in a Petrie dish, in order to avoid contamination of the microscope stage with fluid leaking from the slide. After the end of the

work, the Petrie dishes and slides are submerged in disinfectant. In order to avoid splashing of fluid during pulverizing of ticks to make a suspension, it is necessary to cut up the ticks with a scissors before pulverizing, under a cover from the Petrie disk or a large funnel.

3.8.13. Skinning and preparation of collection carcasses from animals trapped in regions where an epizootic disease is possible or is occurring, are done as follows:

a) When preparing the collection carcasses of animals for training purposes, it is necessary to keep them in 10% formalin. The time they are kept in formalin is determined based on the size of the animal and the speed of penetration of the formalin in the tissue (1 cm per day). Work with animals fixed in formalin can be done in any work area; the protective suit is not regulated.

b) During preparation of carcasses for scientific purposes, when the action of formalin cannot be allowed, before removal of the skin the animal is submerged for 10-15 minutes in a 5% solution of Lysol, and the removed skin is again submerged for 3 hours in the Lysol solution, after which the fat is removed, it is washed and treated on the inside with sodium arsenide. The skull is either kept in formalin or disinfected by boiling. The removal of the skin from a rodent is done in compliance with the working regime in the areas within the block for working with infected animals or in another especially allocated area (tent, separate room, individual work space in laboratory etc.). All the work of collecting and packing collection carcasses may be done with hands in gloves, since it is not possible with instruments alone. The work of removing the skins is done in a type I protective suit. Work of packing carcasses from sinks kept in Lysol requires type II suits.

3.8.14. Collection of the droppings of raptors and excrements of animals are done after they are kept for 12-18 hours in a 1% solution of formalin in any work area; the protective suit is not regulated.

### **3.9. Procedures for Trapping, Transporting and Keeping Wild Vertebrates and Arthropods During Experimental Work**

3.9.1. In enzootic plague regions, the trapping and removal of wild animals and arthropods is authorized for antiplague institutions serving specific natural foci, and by agreement with them for departments of especially hazardous infections of State Sanitation and Epidemiological Oversight Centers. In necessary cases, the state centers, by agreement with antiplague institutions, may authorize other organizations (private individuals) to trap.

On plague non-enzootic territory, each institution of a medical or biological nature can trap and keep vertebrates and blood-sucking arthropods, in strict compliance with these

regulations.

Trapping and removal of vertebrates and arthropods from enzootic territory for hemorrhagic fevers (GLPS, Crimea, Omsk) are authorized for departments of especially hazardous infections of State Sanitation and Epidemiological Oversight Centers, antiplague institutions and medical and biological institutions conducting observations of specific natural foci.

Any material is considered potentially hazardous with respect to possible infection of natural foci illnesses inherent in the particular landscape zone where it was collected.

3.9.2. Before the start of work at trapping wild animals, the chief of the expedition must obtain a reference from the State Sanitation and Epidemiological Oversight Center regarding the absence of epizootic diseases in the last 3 years, or cases of illnesses in people from natural foci diseases in the region of the proposed trapping of animals or arthropods.

3.9.3. Responsibility for trapping of wild animals and keeping them belongs to the leader (chief) of the epidemiological team or expedition. All the staff of the detachment or expedition must be acquainted with the work safety regulations for prevention of infection by natural-foci infections circulating in the given territory.

When working in plague enzootic regions, each associate must take his temperature daily, and the results are entered in a log.

3.9.4. Live wild animals and arthropods caught in nature, before removal to scientific and other institutions, must be kept in quarantine. The quarantine vivarium may be organized on the basis of the temporary epidemiological detachment (expedition) or stationary institution. The duration of the quarantine is 1 month.

3.9.5. Work areas for the quarantine vivarium and the work with arthropods (insectarium) must be isolated from the other areas and protected from penetration of rodents and insects.

3.9.6. Responsibility for compliance with work safety regulations in the quarantine vivarium and area for working with arthropods lies with the chief of the epidemiological detachment (expedition), or the department or laboratory chief where the vivarium is organized.

3.9.7. Wild vertebrates are delivered to the quarantine vivarium in containers or wooden boxes lined on the inside with tin, which after each use is treated with disinfectant.

Arthropods are delivered in test tubes with cotton gauze plugs (wet chambers), and placed in metal cases, or in thick-walled flasks with plugs, placed in cloth sacks (ticks, fleas, lice). Mosquitoes, flies, horseflies and other two-winged blood-sucking insects are delivered alive in enclosures of very fine gauze (double walled) or anesthetized and placed in glass test tubes or penicillin flasks closed by rubber plugs, which are transported in thermal containers with dry ice or liquid nitrogen.

3.9.8. Transport of animals to the quarantine is in special transport escorted by an associate authorized to work with group I and II biological material. It is forbidden to transport field material by public transport.

Material obtained by the zoological groups of antiplague or other institutions (rodents, arthropods, environmental objects) regardless of the enzootic situation on the territory with regard to natural focal infections, are considered to be infected and is delivered with every precautionary measure. Rodents are packed in cloth sacks which are placed in oilcloth sacks, and then in special metal containers, steam sterilizers, or containers.

3.9.9. The animals delivered to the quarantine vivarium must be rid of ectoparasites and transferred to clean metal or glass containers with tight screen covers. The individual combing the animals and caring for them during quarantine must wear a type II protective suit in full compliance with the requirements of work safety.

3.9.10. For animals delivered from natural plague foci, at the quarantine vivarium blood is drawn from the paw or tail for bacteriological and serological investigations. Detection of specific antibodies in the animals testifies to plague epizooty, and detection of the pathogen or fraction I of the plague microbe indicates that the animal is ill. Such animals are killed and examined..

3.9.11. In the event that a dead animal is found in a container, it is necessary to perform bacteriological (virological) and serological investigations of the carcass.

3.9.12. When an infectious disease is discovered among the animals, the quarantine period is extended by a month, counting from the day of registration of the death of the last animal. If there are mass deaths, all the animals are killed and the vivarium is thoroughly disinfected (attachment 5.4).

3.9.13. Carcasses of animals who died or were killed are disinfected by autoclaving, boiling, burning or submersion in a disinfectant solution.

3.9.14. Health animals, after the period of quarantine, are prepared for transport or delivered too the laboratory.

3.9.15. Insects are kept in a special work area (insectarium) in enclosures or jars to keep them from escaping.

3.9.16. The vessel used when working with arthropods is disinfected by boiling. Wastes are treated with disinfectant solution or burned, and the instruments are boiled or burned in a fire.

3.9.17. In the vivarium or insectarium, the record of movement of vertebrates and arthropods is maintained in a special numbered and sewn log with indication of the place and date of capture, results of investigation and quarantine.

3.9.18. Transfer of vertebrates and arthropods from the vivarium or insectarium to other institutions is possible only with the permission of the institution director; only animals born in a clean vivarium may be transported.

3.9.19. Work areas of the vivarium and insectarium are sealed at the end of the work.

### **3.10. Procedures for Controlling Accidents During Work with Biological Material**

3.10.1. At every antiplague institution and other institution conducting scientific research work with pathogens of plague, cholera, glanders, melioidosis, and deep mycoses, with highly contagious group I viruses, there must be an isolation ward.

3.10.2. Associates who have come into contact with material are placed in the isolation ward if they show symptoms of the diseases caused by the group I and II pathogens listed in para. 3.10.1, as well as persons who caused an accident.

3.10.3. When a disease is identified, or suspected in an associate, he is placed in the isolation ward. Material is drawn for laboratory investigation, and a course of specific treatment is carried out.

If it is necessary to hospitalize the associate in an infectious disease clinic, this is done in accordance with the integrated plan of measures for localization and elimination of the foci of especially hazardous infections for the city (region).

3.10.4. Physicians who serve the isolation ward must have clinical training and be authorized for work with biological material. If necessary, other physicians, laboratory assistants, disinfection specialists and lab workers from among the associates of the institution with the necessary clearance may also be recruited to work in the isolation ward.



Experienced infectious disease specialists and other specialists, without clearance to work with group I and II pathogens, may also be recruited for consultation if they have first been briefed on matters of work safety and if they have donned the appropriate protective clothing. During the visit to the patient, they are escorted by the isolation ward physician of the institution. Medical observation of the consultants is set up (without isolation) for the incubation period; for this period they are not allowed to travel to other populated areas).

3.10.5. The isolation ward must have a store of the main and backup specific medicinal preparations, a store of medications for provision of aid per vital signs (cardiological, anti-shock, antibiotics, etc.). The isolation ward physician is responsible for seeing that the medicine chest contains modern and effective preparations.

3.10.6. When there are accidents during work with biological material, the work is immediately halted and the emergency warning system is activated.

A. If the accident involves splashing of infectious material, i.e. the possible formation of an aerosol (breakage of a test tube, flash, tube with a liquid culture, dish, test tube with culture in an agar with condensate, splashing of a bacterial suspension from a pipette or syringe, tissue fluid during the dissection of infected bodies of animals or people, an emergency with the vacuum unit during the process of drying virulent cultures, or in other types of accidents leading to contamination of the air or surrounding objects), all those in the room will immediately cease work, and while holding their breath, will exit the room where the work was performed, into the pre-cabinet, close the door, wash their hands with disinfectant solution or alcohol, and if the face was not protected, wash it carefully with 70° ethyl alcohol, then wet down their protective clothing copiously with ethyl alcohol (starting with the kerchief or helmet), remove it, submerge it in disinfectant solution or place it in the steam sterilizer (tank) for autoclaving. After this, wipe the exposed parts of the body with 70° ethyl alcohol, change into other work clothing and treat the mucous membrane of the eyes, nose and mouth with preparations from the emergency chest. The mouth and throat are rinsed with 70° ethyl alcohol, and a 1% solution of Protargol is instilled in the nose. If botulism toxin has gotten onto exposed sections of the skin, wash it with a large quantity of soap and water (the waste water is autoclaved).

Antibiotic solutions to which the pathogen is sensitive are dripped into the eyes (may also be used in the nose instead of Protargol). When working with the plague or tularemia pathogen, drop a solution of streptomycin into the eyes (25 mg/ml), gentamycin (4 mg/ml), sizomycin (5 mg/m), tobramycin (4 mg/ml), amycacin (4 mg/ml), or netilmycin (4 mg/ml). When working with the cholera pathogen, tetracycline (20 mg/ml); with anthrax, penicillin or streptomycin (20 mg/ml), with brucellosis or glanders, tetracycline or chlortetracycline (20 mg/ml); with melioidosis, chlortetracycline or levomycitine (20 mg/ml), with Q fever and

ornithosis, with oxytetracycline (15 mg/ml); with Legionella, tetracycline (5-10 mg/ml) or gentamycin (1-5 mg/ml).

If there is an accident when working with viruses, wash the eyes with a 1% solution of boric acid or a stream of water; infiltrate 1% Protargol in the nose,; gargle with 70deg\* ethyl alcohol for the nose and throat, or a 0.5% solution of potassium manganate, or 1% boric acid solution; if there are immunospecific medications (gamma globulin, reconvalescent serum), intramuscular injection per instruction.

If there is an accident when working with deep mycoses pathogens, infiltrate the eyes and nose with 1% boric acid, and gargle with 70deg\* ethyl alcohol for the mouth and throat.

When there is an accident with botulism toxin, cleanse the eyes and mouth with water and antitoxin serum diluted to 10 ME/ml; administer the serum or antitoxin depending on vaccination dates (revaccination).

If an accident occurred while working an unknown pathogen, use a combination of antibiotics from the aminoglycoside group (streptomycin, canamycin, monomycin in a concentration of 200 mg/ml) with the tetracycline group (chlortetracycline, oxytetracyclin in a concentration of 10-200 mg/ml). It is also possible to use other tested antibiotics for each infection.

B. If there was an accident without splashing of biological material (touching of the edge of a dish, test tube, flask, crystallizer, crack in the Petrie dish, test tube, or flask of biological material, dropping a solid particle on the table when incinerating the loop after sowing, touching the surface of a culture on a solid nutritive medium and so forth), without leaving the premises a cotton wipe with disinfectant is placed on the point of contamination with biological material on the surface of the object, the supervisor or his assistant is called, and disinfection treatment of the accident area is continued. After this the associate leaves the area where the accident occurred, and removes his protective clothing and submerges it in disinfectant. Exposed areas of the body are treated with disinfectant or 70 deg\* alcohol.

C. If the accident occurred in a functional safety cabinet, work is halted, the spirit lamp extinguished, equipment turned off (centrifuges, rockers\*, and so forth, without opening them), wipes copiously wetted with disinfectant are placed over the accident site. The bactericidal lamps are turned on for 30 minutes in the safety cabinet and in the area. The warning signals are turned on. The cabinet is disinfected. Two hours after completion of the disinfection, work may be continued in the safety cabinet. The exhaust ventilation must be left on during the accident and disinfection.

D. If the accident was associated with a wound or other damage to skin integrity, the work is halted, the hands treated with disinfectant, and gloves removed. Blood is pressed from the wound into a disinfectant solution. A compress is with disinfectant or 70 deg\* alcohol is placed onto the wound site for 4-5 minutes. When working with anthrax, the wound site is thoroughly washed with soap and water and wiped with iodine, without using disinfectants.

When there is an accident with the pathogens of deep mycoses, the wound location is treated with the appropriate disinfectant (attachment 5.4), washed with soap and water, and treated with iodine.

When working with group I-II viruses, the blood is pressed into a dry sterile cloth and the wound is treated with iodine, without the use of disinfectant.

If the accident was with botulism toxin, the site of the wound is washed with water and diluted antitoxin serum (10 ME/ml).

E. If the accident occurred during material transport (to the autoclave and between sections), the personnel hold their breath and, leaving the portable containers where they are, they leave the hazard zone, closing the approaches to it. The escort reports the incident to the section chief. Those involved in the accident undergo sanitary and prophylactic treatment in the scope indicated above (section A). Associates entering the accident site undergo disinfection.

3.10.7. At the "accident" signal, any associate who receives it immediately informs the section leader or his assistant. The latter reports to the chairman (member) of the board and the institute director. The supervisor and board members, upon arrival at the accident site, evaluate the situation, determine the scope of measures required to control the event, and organize and supervise the actions of the associates.

3.10.8. Depending on the nature of the accident, a different scope of disinfection measures may be carried out, from treatment of the accident site to complete treatment of the area.

Complete treatment of the area is carried out in anti-plague suite type I, or anti-plague suite type I with filtration gas mask; or in KZM-1 isolation suite with filtration gas mask. Personnel enter the area, spraying disinfectant before them from the hydraulic unit, irrigating the air, ceiling, walls, all objects and the floor. At the end of disinfectant spraying, the fixed or portable bactericidal lamps are turned on for 30-40 minutes in the area. Protective suits are disinfected. Two hours after disinfection, the area is cleaned and work may be continued in it.

An alternative option is to use the gas disinfection method with vapors of formaldehyde. Here personnel must be clothed in a KZM-1 suit or in a type I protective suit with filtration gas mask. After the appropriate exposure, the gas is neutralized and the area is wet-mopped.

3.10.9. The laboratory supervisor reports the accident and measures taken to the institute director, with a detailed account of the nature of the accident, the properties of the pathogen, and information about vaccination of victims. The institute director, upon recommendation from the board, decides whether medical observation, quarantine with the right to go to work, quarantine without the right to go to work, quarantine with preventive treatment, or preventive treatment without quarantine is indicated.

3.10.10. Associates must report All accidents or errors committed by themselves or others during work with biological material to the section chief or board chairman. For concealing accidents, the culprits will be relieved from working with group I and II biological material.

The section chief may temporarily (before a decision by the institute director) relieve from work with biological material those persons who violate these rules and work inattentively or negligently.

Persons who systematically violate these rules may be relieved from working with biological material by order of the institute director.

3.10.11. When working with group I and II biological material it is necessary to report all cases of accidents which require preventive treatment of a victim to the Russian State Committee for Sanitation and Epidemiological Oversight.

3.10.12. Information about all cases of laboratory contamination with group I and II pathogens must be immediate sent to the Russian State Committee for Sanitation and Epidemiological Oversight, the local epidemiological center, and the health organs.

### **3.11. Work in Hospitals, Isolation Wards, and Observation Wards**

3.11.1. Patients or persons suspected of having infectious diseases caused by group I and II microorganisms (plague, cholera, the pulmonary form of anthrax, group I viruses), as well as persons who have come into contact with them and left the quarantine zone, are placed in special treatment institutions.

3.11.2. Patients suspected of having plague, cholera or highly contagious fevers caused

by group I viruses are hospitalized in a temporary\* hospital or division located in a separate building or isolation ward of a specialized hospital with separate entries for patients and servicing personnel.

3.11.3. A hospital for patients with plague, cholera, or highly contagious fevers caused by group I viruses may be organized on the basis of an infectious-disease or multiprofile hospital. It is permissible to organize a temporary hospital in isolated premises such as school buildings, communal dwellings etc. as well as in wards with mandatory assignment of separate service personnel and compliance with these rules.

3.11.4. Persons with other infections may be hospitalized in the infectious disease ward of any hospital. Patients suffering from anthrax, glanders, melioidosis, Crimean hemorrhagic fever (KGL), deep mycoses, ornithosis or Q fever with involvement of the lungs are placed in isolation wards or rooms.

3.11.5. The infectious disease section of a hospital must have:

- a) a reception and triage section with individual entry for patients and a wardrobe for storage of patient's clothing before it is sent to the disinfection chamber;
- b) a section for patients, which must include wards (rooms) for separate accommodation of patients based on time of onset, clinical forms and degree of severity of the disease;
- c) a food service area;
- d) a room for disinfection of infectious material (patient secretions, vessels, bed-linens, etc.);
- e) baths and toilets;
- f) a treatment room;
- g) patient sign-out area with medical checkpoint;
- h) medical checkpoint for personnel (rooms for donning and removing protective clothing, shower);
- i) in a cholera hospital, a registration room;

j) in a plague hospital, an x-ray room equipped with portable apparatus.

3.1.1.6. In the reception and triage section, they examine arriving patients, provide emergency assistance, take material for bacteriological (virological) examination, perform sanitary treatment, change the patient's clothes, prepare the patient's clothing to be sent to the disinfection chamber, begin specific treatment and write up the initial documents for the arriving patient. The reception room is equipped in accordance with its purpose and the need to conduct routine and final disinfection.

Clothing is stored in the wardrobe in individual bags, folded in chests or polyethylene sacks, the inside surface of which has been treated with insecticide solution.

3.1.1.7. The hospital division must include wards for patients with mixed infections, for pregnant women and newborns, as well as all equipment and instruments to provide emergency surgical obstetric and gynecological assistance. The patient wards must offer all conditions for their treatment, an individual supply of personal items, and items for routine and final disinfection.

Patients must not use the common toilets. Baths and toilets must be constant locked and the key must be kept by the person responsible for biological safety. The toilets are opened for drainage of decontaminated solutions, and the baths for sanitary treatment of persons being released.

Patient food is delivered in a kitchen vessel to the service entrance of the non-infectious block and there it is transferred from the kitchen vessel to a vessel of the hospital canteen. In the canteen the food is transferred to vessels of the sections and sent to the section distribution point, where the food is distributed by portions and delivered to the wards. The vessel in which the food entered the section is decontaminated by boiling, after which the chest of dishes is transferred to the canteen, where it is washed and stored for next distribution. The distribution point must be equipped with everything needed to decontaminate food remnants. Individual vessels are decontaminated by boiling.

3.1.1.8. In the non-infectious half, there are areas for service personnel:

- a) a wardrobe for outer wear;
- b) a medical checkpoint (preferably separate ones for males and females;
- c) toilets;

- d) canteen;
- e) linen storeroom;
- f) rooms for duty personnel (for filling out patient histories and other documentation, and for breaks);
- g) other support facilities (pharmacy, etc.).

3.11.9. The hospital grounds are equipped with a drainage system and pit for disinfecting the vehicles used to transport patients.

3.11.10. Bed lines of persons released from the convalescent hospital are turned in to the disinfection chamber; the bed and bedside table are decontaminated (attachment 5.4).

3.11.11. Before the patient is released, he undergoes sanitary treatment. The terms of patient release from the hospital are indicated in the relevant instructions for each infection.

3.11.12. A barracks situation is mandatory for personnel who serve individuals with the pulmonary form of plague and highly contagious forms fevers caused by group I) viruses, or who are suspected of having these diseases or have come in contact with them. All personnel who serve the above contingent must be under systematic medical observation.

3.11.13. All persons participating in evacuation of individuals suspected of having plague, highly contagious fevers caused by group I) viruses, Crimean hemorrhagic fever and the pulmonary form of glanders will don type I) protective suits; when evacuating cholera patients, type IV suits.

When evacuating patients suspected of having diseases caused by the other group II microorganisms, personnel will use the protective suit provided for the evacuation of infected patients.

3.11.14. Patient transport to a hospital is carried out by a team of evacuation specialists with specially allocated vehicles. The team will include a physician or mid-level medical worker familiar with safety procedures, two medics and a driver. The driver of the evacuation team, if there is an isolated cab available, must wear overalls, or if there is no isolated cab, the same suit as the other team members.

3.11.15. After delivery of the patient to the hospital, the vehicle and objects used during transport are decontaminated in a specially equipped area by the evacuation team

members. At the end of each trip, the personnel who have accompanied the patient must disinfect their footwear and hands (in gloves), and polyethylene (oilcloth) aprons, which are also worn in mass transports. All team members must undergo sanitary treatment their shift.

When transporting patients with the pulmonary form of plague or glanders, and highly contagious fevers cause by group I) viruses, Crimean hemorrhagic fever or suspected of having these diseases, the evacuation team will change their protective clothing after each patient.

3.11.16. All work in the hospital involving care and treatment of patients will be carried out while wearing protective clothing:

a) In the hospital with patents suffering from the pulmonary or septic forms of plague, highly contagious fevers caused by group I) viruses, Crimean hemorrhagic fever and the pulmonary form of glanders will wear a type I) suit. The period of time spent in a type I) suit must not exceed three hours. In hot weather the duration of continuous work is reduced to two hours.

b) In a hospital with patients suffering from bubonic or the cutaneous forms of plague, who are receiving specific treatment, a type III protective suit is used.

c) Before establishment of a diagnosis for patients with bubonic and cutaneous forms of plague, and until the first negative result is obtained from bacteriological examination, all personnel of this section must wear a type I) protective suit.

d) In a hospital with patients suffering from anthrax or cutaneous and nasal forms of glanders, a type III suit will be worn; for patients with ornithosis, and Q fever with pulmonary inflammation, a type II suit.

e) In a hospital with cholera patients, all personnel will work in a type IV suit, and when helping a patient go to the toilet, or when drawing rectal material, will also wear rubber gloves. Junior personnel will also don an oil-cloth (polyethylene) apron, rubber shoes, and when handling patient secretions, a mask.

At the end of work, the protective suit, except for the jumpsuit, must be decontaminated.

3.11.17. In a hospital with patients suffering from tularemia, brucellosis, melioidosis, or other diseases classed in group II, an anti-epidemiological regime is set up as called for by the relevant infection. Personnel of a cholera hospital work in a regime established for



departments with acute gastro-intestinal illnesses.

3.11.18. Patients who are suspected of having plague, cholera, highly contagious fevers caused by group I) viruses and Crimean hemorrhagic fever, and who are subject to temporary hospitalization will be housed individually or in small groups based on time of onset, and preferably on clinical forms and severity of the disease.

3.11.19. The setup, procedure and working regime of the temporary hospital are the same as for an infectious disease hospital.

3.11.20. When the diagnosis is confirmed in the temporary hospital, the patients are transferred to the appropriate departments of an infectious disease hospital.

In the ward of a temporary department, after transfer of a patient, it is disinfected in accordance with the nature of the infection. The other patients (or persons who came in contact) undergo sanitary treatment, receive fresh linens and if possible are transferred to a different ward and begin prophylactic treatment.

When the suspected diagnosis is ruled out, the personnel work in suits in accordance with the diagnosed illness.

3.11.21. The time of release of a patient from a temporary hospital is specified in each case, but must be no sooner than the incubation period of the suspected disease, counting after the appearance of the first case in the hospital.

3.11.22. The setup and regime of an isolation ward is the same as in an infectious-disease hospital.

3.11.23. In an isolation ward which has persons who have come in contact with patients suffering from the pulmonary form of plague, highly contagious fevers caused by group I) viruses and Crimean hemorrhagic fever, all service personnel must work in a type I) protective suit.

In an isolation ward for persons who have come in contact with patients suffering from bubonic, septic or cutaneous forms of plague bacteriologically confirmed, and who are receiving specific prophylactic treatment, as well as cholera patients, the service personnel will work in a type IV protective suit.

3.11.24. In a hospital and isolation ward, especially in patients and isolated individuals, there must be no extraneous objects. The entire hospital and isolation ward situation must

consist of those objects which can be easily disinfected.

3.11.25. The secretions of patients and isolated individuals (sputum, urine, excrement etc.) will undergo mandatory decontamination. The decontamination procedures are in accordance with the nature of the infection (attachment 5.4).

3.11.26. Thorough routine infection will be carried out daily in the hospital and isolation ward, and after they become free, final decontamination.

3.11.27. The biological safety regime form an infectious disease temporary hospital and isolation ward will be under systematic monitoring of an anti-plague institution; where there are no anti-plague institutions they will be monitored by the departments of especially dangerous infections of State Sanitary Epidemiological Oversight centers.

3.11.28. Persons in a quarantine zone for plague can leave the zone after six days of observation with daily thermometry. A document will be issued about the observation in the authorized form.

3.11.29. Persons who are in a quarantine zone for cholera may leave the zone after 5 days of observation. During the observation there will be a one-time examination as a vibrio carrier. Documents regarding the observation will be issued in authorized form.

3.11.30. Entry into a plague quarantine zone is permitted only with the approval of the organ possessed of that right.

3.11.31. Observation wards are set up in equipped premises (administrative buildings, schools, preventive clinics, hotels, children and sports camps, passenger vessels and so forth).

3.11.32. The observation ward premises must have an admissions office, wards for the persons under observation, rooms for medical and service personnel, rooms for drawing materials, storage of personal items of the observed individuals, a canteen, sanitary checkpoint, and support facilities.

3.11.33. Persons visiting the observation ward will undergo medical examination to find persons with a temperature or gastro-intestinal distress. Only healthy individuals will be allowed into the observation ward.

3.11.34. Observation departments and wards are filled one at a time. The observees are accommodated by time of arrival, if possible in small groups, with steps taken to preclude interaction with persons from other areas.

3.11.35. When a patient with a temperature or with acute intestinal disease is found in the observation ward, he is transferred to the temporary hospital. Persons who have come in contact with the patient are isolated on site until the results of bacteriological examination come in.

If there is inadequate separation of observees, the period of observation is renewed from the moment of removal of the last patient and the conduct of final disinfection. If negative results are obtained from the laboratory examination, the observation period is unchanged.

3.11.36. After the observation department is vacated, final disinfection is carried out, after which it may be filled again.

3.11.37. Medical and service personnel of the observation ward are housed in a barracks and must be vaccinated. It is permissible to mobilize medical workers and other service personnel from among the observees to work in the observation ward.

3.11.38. Hospitals are guarded around the clock by military or police detachments.

### **3.12. Medical Observation of the Populace. Disinfection and Pathological/Anatomical Work at the Foci of Especially Dangerous Infections**

3.12.1. When there is an outbreak of diseases caused by group I) and II microorganisms among the populace, house-to-house visits are carried out at the disease focus: at plague foci, under the supervision of a physician from the anti-plague institute, and in foci of cholera and other infections, by an epidemiologist from the State Center for Sanitary and Epidemiological Oversight.

3.12.2. Medical personnel who conduct the house-to-house visits will wear the following protective clothing:

a) at a focus where an individual suffering from the pulmonary form of plague, or from highly contagious fevers caused by group I) viruses, a type I) protective suit.

b) In territory where there is a patient with diagnosed cutaneous, bubonic or septic forms of plague or in territory threatened by plague, as well as in foci of Crimean hemorrhagic fever, a type IV suit, with boots rather than shoes. The investigators are obliged to have rubber gloves, a gauze mask, and protective glasses, which are donned before entering a building which according to preliminary questioning of neighbors might house a suspect patient.

c) At foci of cholera, anthrax, tularemia, brucellosis, and other diseases caused by group II microorganisms, as well as during routine epidemiological observation of a healthy populace in territories potentially endangered by the above infections, a medical smock or kerchief (cap).

Depending on the epidemiological situation, the focus chief can change the authorized type of protective clothing for medical personnel during conduct of house-to-house visits.

3.12.3. Persons engaged in routine or final disinfection and disinfection at foci of the pulmonary forms of plague and glanders, and highly contagious fevers caused by group I) viruses and Crimean hemorrhagic fever, must wear type I) protective suits; in foci of the bubonic form of plague, melioidosis, and other forms of glanders, anthrax, cholera, and Q fever, in type II suits; in foci of diseases caused by group II microorganisms (not named above), in type III suits.

3.12.4. All the bodies of persons who have died of diseases caused by group I and II microorganisms (except for group I viruses) must undergo pathological, bacteriological (virological), and serological examinations. The autopsy must be performed by a pathologist or coroner in the presence of an expert in these diseases.

The autopsy will take place in a special area located if possible on hospital grounds. The dissection area must be sufficiently lighted, protected from flies and other insects, and rodent-proof, with tight floors without cracks and supplied with an adequate quantity of disinfectants and tanks for solutions.

It is categorically forbidden to drain fluid in the process of autopsies into the sewage system. The fluid must be decontaminated.

3.12.5. If there is no autopsy facility, it can be performed at the edge of a grave prepared for burial of the corpse. Personnel participating in the dissection will stand on the upwind side of the corpse.

3.12.6. At the end of the dissection, the corpse will be lowered into the grave and the top layer of soil from the dissection site will be thrown in on top. The burial will be conducted in accordance with local customs.

3.12.7. The instruments and vehicle in which the corpse was transported, the protective suits of personnel and all objects which came in contact with the corpse must undergo thorough disinfection (attachment 5.4).

3.12.8. The corpse of someone who has died of plague, hemorrhagic fevers caused by group I viruses, anthrax, or melioidosis can be transported to the burial site by any type of transportation, in a metal or tightly closed wooden coffin lined with oilcloth on the inside. In order to avoid leakage of fluid from the body, the seams in the oilcloth must run from top to bottom and be on the sides of the coffin. A corpse placed in the coffin is covered over with lime and the coffin is tightly sealed.

3.12.9. As a rare exception, may be buried without a coffin, wrapped in a shroud that was been wetted with a disinfectant solution.

3.12.10. Persons participating in the autopsy of a human being or camel dead from plague, as well persons who have died of Crimean hemorrhagic fever, must wear a type I) protective suit.

Bodies of persons who have died of cholera, anthrax, melioidosis, or glanders will be dissected and buried by persons wearing a type II suit.

During the autopsy, an oilcloth or polyethylene suit will also be worn, as well as sleeves and a second pair of gloves.

3.12.11. The corpses of persons who have died of other diseases classed as group II will be buried in the normal way.

### **3.13. Procedures for Exiting from Institutions Working with Biological Material**

3.13.1. Associates who work directly with plague enzootic territory (physicians, zoologists, entomologists, laboratory personnel, disinfection specialists, pharmacists, sanitation workers, and seasonal workers) can travel to other populated areas only after a six-day observation period with daily thermometry.

When they work with the cholera pathogen, the observation period is 5 days.

When they work in a facility which handles both plague and cholera pathogens, the observation period is 6 days.

When working with highly contagious group I) viruses, 21 days.

During the observation, the associates are under medical monitoring.

3.13.2. During the entire observation period, they are not allowed to visit the rooms

where work with biological material is being conducted.

3.13.3. During pre-exit observation, if an associate has contact with a person who works with biological material, and who has an elevated temperature at the moment of contact, or shows symptoms of acute gastro-intestinal illnesses, the departure of the associate under observation is put off until the suspected diagnosis of the ill person is disproved. In this case the observation period of the associate is not extended, and is considered over at 5 or 6 days from the start.

3.13.4. In the event that the subject proves to be sick, his departure is put off until he recovers.

3.13.5. For persons who do not work with group I) or II biological material (associates of the bookkeeping department, administrative apparatus, etc.), they may depart without observance of the above restrictions, as long as they have not had contact with laboratory workers with an elevated fever or symptoms of acute gastrointestinal illness of uncertain etiology, and provided that they have not visited facilities where work was being done with these materials.

3.13.6. Documents recording the course of the observation (before vacation, business trips, discharge and so forth) and the permission to exit is drawn up as follows:

a) Observation periods are established by order for the institution, with notification of the subject;

b) Permission to exit is drawn up as a certificate in the following form, and handed over to the individual:

### CERTIFICATE

(Name) \_\_\_\_\_ holding the post of \_\_\_\_\_  
in accordance with para. \_\_\_\_\_ of the Regulations "Safety in Working with  
Group I) and II Pathogenicity Biological Material," approved \_\_\_\_\_ 199\_, is  
allowed to depart for \_\_\_\_\_ on \_\_\_\_\_ 199\_.

Stamp

Signature of institute director.

3.13.7. In those populated areas where there are anti-plague institutions, an arriving associate will turn over his certificate to the director of that institution, and will receive

another, analogous one when he departs. Upon arrival of an associate sent to Moscow, he must report within one day to the Antiplague Center of the Russian State Committee for Sanitation and Epidemiological Oversight, and turn over his certification of his right to travel. In all other cases, he will keep the certificate with him until his return to the institution.

3.13.8. Release of associates who work with pathogens of plague, cholera, and group I) viruses without observation is possible with permission of the Russian State Committee for Sanitation and Epidemiological Oversight, when at least two go together.

All members of the group, during they time they are en route and upon arrival at their work destination, are obliged to continue their observation. If any member of the group shows an elevated temperature or symptoms of acute intestinal disease, the sock individual must immediately be isolated in the nearest antiplague or general medical institution with urgent notification of his work place.

3.13.9. Travels by associates of antiplague institution in a zone served by that or some other antiplague institution (except for Moscow and St. Petersburg) may be conducted without preliminary observation, if the travel time between the populated areas where the antiplague institutions are located does not exceed 24 hours, including wait times at transfer points.

When associates of anti-plague institutions go on leave, they undergo observation in accordance with paragraphs 3.13.1-3.13.5 of these regulations. The procedures for travel indicated in paragraphs 3.13.1-3.13.6 also apply to persons on business in an anti-plague institutions.

3.13.10. Associates working only with non-contagious group II pathogens may travel without undergoing observation.

#### **4. Organization of Monitoring**

4.1. Sanitation and epidemiological oversight of execution of the requirements of these regulations must be carried out by the institutions of the Russian State Committee for Sanitation and Epidemiological Oversight:

- The Anti-Plague Center - in institutions which work with group I) biological material on the territory of Russia;

- Anti-plague institutions (Anti-plague center, anti-plague stations, scientific research anti-plague institutes) - at Russian State Committee for Sanitation and Epidemiological Oversight centers working with group II biological material, on assigned administrative

territory;

- Russian State Committee for Sanitation and Epidemiological Oversight centers - in institutions working with group II material on served territory.

4.2. Each institution working with group I) and II biological material must have a board to monitor compliance with the requirements of biological safety. Organization of their work is specified by the "Statute . . ." (Attachment 5.2).

4.3. The procedural leadership on matters of sanitation and epidemiological oversight of compliance with the biological safety regulations when working with group I) and II biomaterial and analysis of the operations in this field is provided by the Anti-Plague Center of Russian State Committee for Sanitation and Epidemiological Oversight.

4.4. Sanitation and epidemiological oversight of compliance with general sanitation norms and regulations in divisions working with group I) and II biomaterial must be provided by the territory Russian State Committee for Sanitation and Epidemiological Oversight Center.



### Classification of Microorganisms Pathogenic to Man

#### Bacteria

##### Group I

1. *Yersinia pestis* - plague

##### Group II

1. *Bacillus anthracis* - anthrax
2. *Brucella abortus* - brucellosis  
*Brucella melitensis*  
*Brucella suis*
3. *Francisella tularensis* - tularemia
4. *Legionella pneumophila* - legionellosis
5. *Pseudomonas mallei* - glanders
6. *Pseudomonas pseudomallei* - melioidosis
7. *Vibrio cholerae* 01 toxigenic - cholera
8. *Vibrio cholerae* non 01 toxigenic - cholera

##### Group III

1. *Bordetella pertussis* - whooping cough
2. *Borrelia recurrentis* - relapsing typhus
3. *Campylobacter fetus* - abscesses, septicemia
4. *Campylobacter jejuni* - enteritis, cholecystitis, septicemia
5. *Clostridium botulinum* - botulism
6. *Clostridium tetani* - tetanus
7. *Corynebacterium diphtheriae* - diphtheria
8. *Erysipelothrix rhusiopathiae* - erysipeloid

- |     |   |   |
|-----|---|---|
| 9.  | <i>Helicobacter pylori</i>  | - gastritis, gastric and duodenal ulcers      |
| 10. | <i>Leptospira interrogans</i>   | - leptospirosis                               |
| 11. | <i>Listeria monocytogenes</i>   | - listeriosis                                 |
| 12. | <i>Mycobacterium leprae</i>   | - leprosy                                     |
| 13. | <i>Mycobacterium tuberculosis</i><br><i>Mycobacterium bovis</i><br><i>Mycobacterium avium</i> | - tuberculosis                                |
| 14. | <i>Neisseria gonorrhoeae</i>  | - gonorrhea                                   |
| 15. | <i>Neisseria meningitidis</i>   | - meningitis                                  |
| 16. | <i>Nocardia asteroides</i>  | - nocardiosis                                 |
| 17. | <i>Proactinomyces israelii</i>  | - actinomycosis                               |
| 18. | <i>Salmonella paratyphi A</i>   | - paratyphus A                                |
| 19. | <i>Salmonella paratyphi B</i>   | - paratyphus B                                |
| 20. | <i>Salmonella typhi</i>   | - typhoid fever                               |
| 21. | <i>Shigella spp.</i>  | - dysentery                                   |
| 22. | <i>Treponema pallidum</i>   | - syphilis                                    |
| 23. | <i>Yersinia pseudotuberculosis</i>  | - pseudotuberculosis                          |
| 24. | <i>Vibrio cholerae</i> 01 non-toxigenic   | - diarrhea                                    |
| 25. | <i>Vibrio cholerae</i> non 01 toxigenic   | - diarrhea, wound infections, septicemia etc. |

#### Group IV

- |    |                             |                                   |
|----|-----------------------------|-----------------------------------|
| 1. | <i>Aerobacter aerogenes</i> | - enteritis                       |
| 2. | <i>Bacillus cereus</i>      | - food poisoning                  |
| 3. | <i>Bacteroides spp</i>      | - pulmonary abscesses, bacteremia |
| 4. | <i>Borrelia spp.</i>        | - tick-borne spirochetosis        |

- |     |   |  |
|-----|---|--|
| 5.  | <b>Bordetella bronchiseptica</b><br><b>Bordetella parapertussis</b>   | - bronchosepticosis<br>- para-whooping cough           |
| 6.  | <b>Campylobacter spp</b>  | - gastroenteritis, gingivitis, periodontitis           |
| 7.  | <b>Citrobacter spp</b>  | - local inflammatory processes, food poisoning         |
| 8.  | <b>Clostridium perfringens</b><br><b>Clostridium novyi</b><br><b>Clostridium septicum</b><br><b>Clostridium histolyticum</b><br><b>Clostridium bifermentans</b> | - gas gangrene   |
| 9.  | <b>Escherichia coli</b>   | - enteritis  |
| 10. | <b>Eubacterium endocarditidis</b>   | - septic endocarditis                                  |
| 11. | <b>Eubacterium lentum</b><br><b>Eubacterium ventricosum</b>   | - secondary septicemia                                 |
| 12. | <b>Flavobacterium meningosepticum</b>   | - meningitis, septicemia                               |
| 13. | <b>Haemophilus influenza</b>  | - meningitis, pneumonia, laryngitis                    |
| 14. | <b>Hafnia alvei</b>   | - cholecystitis, cystitis                              |
| 15. | <b>Klebsiella ozaenae</b>   | - ozena  |
| 16. | <b>Klebsiella pneumoniae</b>  | - pneumonia  |
| 17. | <b>Klebsiella rhinoscleromatis</b>  | - rhinoscleroma  |
| 18. | <b>Photochromogens</b><br><b>Scotochromogens</b><br><b>Nonphotochromogens</b><br><b>Rapid growers</b>   | - microbacteriosis                                     |
| 19. | <b>Mycoplasma hominis 1</b><br><b>Mycoplasma hominis 2</b><br><b>Mycoplasma pneumonia</b>   | Local inflammatory processes, pneumonia                |
| 20. | <b>Propionibacterium avidum</b>   | - sepsis, abscesses                                    |
| 21. | <b>Proteus spp.</b>   | - food poisoning, sepsis, local inflammatory processes |

- |     |   |   |
|-----|---|---|
| 22. | <i>Pseudomonas aeruginosa</i>   | - local inflammatory processes, sepsis                        |
| 23. | <i>Salmonella</i> spp   | - salmonellosis   |
| 24. | <i>Serratia marcescens</i>  | - local inflammatory processes, sepsis                        |
| 25. | <i>Staphylococcus</i> spp.  | - food poisoning, septicemia, pneumonia                       |
| 26. | <i>Streptococcus</i> spp.   | pneumonia, tonsillitis, polyarthritits, septicemia            |
| 27. | <i>Vibrio</i> spp.<br><i>Vibrio parahaemolyticus</i><br><i>Vibrio mimicus</i><br><i>Vibrio fluvialis</i><br><i>Vibrio vulnificus</i><br><i>Vibrio alginolyticus</i> | - diarrhea, food poisoning, wound infections, septicemia etc. |
| 28. | <i>Yersinia enterocolitica</i>  | - enteritis, colitis  |
| 29. | <i>Actinomyces albus</i>  | - actinomycosis   |

**Rickettsia****Group II**

- |    |                          |                                       |
|----|--------------------------|---------------------------------------|
| 1. | <i>R. provazekii</i>     | - epidemic typhus and Brill's disease |
| 2. | <i>R. typhi</i>          | - murine typhus                       |
| 3. | <i>R. rickettsii</i>     | - Rocky Mountain spotted fever        |
| 4. | <i>R. tsutsugamushi</i>  | - Tsutsugamushi fever                 |
| 5. | <i>Coxiella burnetii</i> | - coxiellosis (Q fever)               |

**Group III**

- |    |                     |                                      |
|----|---------------------|--------------------------------------|
| 1. | <i>R. sibirica</i>  | - tick typhus of Northern Asia       |
| 2. | <i>R. conorii</i>   | - Mediterranean spotted fever        |
| 3. | <i>R. sharoni</i>   | - Israeli fever                      |
| 4. | <i>R. sp.now?</i>   | - Astrakhan fever                    |
| 5. | <i>R. acari</i>     | - Venezuelan rickettsiosis           |
| 6. | <i>R. australis</i> | - tick typhus of Northern Queensland |

- |    |                                 |  |
|----|---------------------------------|--|
| 7. | <i>R. japonica</i>              | - Japanese spotted fever               |
| 8. | <i>R.sp.now?</i>                | - African fever                        |
| 9. | <i>R.sp.now?</i> , strain "TTT" | - tick-borne rickettsiosis of Thailand |

**Ehrlichiae (subfamily Ehrlichiae, family Rickettsiaceae)**

**Group III**

- |                            |                    |
|----------------------------|--------------------|
| <i>Ehrlichiae sennetsu</i> | - sennetsu disease |
| <i>E. canis</i>            | - no name          |
| <i>E. chaffeensis</i>      | - no name          |

**Fungi**

**Group II**

- |    |  |                  |
|----|--|------------------|
| 1. | <i>Blastomyces brasiliensis</i><br><i>Blastomyces dermatidis</i> | - blastomycosis  |
| 2. | <i>Coccidioides immitis</i>                                      | - coccidioidosis |
| 3. | <i>Histoplasma capsulatum</i>                                    | - histoplasmosis |

**Group III**

- |    |   |                  |
|----|---|------------------|
| 1. | <i>Aspergillus flavus</i><br><i>Aspergillus fumigatus</i> | - aspergillosis  |
| 2. | <i>Candida albicans</i>                                   | - candidosis     |
| 3. | <i>Cryptococcus neoformans</i>                            | - cryptococcosis |

**Group IV**

- |    |   |                 |
|----|---|-----------------|
| 1. | <i>Absidia corymbifer</i>                               | - mycorosis     |
| 2. | <i>Aspergillus niger</i><br><i>Aspergillus nidulans</i> | - aspergillosis |

- |     |                                   |                        |
|-----|-----------------------------------|------------------------|
| 3.  | <i>Candida brumptii</i>           | - candidosis           |
|     | <i>Candida crusei</i>             |                        |
|     | <i>Candida intermedia</i>         |                        |
|     | <i>Candida pseudotropicalis</i>   |                        |
|     | <i>Candida tropicalis</i>         |                        |
|     | <i>Candida guilliermondii</i>     |                        |
| 4.  | <i>Cephalosporium acremonium</i>  | - cephalosporiosis     |
|     | <i>Cephalosporium cinnabarium</i> |                        |
| 5.  | <i>Epidermophyton floccosum</i>   | - epidermophytia       |
| 6.  | <i>Geotrichum candidum</i>        | - rheotrichosis        |
| 7.  | <i>Microsporum spp</i>            | - microsporia          |
| 8.  | <i>Mucor musedo</i>               | - mycorosis            |
| 9.  | <i>Penicillium crustosum</i>      | - penicilliosis        |
|     | <i>Penicillium luteo-viride</i>   |                        |
|     | <i>Penicillium notatum</i>        |                        |
| 10. | <i>Pityrosporum orbiculare</i>    | - variegated lichen    |
| 11. | <i>Rhizopus nigricans</i>         | - mycorosis            |
| 12. | <i>Trichophyton spp</i>           | - tegular mycosis      |
| 13. | <i>Trichosporon cerebriform</i>   | - nodular trichosporia |

#### Protozoa

- |    |                              |                              |
|----|------------------------------|------------------------------|
| 1. | <i>Leishmania donovani</i>   | - visceral leishmaniasis     |
| 2. | <i>Plasmodium vivax</i>      | - malaria                    |
|    | <i>Plasmodium malariae</i>   |                              |
|    | <i>Plasmodium falciparum</i> |                              |
| 3. | <i>Trichomonas vaginalis</i> | - urogenital trichomonadosis |

#### Group IV

- |    |                                 |                       |
|----|---------------------------------|-----------------------|
| 1. | <i>Acanthamoeba culbertsoni</i> | - meningoencephalitis |
|    | <i>Acanthamoeba spp</i>         |                       |
| 2. | <i>Babesia caucasica</i>        | - babesiosis          |

- |    |   |                           |
|----|---|---------------------------|
| 3. | <b>Balantidium coli</b>                               | - balantidiosis           |
| 4. | <b>Entamoeba histolytica</b>                          | - amebiasis               |
| 5. | <b>Isospora belli</b><br><b>Lambliia intestinalis</b> | - enteritis               |
| 6. | <b>Naegleria spp</b>                                  | - meningoencephalitis     |
| 7. | <b>Pentatrichomonas hominis</b>                       | - colitis                 |
| 8. | <b>Leishmania major</b><br><b>Leishmania tropica</b>  | - cutaneous leishmaniasis |
| 9. | <b>Toxoplasma gondii</b>                              | - toxoplasmosis           |

### Viruses

(In connection with the lack of binomial nomenclature for viruses,  
designations are given in Russian translation)

#### Group I)

- |     |   |   |
|-----|---|---|
| 1.  | <b>Filoviridae: Marburg and Ebola viruses</b>         | - hemorrhagic fever                       |
| 10. | <b>Arenaviridae: Lassa, Junin and Machupo viruses</b> | - hemorrhagic fever                       |
| 11. | <b>Poxvirus: smallpox virus</b>                       | - human smallpox                          |
| 12. | <b>Herpesviridae: simian virus B</b>                  | - chronic encephalitis and encephalopathy |

#### Group II

- |    |  |   |
|----|--|---|
| 1. | <b>Togaviridae: equine encephalomyelitis viruses (Venezuelan, Eastern, Western)</b>                        | - mosquito-borne encephalitis, encephalomyelitis, encephal meningitis |
|    | <b>Semliki, Chikungunya, O'Nong-Nong, Karelian, Sindbis, Ross River, Miyaro, and Mucambo fever viruses</b> | - febrile diseases  |

2. **Flaviviridae: viruses of the tick-borne encephalitis complex: tick-borne encephalitis, Alma-Arasan, Apon, Langat, Negishi, Powassan, Scottish ovine encephalomyelitis** - encephalitis, encephalomyelitis
- Kyasanur forest disease, Omsk hemorrhagic fever** - hemorrhagic fever
- viruses of the Japanese encephalitis group, Western Nile, Ilwes, Rosio, St. Louis, Usutu encephalitis, Murray Valley encephalitis** - encephalitis, meningoencephalitis
- Karshi, Kunzhin, Sepik, Wesselborn** - febrile diseases
- Yellow fever** - hemorrhagic fever
- hepatitis C virus** - parenteral hepatitis, hepatocellular liver cancer
3. **Bunyaviridae**
- Bunyavirus* species: California encephalitis group, La-Crosse, Jamestown Canyon, Zaytsev-Belyakov, Inko, Tyaginya** - encephalitis, encephalomyelitis, meningoencephalitis, and febrile diseases with meningeal syndrome and arthritis
- C-virus group Apey, Madrid, Oribona, Ossa, Restan et al** - febrile diseases with myositis and arthritis
- Phlebovirus* species: sandfly fever viruses, Sicilian, Naples, Rift Valley, Tuscan etc.** - encephalitis and febrile diseases with arthritis and myositis
- Nairovirus* species: Crimean hemorrhagic fever, Congo, Nairobi ovine disease, Ganjam, Dugbe** - hemorrhagic fever with meningeal encephalitis syndrome
- Hantavirus* species: Hantaan, Seoul, Puumala etc.** - hemorrhagic fever with renal syndrome



- |     |   |   |
|-----|---|---|
| 4.  | <b>Reoviridae:</b><br><i>Orbivirus</i> : Kemerovo, Colorado tick fever, ovine blue tongue, Changwinola, Orungo etc. | - fevers with meningeal syndrome and arthritis  |
| 5.  | <b>Rhabdoviridae:</b><br><i>Lyssavirus</i> species: rabies virus<br><br>pseudo-rabides, Lagos-bat                   | - rabies<br><br>- pseudorabies and encephalopathy   |
| 6.  | <b>Picornaviridae:</b><br><i>Aphthovirus</i> species: foot and mouth virus  | - foot and mouth  |
| 7.  | <b>Arenaviridae:</b> viruses of lymphocytic choriomeningitis, Tokaribe, Pichinde                                    | - asthenic meningitis and meningoencephalitis   |
| 8.  | <b>Hepadnaviridae:</b> hepatitis B and D viruses  | - parenteral hepatitis  |
| 9.  | <b>Retroviridae:</b> human immune-deficiency viruses (HIV-1, HIV-2)<br><br>Human T-cell leukemia virus (HTLV)       | - AIDS<br><br>- human T-cell leukemia   |
| 10. | <b>Unconventional agents:</b> slow neural infection pathogens:  | - Creutzfeld-Jakob, Kuru, scrapie, aminotrophic leukospongiosis, olivopontocerebral atrophy, Herstmann-Straussler-Scheinker syndromes |

### Group III

- |    |  |             |
|----|--|-------------|
| 1. | <b>Orthomyxoviridae:</b> influenza A, B, and C viruses | - influenza |
|----|--|-------------|

- |    |  |  |
|----|--|--|
| 2. | <b>Picornaviridae</b><br><i>Enterovirus</i> species: poliomyelitis strains<br><br>hepatitis A and B viruses<br><br>acute hemorrhagic conjunctivitis virus                    | - poliomyelitis<br><br>- enteral hepatitis<br><br>- hemorrhagic fever  |
| 3. | <b>Herpesviridae:</b><br>Herpes simplex 1 and 2 viruses<br><br>varicella-zoster herpesvirus<br><br>herpes 6 (HVLV-HHV6) -<br><br>cytomegaly virus<br><br>Epstein-Barre virus | - herpes simplex<br><br>- chicken pox, shingles<br><br>- involvement of human B lymphocytes, roseola infantum, lymphoproliferative diseases<br><br>- cytomegaly<br><br>- infectious mononucleosis, Burkitts lymphoma, nasopharyngeal carcinoma |

#### Group IV

- |    |  |  |
|----|--|--|
| 1. | <b>Adenoviridae: adenoviruses of all types</b>   | - ARVI, pneumonia, conjunctivitis  |
| 2. | <b>Reoviridae: <i>Reovirus</i> species: human retroviruses</b><br><br><i>Rotovirus</i> species: human rotaviruses, Nebraska Calf Diarrhea Virus (NCDV)                       | - rhinitis, gastroenteritis<br><br>- gastroenteritis and enteritis   |
| 3. | <b>Picornaviridae: group A and B coxsackieviruses</b><br><br>ECHO viruses<br><br>enteroviruses - types 68-71<br><br><i>Rhinovirus</i> species: human rhinoviruses, 120 types | - serous meningitis, diarrhea, ARVI, Bornholm's disease, herpangina, polyneuritis<br><br>- serous meningitis, diarrhea, ARVI, polyneuritis, uveitis<br><br>- serous meningitis, conjunctivitis, ARVI<br><br>- ARVI, polyneuritis, herpangina, conjunctivitis |

- |    |   |  |
|----|---|--|
|    | <i>Cardiovirus</i> species: encephalomyocarditis and Mengo virus          | - ARVI, polyneuritis, encaphalomyocarditis, pericarditis |
| 4. | Coronaviridae: Human coronaviruses  | - ARVI (profuse cold without temperature), enteritis     |
| 5. | Caliciviridae: Norfolk virus  | - acute gastroenteritis                                  |
| 6. | Paramyxoviridae: human parainfluenza viruses type 1-4                     | - ARVI, bronchial pneumonia                              |
|    | respiratory-syncetial virus (RS-virus)                                    | - pneumonia, bronchitis, bronchiolitis                   |
|    | epidemic parotitis virus  | - epidemic parotitis                                     |
|    | measles virus   | - measles  |
|    | Newcastle disease virus   | - conjunctivitis   |
| 7. | Togaviridae:<br><i>Rubivirus</i> species: rubella virus                   | - rubella  |
| 8. | Rabdoviridae:<br><i>Vesiculovirus</i> species, vesicular stomatitis virus | - vesicular stomatitis                                   |
| 9. | Poxviridae:<br>cowpox virus   | - cow pox  |
|    | ectromelia virus  | - mouse ectromelia                                       |
|    | milkmaid's ganglion virus   | - chronic disease of milker's hands                      |
|    | orphivirus  | - contagious pustular dermatitis                         |
|    | Tana and Yaba viruses   | - Yaba disease   |

#### Chlamydia Group II

- |    |                           |                                     |
|----|---------------------------|-------------------------------------|
| 1. | <i>Chlamydia psittaci</i> | - ornithosis - psittacosis          |
| 1. | <i>C. tracomatis</i>      | - trachoma, urogenital chlamydiosis |
| 2. | <i>C. paratrachomatis</i> | - trachoma-like conjunctivitis      |

3. **C. veneral lymphagranulema** - veneral lymphagranulema, involvement of the inguinal lymph nodes.

**Toxins of Biological Origin  
Group II**

1. **Botulism toxins of all types**
2. **Tetanus toxin**
3. **Karakurt spider toxin**

**Group III**

1. **Mycotoxins** - mycotoxicosis
2. **Diphtheria toxin**
3. **Streptococcus toxin, group A**
4. **Staphylococcus toxins**
5. **Snake poisons (cobras etc.)**

**Notes:**

**1. Attenuated strains of group I) and II pathogens are classified as group III pathogenicity microorganisms. Attenuated group III-IV strains are classed as group IV pathogenicity.**

**2. Among the sources of human and animal diseases caused by group I)-IV microorganisms we should include: infected humans, warm-blooded animals, carries, and environmental objects.**

Statute on a Board to Monitor Compliance  
with the Biological Safety Requirements  
at an Institution (Enterprise)

1. The board to monitor compliance with biological safety requirements (henceforth "Board") is an executive/consultative organ which monitors procedures for working with biological material in diagnostic, scientific-research and industrial laboratories.

2. The board will be created at institutions (enterprises) which conduct any sort of work (diagnostic, research, production) with group I) and I) biological material.

3. The board, consisting of 3 to 5 persons, competent in questions of biological material work safety, is appointed by institution order for a period of 5 years.

The chairman of the board will be the assistant institution director for epidemiological matters (science) or a specialist with the relevant knowledge and experience.

4. In its operations the commission will be guided by these Sanitation Regulations and by the other normative documents to assure biological safety and by the instructions of the institution director.

5. Administratively the board is subordinate to the institution director, who is responsible for the state of biological material work safety.

6. In order to assure biological material work safety during conduct of diagnostic, research and industrial tasks, the board will do the following:

- organize and conduct continuous monitoring of compliance with regulations assuring biological safety at the institution;

- organize and conduct a group of measures directed toward preventing emergency situations and controlling their consequences;

- monitor the preparedness of personnel to work with infectious material, and organize health monitoring;

- monitor compliance with the requirements of the relevant normative documents, as well as the orders of the institution director and proposals of the institution board;

- analyze the status of biological safety and develop a group of measures to improve it;
- prepare reports and other documents on biological safety questions.

7. In accordance with its assigned tasks, the board will conduct the following group of measures:

- conduct scheduled and periodic unscheduled checks of compliance with regulations to assure biological safety;
- ensure the timely hospitalization of personnel, ensure a regular procedure for immun.-prophylaxis, maintain a file of persons with high sensitivity to antibiotics or contraindications to vaccination;
- in the event of an "accident" in work with biological material, develops and provides to the institution leadership a plan for controlling its consequences;
- analyzes identified violations of safety rules, and precursors to them, and the causes of accidents, and provides the institution director with a plan to raise the effectiveness of the biological safety system;
- formulates the necessary documents to obtain (extend) permission to conduct work with biological material;
- checks knowledge on questions of assuring biological safety among personnel working with biological material;
- monitors the established procedures for travel of associates, and issues and receives observation certificates;
- prepares a report on board work for th year and presents it to the institutions which have oversight functions (para. 4 of these regulations);
- has a work plan approved by the institution leadership, and normative and other documents, the need for which is determined by its tasks and functions.

8. For effective implementation of its missions, the board has the following rights:

- to demand unconditional compliance with biological safety rules from subsection and section supervisors, and to appeal to the institution director to eliminate existing violations;

- to conduct independently or with the assistance of other qualified specialists, scheduled and unscheduled checks of compliance with the rules of biological safety in the institution;

- to appeal to the institution director to halt work with biological material if it is impossible to comply with biological safety rules, or when there are systematic violations, and to halt or cancel access of individuals to work with biological material;

- to submit a reasoned appeal to the regulating institution to halt the use or ban the introduction of new laboratory procedures, equipment types, disinfectants and so forth which do not assure the necessary level of biological safety;

- to examine documents and issue conclusions;

- to hear section supervisors and institution associates at board sessions;

- to appeal to the institution director to file administrative charges against persons who violate the established biological material work safety regulations.

## Means and Methods of Disinfection Used in Work with Pathogenic Microorganism

### I). Non-Spore-Forming Bacteria

1. Chloramine B or CB (contents of active chlorine AC no less than 26%) 0.5%, 1%, 2%, 3% solutions of preparation
2. Lime (AC content no less than 25%), dry compound  
0.5%, 1%, 2% (of preparation) clarified solutions  
10% (preparation) clarified and non-clarified solutions  
20% (preparation) lime milk
3. Heat-resistant bleaching powder (AC content no less than 25%)  
dry compound  
0.5%, 1%, 2% (preparation) clarified solutions  
10% (preparation) clarified and non-clarified solutions
4. Neutral potassium hypochlorite NGK (AC content no less than 52% for quality A and no less than 24% for quality B)  
dry compound  
0.15% (AC) solution  
0.25%, 0.5%, 1% (AC) clarified solutions  
5% (preparation) clarified and non-clarified solutions
5. Technical potassium hypochlorite GKT (AC content no less than 35%)  
dry compound  
0.4% (AC) clarified solution  
1.5% (preparation) clarified solution  
5% (preparation) clarified and non-clarified solution
6. Two-third basic potassium hypochlorite salt - DTS GK  
(AC content no less than 47%)  
dry compound  
0.15%, 0.5% (AC) solution  
0.25%, 1% (preparation) clarified solutions  
5% (preparation) clarified solution and non-clarified solution
7. Dibasic potassium hypochlorite salt -- DSGK (AC content no less than 30%)



- dry compound  
1% (preparation) clarified solution  
5% (preparation) clarified solution and non-clarified solution
8. Sodium hypochlorite (AC content no less than 14%)  
1% (AC) solution
9. Sodium hypochlorite obtained on ELMA-1<sup>1</sup> or EDR-01<sup>2</sup>, 0.125%, 0.25% (AC) solutions
10. Anolyte produced on the STEL<sup>3</sup> unit, with 0.5% AC content.
11. c with 0.06% and 0.09% AC
12. Anolyte produced on the EKHA-30<sup>4</sup>, with contents of 0.02%, 0.03%, 0.04% and 0.05% AC
13. c (AC content no less than 15%).
14. DP-2 (containing no less than 35% AC)  
0.1%, 0.2% (preparation) solutions
15. Desoxon-1 or desoxon-4 (no less than 5% peracetic acid - NUK);  
1%, 2% (preparation) solutions
16. Perhydrol (hydrogen peroxide content 30%)  
3% hydrogen peroxide solution

---

<sup>1</sup>The ELMA-1 and EDR-01 units are produced by the "Sintez" Scientific Research Institute and the "Aspo" MP, Moscow.

<sup>2</sup>The ELMA-1 and EDR-01 units are produced by the "Sintez" Scientific Research Institute and the "Aspo" MP, Moscow.

<sup>3</sup>STEL and STEL-MT-1 units are produced by the "Ekran" Scientific Production Association, Moscow

<sup>4</sup>The EKHA-30 unit is produced by the "Medfizpripor" production association, Kazan.

17. Hydrogen peroxide with detergent<sup>1</sup> (3% solution of hydrogen peroxide with 0.5% detergent)
18. Potassium fluoride peroxyhydrate (PFK-1)  
(hydrogen peroxide content no less than 35%)  
3%, 6%, 7%, 9% (preparation) solutions
19. Polysept<sup>2</sup>  
1% (preparation) solution
20. Amfolan<sup>3</sup>  
1%, 2%, 3% (preparation) solutions
21. Lysol A (phenol content 5%)  
2%, 3%, 10% (preparation) solutions
22. I)-chlor-be\*-naphthol  
0.5%, 2% (preparation) solutions
23. Industrial wastes or semifinished products containing:  
3% cresol or 1% AC, or 2% alkali, or 1% acid with anion-type Surfactant\* in a ratio of 1:1 or 0.5:1.
24. Caustic soda  
10% (preparation) solution
25. Formalin  
10%, 20%, 40% (formaldehyde) aqueous solutions
26. Ammonia  
10% aqueous solution (for neutralization of formaldehyde in a ratio of 1:1)
27. Boiling

---

<sup>1</sup>Added to hydrogen peroxide solutions to impart detergent properties.

<sup>2</sup>Polysept is based on surfactant materials produced by the Pokrov biopreparations plant in Vladimir Oblast

<sup>3</sup>Amfolan is based on surfactants and is produced by the "Sinteko" MP in the city of Kiev.

2% soda solution

28. Treatment with an steam under pressure (autoclaving)

0.20 MPa (2.0 kgf/cm<sup>2</sup>), 132 ± 2 °C

0.15 MPa (1.5 kgf/cm<sup>2</sup>) 126 ± 2 °C

0.11 MPa (1.1 kgf/cm<sup>2</sup>), 120 ± 2 °C

29. Alcohol 70 deg\*

30. Incineration

31. Baking

32. Treatment in disinfection chambers: steam method, formalin steam

33. Aerosol method of decontamination

34. Ultraviolet irradiation

## II. Spore-forming Bacteria

1. Chloramine B or CB  
1-4% activated solutions containing 0.25-1% active chlorine
2. Lime or heat-resistant bleaching powder  
dry compound  
20% clarified solutions containing no less than 5% AC  
4% activated clarified solutions containing no less than 1% AC
3. Two-third basic potassium hypochlorite salt (DTS GK) or neutral potassium hypochlorite (NGK)  
dry compound  
15% clarified solutions containing no less than 5% AC  
2% activated clarified solutions containing no less than 1% AC
4. Dibasic potassium hypochlorite salt (DSGK)  
4% activated solutions containing no less than 1.2% AC
5. Caustic soda  
10% solution (70 deg\* C)
6. Perhydrol containing 30-35% hydrogen peroxide  
6% solution of hydrogen peroxide with 0.5% detergent  
("Progress," "Novost," "Lotos," "Astra")  
3% solution of hydrogen peroxide with 0.5% detergent at solution temperature of 50 deg C
7. Desoxon-1 or desoxon-4  
solution containing 1% peracetic acid
8. Formalin  
20%, 40% (formaldehyde) aqueous solutions
9. Boiling
10. Baking
11. Incineration
12. Dry hot air (180 deg\* C)

13. Steam treatment at pressure of 2.0 kgf/cm<sup>2</sup> (132 ± 2 °C)
14. All containers which are used for disinfection must be closed with a cover, including containers for animals
15. Treatment in chambers: steam and formalin steam
16. Aerosol decontamination method

### III. Viruses and Chlamydia

1. Chloramine (AC content no less than 26%)  
1%, 3% (preparation) solutions  
0.5%, 1.5% (preparation) activated chloramine solutions
2. Lime (AC content no less than 25%)  
dry compound  
3%, 10% (preparation) clarified and non-clarified solutions  
20% (preparation) lime milk
3. Heat resistant bleaching powder (AC content no less than 25%)  
dry compound  
3%, 10% (preparation) clarified and non-clarified solutions
4. Two-thirds basic potassium hypochlorite salt -DTSGK  
(AC content no less than 47%)  
dry compound  
1.5%, 5% (preparation) solutions
5. Neutral potassium hypochlorite (AC content no less than 52% and 24% for types A and B)  
dry compound  
1.5%, 5% (preparation) solutions
6. Technical potassium hypochlorite -GKT (AC content no less than 35%)  
dry compound  
1%, 1.5%, 5% (preparation) solutions
7. Dibasic potassium hypochlorite salt (AC content no less than 30%)- DSGK  
1%, 1.5%, 5%, 7% (preparation) clarified and non-clarified solutions

8. Sulfochlorantin and Sulfochlorantin M (AC content 15.6%)  
0.1%, 0.2% (preparation) solutions
9. DP-2 (AC content 40%)  
0.1%, 0.2%, 0.5% (preparation) solutions
10. Anolyte produced in EKHA-30 unit with AC content of 0.04% and 0.05%
11. Anolyte produced in STEL-MT-1 unit with AC content of 0.06% and 0.09%
12. Anolyte produced in STEL unit with AC content of 0.05%.
13. Perhydrol with AD content of 30-35%  
6% (ADV) hydrogen peroxide solution  
6% hydrogen peroxide solution with 0.05% detergent
14. Potassium fluoride peroxyhydrate - PFK-1  
4% (preparation) solution
15. Desoxon-1 or desoxon-4 with NUK content of 5%  
0.1%, 0.5% (NUK) solutions
16. Lysol A  
5% and 8% solutions
17. Formalin  
40% (formaldehyde) aqueous solution
18. Ammonia  
10% (ADV) aqueous solution  
For neutralization of formaldehyde, ratio of 1:1 is used
19. Caustic soda  
10% (preparation) solution
20. 70 deg\* ethyl alcohol solution
21. Bicarbonate of soda  
2% solution

22. Soda ash  
2% solution
23. Disinfection with water steam at a positive pressure in a vapor sterilizer (autoclave)  
1.1 kgf/cm<sup>2</sup> (0.11 MPa), 120 °C  
1.5 kgf/cm<sup>2</sup> (0.15 MPa) 126 ± 2 °C  
2.0 Kgf/cm<sup>2</sup> (0.20 MPa)m 132 ± 2 °C
24. Disinfection with dry heat in an air sterilizer, 180 °C, 60 min
25. Boiling
26. Incineration
27. Treatment in disinfection chamber: steam or formalin steam method
28. Aerosol method of disinfection
29. Gas method (disinfection by formaldehyde vapors)
30. UV irradiation

*Note:*

*Ammonium salts (ammonium chlorite, sulfite or nitrate) in a ratio of 1:1 with active chlorine may be used as the active ingredient of chlorine preparations, or in a ratio of 1:2, or ammonia in a 1:8 ratio with active chlorine (8 times more chlorine than ammonia).*

**IV. Rickettsia**

1. Lime or bleaching power  
dry compound  
20% clarified and non-clarified solutions containing no less than 5% active chlorine  
3% clarified solutions containing no less than 1% active chlorine
2. Chloramine B or CB  
3% solutions containing no less than 0.6% active chlorine  
0.5% activated chloramine solution
3. Two-third basic potassium hypochlorite salt (DTS GK) or neutral potassium hypochlorite

- (NGK), technical potassium hypochlorite (GKT)  
dry compound  
15% clarified or non-clarified solutions containing no less than 5% active chlorine  
1.5% solution containing no less than 0.5% active chlorine
4. Perhydrol containing around 30-35% hydrogen peroxide  
6% hydrogen peroxide solution  
3% hydrogen peroxide solution with 0.5% detergent
5. Formalin - 20% formaldehyde solution
6. Caustic soda  
3% - 5% - 10% solutions
7. Lysol A  
8% solution
9. Incineration
10. Dry hot air at 180 °C
11. Treatment with steam under pressure (autoclaving)  
2.0 kgf/cm<sup>2</sup> (132 ± 2 °C)  
1.1 kgf/cm<sup>2</sup> (120 ± 2 °C)
- 11 Alcohol 70 °
12. Chamber treatment:  
a) water steam method  
b) formalin steam method

### V. Fungi

1. Chloramine B or CB (containing active chlorine AC, no less than 26%)  
5% preparation solution
2. DTSGK: 2% solution of two-thirds basic potassium hypochlorite salt
3. Sulfochlorantin or Sulfochlorantin M (AC no less than 15%)  
3% solution



4. Benzylphenol solutions 2-2.5%
5. Lysol solutions 5-10%
6. Formalin solutions 5-10%
7. Iodonate: 1% solution
8. Boiling
9. Pressure steam treatment, 1.1 kgf/cm<sup>2</sup> - 2 kgf/cm<sup>2</sup> (120 ± 2 °C, 126 ± 2 °C , 132 ± 2 °C)
10. Incineration
11. Baking
12. UV lamp treatment
13. Aerosol disinfection
14. Chamber treatment
  - a) water steam treatment
  - b) formalin steam treatment

Modes of decontamination of various objects which have been infected by pathogenic organisms  
Attachment 5.4 (mandatory)

1	2	3	4	5	6	
<b>I). Non-spore-forming bacteria</b>						
1.	Limited sections of soil (road)	irrigation	10% clarified or unclarified solution of lime or heat-resistant bleaching powder	60		2 l/m <sup>2</sup>
			5% solution of DTSGK or NGK, or DSGK, or GKT	"		"
			5% solution of Lysol A	"		"
			10% solution of caustic soda	"		"
			1% solution (AC) of sodium hypochlorite	"		"
			Industrial wastes or semifinished products with 3% cresol or 1% AC, or 2% acid or 2% alkali, or 1% acid with anion-type surfactant in a ratio of 1:1 or 0.5:1.	"		"
			1% B or Khr. chloramine solution	"		300 ml/m <sup>2</sup>
2.	Surfaces of interiors (floor, walls), furniture, equipment, work table, individual lockers and other furnishings, vivarium	irrigation or wiping with subsequent wet mopping	1% clarified or unclarified solution of lime or heat-resistant bleaching powder	"		"

LN069-99

		0.5% clarified solution (AC) NGK or DTSGK	"	"	"
		0.4% (AC) clarified solution of GKT	"	"	"
		1% clarified solution DSGK	"	"	"
		0.25% sodium hypochlorite solution produced on ELMA-1 or EDR-01 units	"	"	"
		1% AC sodium hypochlorite sodium hypochlorite solution	"	"	"
		0.1% DP-2 solution	"	"	"
		0.2% Sulphochlorantin or Sulphochlorantin M	"	"	"
	double wiping with 30 min interval	Anolyte produced on the EKHA-30 containing 0.03% AC	"	"	80 ml/m <sup>2</sup>
	wiping or irrigation	Anolyte produced on STEL-MT-1 unit containing 0.06% AC	"	"	200 ml/m <sup>2</sup>
	wiping	1% solution of desoxon-1 or desoxon-2	30		300 ml/m <sup>2</sup>
		9% solution PFK-1	30		200 ml/m <sup>2</sup>
		1% solution Polysept	60		100 ml/m <sup>2</sup>
		3% solution ampholan	30		150 ml/m <sup>2</sup>
	irrigation	5% solution Lysol A	20		500 ml/m <sup>2</sup>
		3% solution Lysol A	60		
		0.5% solution 1-Cl-β-Naphthol	60		100 ml/m <sup>2</sup>
<p>Note: in the event of an accident, pour one of the above solution on the infected surfaces for 2 hours.</p>					

	For extreme situations, provided that the premises are sealed.	Evaporation of the solution with subsequent ventilation of the premises	40% solution of formaldehyde with subsequent neutralization with ammonia (25% solution with expenditure norm of 10 ml/m <sup>3</sup> )	12 hours	c
3.	Personnel protective clothing (smocks, caps, masks, kerchiefs), patient linen without visible contamination	Aerosol method of disinfection using pneumatic (PVAN) or turbulent (TAN) aerosol nozzles. Compressed air source for atomizing is a compressor with flow rate of no less than 300 m <sup>3</sup> /h at working pressure of 3-4 atm, or aerosol generator (AGP)	40% aqueous solution of formaldehyde with subsequent neutralization with ammonia (expenditure norm of 10 ml/m <sup>3</sup> )	12 h	formalin 12.5 ml/m <sup>3</sup> (5 g/m <sup>3</sup> formaldehyde)
			3% solution of hydrogen peroxide with 0.5% detergent	120 min	200 ml/m <sup>3</sup> , double treatment with 60-min interval
		Steam sterilizer (autoclave)	Saturated steam at a pressure of 1.1 kgf/cm <sup>2</sup> (120 ± 0.11 MPa)	30	
		boiling	2% soda solution or 0.5% solution of any detergent	15	5 l per 1 kg of dry linen
		soaking in one of the disinfectants with subsequent laundering and rinsing	0.5% solution of chloramine B or KHB.	60	
			0.1% solution of Sulphochlorantin or Sulphochlorantin M	60	
			0.2% solution of Sulphochlorantin or Sulphochlorantin M	30	
			0.1% DP-2 solution	30	
			0.125% solution with respect to AC of sodium hypochlorite produced on the ELMA-1 or EDR-01 unit	60	



			Anolyte produced on the STEL unit and containing 0.05% AC	30
			0.2% solution of Sulphochlorantin or Sulphochlorantin m	120
			2% solution of desoxon-1 or desoxon-4	60
			3% solution of hydrogen peroxide with 0.5% detergent	120
			7% solution of PFK-1	60
			3% solution of ampholan	60
			1% solution of Polysept	60
			3% solution of Lysol A	120
			2% solution of 1-Cl- $\beta$ -Naphthol	60
			aqueous saturated steam at a pressure of 1.1 kgf/cm <sup>2</sup> (MPa 0.11), 120 $\pm$ 2 $^{\circ}$ C	30
5.	Gloves	Vapor sterilizer (autoclave)	Aqueous saturated steam at a pressure of 1.1 kgf/cm <sup>2</sup> (MPa 0.11), 120 $\pm$ 2 $^{\circ}$ C	45
		boiling	2% soda solution	15
		submersion	3% solution of Lysol A	120
			1% solution of chloramine B or KHB.	120
			3% solution of hydrogen peroxide with 0.5% detergent	30
			0.2% solution of Sulphochlorantin or Sulphochlorantin M	120
			0.25% solution (in AC) of sodium hypochlorite produced on ELMA-1 or EDR-01 units	60

			0.2% solution of DP-2		60	
			0.1% solution (NUK) of desoxon-1 or desoxon-4		15	
6.	Glasses, phonendoscope etc.	double washing with interval of 15 min with subsequent water rinse	3% solution of hydrogen peroxide with 0.5% detergent		30	
		submersion	3% solution of hydrogen peroxide		30	
7.	Rubber and fake leather shoes, leather boots and leather replacements	wiping	70 ° alcohol	disinfectant solutions indicated in para. 2	30	
8.	cotton jackets, trousers	chamber decontamination	steam-air mixture 80-90 ° C		20	10 sets (60 kg/m <sup>2</sup> )
	bedding accessories	chamber decontamination	steam-air mixture 80-90 ° C		45	
9.	hats, caps, leather and fur footwear, boots	chamber decontamination	steam-air mixture 57-59 ° C		45	formalin 75.0 ml/m <sup>3</sup> , temperature 57-59 ° C, 5 sets (30 kg/m <sup>2</sup> )
10.	Laboratory vessels (pipettes, test tubes, retorts, Petric dishes, smear seals, racks for drying cultures, syringes etc).	vapor sterilizer (autoclave)	aqueous saturated steam at a pressure of 1.5 kgf/cm <sup>2</sup> (126 ± ° C) 0.15 MPa		60	
		submersion	2% soda solution		30	
		submersion	3% chloramine B or KHB. solution		60	full submersion
			0.25% solution (AC0 of sodium hypochlorite produced on ELMA-1 and EDR-01 units		120	

			Anolyte produced EKHA-30 unit containing 0.05% AC	60		full submersion
			Neutral Anolyte produced on STEL-MT-1 unit containing 0.09% AC	120		
			0.2% solution of Sulphochlorantin or Sulphochlorantin M	60		
			2% solution of desoxon-1 or desoxon-4	60		
			3% solution of PFK-1			
			3% solution of hydrogen peroxide with 0.5% detergent	60		
			2% amphotan solution	60		
			3% Lysol solution	60		
			2% soda solution	15*		
			Disinfectant solution indicated in para. 4 (except for Anolyte, which is produced on the STEL unit, and Polysept)			2 l per 1 set of dishes
			6% solution of PFK-1	60		
			2% soda solution	15		full submersion
			0.5% solution of clarified lime or bleaching powder	60		full submersion
11.	Patient dishes	boiling				
		submersion in disinfectant solution with subsequent careful washing with hot water				
12.	Toys	boiling (except for plastic)				
		submersion or wiping with rage dipped in solution with subsequent washing				

or wiping (200 ml/m<sup>2</sup>) with subsequent careful wiping with warm water



			0.25% clarified NGK or DT SGK solution	60	
			0.1% solution of Sulphochlorantin or Sulphochlorantin M	60	
			0.1% solution of DP-2	30	
			1% solution of ampholan	60	
			0.125% (AC) solution of sodium hypochlorite produced on EDR-01 or ELMA-1 unit	60	
			neutral Anolyte produced on STEL-MT-1 unit containing 0.05% AC	30	
			Anolyte produced on EKHA-30 unit containing 0.02% AC	15	
			3% solution of hydrogen peroxide with 0.5% detergent	15	
			1% solution of desoxon-1 or desoxon-4	30	
13.	bacteriological cultures	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 1.5 kgf/cm <sup>2</sup> (126 ± 2 ° C), MPa 0.15	60	
	If it is not possible to decontaminate in a steam sterilizer, submerge for 24 hours in one of the disinfectant solutions indicated in para. 4.				
		boiling	temperature of boiling water 100 ° C	30*	
14.	rubber plugs, hoses, bulbs or pipetting infective material	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 1.5 kgf/cm <sup>2</sup> (126 ± 2 ° C), MPa 0.15	60	
		boiling	temperature of boiling water 100 ° C	30	
15	loops for replanting infective material	incineration in burner flame			

16.	tools after dissection of laboratory animals	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 1.5 kgf/cm <sup>2</sup> (126 ± 2 ° C), MPa 0.15	30	
			boiling	2% soda solution	15*
			submersion	temperature of boiling water 100 ° C	30*
				1% solution of chloramine	30
				3% solution of hydrogen peroxide	80
				3% solution of formalin (with respect to formaldehyde)	30
				0.1% solution (NUK) of desoxon-1 or desoxon-4	15
				0.1% solution of s18 or Sulphochlorantin M	30
				3% solution of hydrogen peroxide with 0.5% detergent	80
				disinfectant solution indicated in para. 5	2
17.	hands in rubber gloves	submersion and washing	0.5% solution of chloramine B or KHB., 70 ° alcohol	2	
18.	unprotected areas of skin and hands	Wash or wipe with swabs dipped in solution, then wash with warm water with individual toilet soap, wipe with individual towel		2	
When exposed to infective material in an accident use:					

			1% solution of chloramine B or KHB.	10	
			70 ° ethyl alcohol	2 times in 3 min.	
19.	bottles and barrels for animals, bedding material, animal excretions, food remnants	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 1.5 kgf/cm <sup>2</sup> (126 ± 2 ° C), MPa 0.15	60	
		wet to edges and wipe outside with rag dipped in disinfectant solution	3% solution of chloramine B or KHB.	24 h	
			1% solution of DTS GK or NGK	24 h	
			5% solution of Lysol A	24 h	
			0.2% solution of DP-2	24 h	
			0.2% solution of Sulphochlorantin or Sulphochlorantin M	24 h	
20.	metal boxes, cages, barrels for dissected animals and trapping gear	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 1.5 kgf/cm <sup>2</sup> (126 ± 2 ° C), MPa 0.15	30	
		air sterilizer	dry heat at a temperature of 160 ° C	60	
		submersion	3% solution of chloramine B or KHB.	120	
			5% solution of Lysol A	120	
		irrigation	3% solution of chloramine B or KHB.	60	
			5% solution of Lysol A	120	

			3% solution of hydrogen peroxide with 0.5% detergent	60	
			0.2% solution of DP-2	60	
			0.2% solution of Sulphochlorantin or Sulphochlorantin M	60	
21.	air bacterial filters	Irrigate, extract, place in polyethylene package, tie, burn	For irrigation, use systems and modes indicated in para. 2.		
		submersion	Use systems indicated in para. 2.	48 h	
		steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 2 kgf/cm <sup>2</sup> (132 ± 2 ° C), MPa 0.2	60	
22.	animal carcasses, bedding material, animal excretions	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 1.5 kgf/cm <sup>2</sup> (126 ± 2 ° C), MPa 0.15	60	
		incineration			
		surface decontamination of carcasses by submersion	5% Lysol solution	24 h	
			5% solution of chloramine B or KHB.	24 h	
		Add one of preparations to container of pathological material	5% Lysol A solution	60	ratio 1:2 (for liquid excretions)
			10% Lysol B solution	60	ratio 1:2
23.	Liquid wastes, waste water	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 1.1 kgf/cm <sup>2</sup> (120 ± 2 ° C), MPa 0.11		
		boiling		30	

		pour and mix	dry lime or bleaching power, or DTS GK, or NGK	60	200 g/l
24.	Patient excretions: sputum, fecal matter, mixed with urine or water in a ratio of 1:5, liquid fecal matter, vomitus, food remnants	pour out and dilute	dry lime or bleaching power, DTS GK or DSGK	60	200 g/l
			NGK	120	150 g/l
			GKT	30	200 g/l
				120	200 g/l A
			5% Lysol A solution	60	250 g/l B
			10% Lysol B solution	60	ratio of 2 parts disinfectant solution and 1 part excretion
25.	urine, rinse water	wet	2% lime or bleaching power	60	ratio 1:1
			2% Lysol solution	60	ratio 1:1
			1% DTS GK or NGK solution	60	ratio 1:1
			2% solution of chloramine B or KHB.	60	ratio 1:1
			lime or bleaching powder	15	10 g/l
			NGK	15	5 g/l
	GKT	15	10 g/l		

26.	vessels for patient excretions (pots, bedpans, urinals), swabs used for washing vessels, stored in special container after disinfection	submersion in one of disinfectant solution with subsequent washing	1% clarified lime or bleaching powder solution	30	
			0.5% NG1% solution	30	
			1% solution of chloramine B or KHB.	60	
			3% solution of chloramine B or KHB.	30	
			5% Lysol A solution	60	
			1.5% GKT solution	30	
			0.2% Sulphochlorantin or Sulphochlorantin M solution	30	
			1% solution (ACO sodium hypochlorite	30	
			0.2% DP-2 solution	30	
			disinfectants indicated in para. 2	60	
27.	Sanitation technical equipment	double wiping with rag dipped in disinfectant	Dichlor-1	15	500 ml/m <sup>2</sup>
			Belka	15	
			Blesk-2	25	
			Sanita	15	
			PChD	15	
			Deaus etc.	15	
28.	clean-up material, rags	boiling	15		
		soaking			
		3% solution of chloramine B or KHB. 60			full submersion

		aerosol method in buildings and tents, adopted for accommodation of vehicles. Atomization of solution with pneumatic nozzles or AGP aerosol generator	15% solution of DTS GK or NGK containing 5% active chlorine	60	100 ml/m <sup>2</sup>
			6% solution of hydrogen peroxide	30	400 ml/m <sup>2</sup>
			20% solution of formaldehyde	30	100 ml/m <sup>2</sup>
			10% solution of formaldehyde	30	200 ml/m <sup>2</sup>
32.	Bags for transporting wild rodents	boiling	2% soda solution	30*	
			water	30*	
33.	smear slides, culture smears	submersion	96 ° ethyl alcohol, Nikiforov's mixture with subsequent submersion in disinfectant indicated in para. 10.	20 min	
34.	Items of synthetic materials	chamber disinfection	steam mixture 80-90 °C	30 min	60 kg/m <sup>2</sup>
		submersion	1% solution of chloramine B or KHB.	5 h	
			0.2% formaldehyde solution at temperature of 70 °C	60 min	
35	gasmask filtration box	purging with formaldehyde vapors	formalin 40% (heated). Air containing formaldehyde vapors is passed through the box using the vacuum unit. Residual vapors of formaldehyde are neutralized by ammonia vapors: air is pumped through the box (until ammonia odor disappears).	5 minn	
<b>II. Spore-Forming Bacteria</b>					
1.	Limited areas of soil (roads)	irrigation	4% activated solution of lime or bleaching powder with 1% AC	120	10 l/m <sup>2</sup>
			2% activated solution of DTS GK	120	10 l/m <sup>2</sup>

			2% activated solution of NGK	120	10 l/m <sup>2</sup>
			3% activated solution of GKT	120	10 l/m <sup>2</sup>
			4% activated solution of DSGK	120	10 l/m <sup>2</sup>
			20% clarified solution or non*-clarified solution of lime or bleaching powder	120	10 l/m <sup>2</sup>
			10% solution of caustic soda	120	10 l/m <sup>2</sup>
			2% clarified activated solution of DTS GK or NGK	120	500 ml/m <sup>2</sup>
			4% activated solution of chloramine B or KHB.	120	500 ml/m <sup>2</sup>
			4% clarified activated solution of lime or bleaching powder	120	500 ml/m <sup>2</sup>
			4% activated solution of DSGK	60	500 ml/m <sup>2</sup>
			20% clarified solution of lime or bleaching powder	120	500 ml/m <sup>2</sup>
			15% solution of DTS GK or NGK	120	500 ml/m <sup>2</sup>
			4% clarified activated solution of lime or bleaching powder	120	500 ml/m <sup>2</sup>
			15% solution of DTS GK or NGK	120	500 ml/m <sup>2</sup>
			6% solution of hydrogen peroxide with 0.5% detergent	120	500 ml/m <sup>2</sup>
			4% activated solution of DSGK	120	500 ml/m <sup>2</sup>
2.	Premises (floor, walls, doors, equipment and other furniture)	wipe twice at interval of 30 min and then wet mop			
		double irrigation with interval of 30 min			
In the event of accident, wet contaminated surfaces with one of the above solutions for 2 hours.					



		aerosol method of disinfection using pneumatic (PVAN) or turbulent (TAN) aerosol nozzles	20% aqueous solution of formaldehyde with subsequent neutralizing with 25% solution of ammonia	24 h	200 ml/m <sup>3</sup>
			10% solution of hydrogen peroxide	60	400 ml/m <sup>3</sup>
3.	personnel protective clothing (smocks, kerchiefs, gauze masks, caps, and patient's linen)	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (132 ± 2 °C)		
		boiling	2% soda solution	60*	
		soaking with subsequent laundering and rinsing	1% activated solution of chloramine B or KHB.	120	5 l/kg of dry protective clothing
			3% solution of hydrogen peroxide with 0.5% detergent at temperature of 50 °C	60	5 l/kg of dry protective clothing
			0.2% solution of formaldehyde with 0.2% soap or OP-10 at temperature of 60 °C	60	5 l/kg of dry protective clothing
4.	gloves	boiling	2% soda solution	60*	
		submersion in disinfectant solution	1% activated solution of chloramine B or KHB.	120	
			6% solution of hydrogen peroxide with 0.5% detergent	60	
			0.2% solution of formaldehyde with 0.2% soap or at temperature of 60 °C	60	

5.	glasses, phonendoscope etc.	double wiping with 30 min interval and subsequent washing with water	6% solution of hydrogen peroxide with 0.5% detergent	60	
6.	shoes (leather or fake leather), rubber or fake leather boots	double wiping or washing with wipe at 15 min interval	1% activated solution of chloramine B or KHB.	60	
7.	cotton jackets and trousers, bedding	chamber disinfection	steam method, temperature 97-98 °C	45	60 kg/m <sup>2</sup> of chamber work zone
8.	hats, leather footwear, caps, boots (of fabric)	chamber disinfection	steam method, temperature 57-59 °C	165	250 ml/m <sup>3</sup> of formalin. Weight of submerged items 18 kg/m <sup>2</sup>
9.	laboratory vessels (Petrie dishes, test tubes, pipettes, retorts etc.)	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (132 ± 2 °C)	90	
		boiling	2% soda solution	60A	
		submersion	4% activated solution of chloramine B or KHB.	60	
			6% solution of hydrogen peroxide with 0.5% detergent	60	
			desoxon-1 solution containing no less than 1% acetic acid	60	
10.	patient dishes	boiling	2% soda solution	60A	
		submersion	4% activated solution of chloramine B or KHB.	60	2 l per dish set
			6% solution of hydrogen peroxide with 0.5% detergent	60	2 l per dish set

			4% activated solution of DSGK	60	2 l per dish set
11.	Toys	boiling	2% soda solution	60	
		double wiping with 30 min interval	6% solution of hydrogen peroxide with 0.5% detergent	60	
12.	cultures	steam sterilizer (autoclave)	1% activated solution of chloramine B or KHB.	60	
13.	rubber plugs, bulbs for pipetting infective material	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (132 ± 2 °C)	90	
14.	microbiological loop	boiling	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (132 ± 2 °C)	90	
		incineration in burner flame	2% soda solution	60A	
15.	instruments after dissection of animals	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (132 ± 2 °C)	90	
		boiling	2% soda solution	60A	
16.	hands in rubber gloves	submersion and washing	disinfectant solution indicated in para. 2	2	
17.	unprotected skin and hands <sup>a</sup>	wash or wipe with swab dipped in solution, then wash with warm water with individual soap, dry with individual towel	1% activated solution of chloramine B or KHB.	2	
increase exposure time to five minutes if there is infective material present.					

18.	bottles and barrels for animals (bottles for animals with bedding and animal excretions)	Fill to edges and wipe on the outside twice with 3 hour interval	4% activated solution of chloramine B or KHB.	48 h	
			2% activated DTS GK or NGK solution	48 h	
			4% activated DSGK solution	48 h	
			20% lime or bleaching powder solution	48 h	
			6% hydrogen peroxide with 0.5 detergent	48 h	
			aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (132 ± 2 °C)	90	
19.	metal boxes, cages, screen covers etc.	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (132 ± 2 °C)	90	
		hot air	180 °C	60	
20.	carcasses of laboratory animals	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (132 ± 2 °C)	90	
		incineration			
21.	vivarium area	double irrigation at 30 min interval	one of disinfectants indicated in para. 2		500 ml/m <sup>2</sup> in each irrigation
			10% solution of caustic soda (70 °C)		
22.	bacterial air filters	3 irrigations with 30 min interval, then pack the filter in a polyethylene bag, tie it and incinerate or autoclave	6% solution of hydrogen peroxide with 0.5% detergent	120	500 ml/m <sup>2</sup> in each irrigation
			aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (132 ± 2 °C)	90	
23.	liquid wastes, waste water	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (132 ± 2 °C)	90	

			pour and mix	dry lime or bleaching powder	120	500 g/kg
24.	Patient excretions (urine)		Sprinkle and mix	dry DTS GK, DSGK or NGK	120	400 g/kg
25.	Excrement, food remnants		Sprinkle and mix	dry lime or bleaching powder solution	120	200 g/l
				dry DTSGK, DSGK, or NGK	120	100 g/l
26.	vessels for patient's excretions (urinals, chamber pots, bedpans)		submersion	dry lime or bleaching powder solution	120	500 g/kg
				dry DTSGK, DSGK, or NGK	120	400 g/kg
				4% activated solution of chloramine B or KHB.	120	
				4% activated DSGK solution	120	
				6% solution of hydrogen peroxide with 0.5% detergent	120	
				20% clarified lime solution	120	
				15% solution of DTS GK or NGK	120	
27.	sanitation technical equipment		double wiping at 30 min interval	One of the disinfectants indicated in para. 26.		300 ml/m <sup>2</sup> for each wipe
28.	clean-up material, ranges		boiling	2% soda solution	60A	
			soaking	4% activated solution of chloramine B or KHB.	120	
				2% activated DTS GK or NGK solution	120	
				4% activated DSGK solution	120	
				20% lime or bleaching powder solution	240	
				15% DTS GK or NGK solution	240	

29.	trash	incineration				
30.	outside lavatories	double irrigation at 30-min interval	one of the disinfectants indicated in para. 2			
		sprinkle	one of the dry disinfectants indicated in para. 25			
31.	vehicles	double irrigation at interval of 15 min	4 % activated solution of chloramine B or KHB.	120	500 ml/m <sup>2</sup> for each irrigation	
			2 % activated DTS GK or NGK solution	120	500 ml/m <sup>2</sup> for each irrigation	
			4 % activated DSGk solution	60	500 ml/m <sup>2</sup> for each irrigation	
			6 % hydrogen peroxide solution with 0.5 % detergent	60	500 ml/m <sup>2</sup> for each irrigation	
32.	smear slides, culture smears	submersion	4 % clarified activated lime or bleaching powder solution	120		
			ethyl alcohol with 3 % hydrogen peroxide and subsequent treatment with disinfectant solution (para.) 9	30 min		
<b>III. Viruses and chlamydia</b>						
1.	limited sections of soil (road)	irrigation	10 % clarified activated lime or bleaching powder solution	120 m	2 l/m <sup>2</sup>	
			5 % DTS GK or NGK, DSGK, or GKT solution	120 m		
			8 % Lysol A solution	120 m		
			10/5 caustic soda solution	120 m		

2.	indoor surfaces (walls, doors, window sills, floors), desk, racks, individual lockers etc., furniture, vivaria*	double irrigation at 30 min interval or double wiping at 15 min interval	3% chloramine solution	120 m	500 ml/m <sup>2</sup> for each irrigation
			3% clarified solution of lime or bleaching powder	120 m	
			1.5% DTS GK or NGK, DSGK, or GKT solution	120 m	
			0.2% Sulphochlorantin or Sulphochlorantin M solution	120 m	
			0.5% DP-2 solution	120 m	
			6% hydrogen peroxide solution	120 m	
			4% PFK-1 solution	120 m	
			0.1% desoxon-1 solution or desoxon-4	120 m	
			8% Lysol A solution	120 m	
			*Note: in case of accident, sprinkle the contaminated surface with one of the above solutions.		
		double wiping at 15 min interval	Anolyte produced on EKHA-30 unit containing 0.05% CL	120 m	
			Anolyte produced on STEL-MT-1 unit containing 0.06% AC	120 m	

	<p>for emergencies, provided the premises are sealed</p>	<p>evaporation of solution, neutralization with subsequent ventilation of premises</p>	<p>40% aqueous solution of formaldehyde with subsequent neutralization with ammonia (25% solution with expenditure normal of 100 ml/m<sup>3</sup>)</p>	<p>24 h</p>	<p>formalin 17.5-12.5 ml/m<sup>3</sup> (-5 g of formaldehyde) at temp. of 20-25 °C; formalin 37.5-25 ml/m<sup>3</sup> (15-10 g/m<sup>3</sup> formalde-hyde) at temp. of 15-17° and relative humid. of 60-92%.</p>
<p>3.</p>	<p>personnel protective clothing, linen, smocks, kerchiefs, patient linen (underwear, bedding, towels, handkerchiefs etc.) without visible contamination</p>	<p>boiling steam sterilizer (autoclave) soaking in solution with subsequent rinse and laundering</p>	<p>2% solution of soda or any other detergent aqueous saturated steam at a pressure of 1.1 kgf/cm<sup>2</sup> (MPa 0.11), 110 ± 2 °C 3% chloramine solution 0.5% activated chloramine solution 0.1% Sulphochlorantin or Sulphochlorantin M solution 0.1% DP-2 solution Anolyte produced on EKHA-30 unit containing 0.04% AC Anolyte produced on STEL-MT-1 unit containing 0.06% AC 3% hydrogen peroxide solution with 0.5% detergent at solution temperature of 50 °C</p>	<p>30 min 45 m 30 m 30 m 60 m 120 m 30 m 60 min 30 m</p>	<p>(5 l/kg)</p>



4.	personnel protective clothing, linen, smocks, kerchiefs, patient linen (underwear, bedding, towels, handkerchiefs etc.) contaminated by blood, pus, fecal matter, sputum etc.	boiling	0.1% desoxon-1 or desoxon-4 solution	90 m	from moment of boiling	
			2% solution of soda or other detergent	30A		
			submersion in solution with subsequent rinsing in water and laundering	3% chloramine solution		120
				0.5% activated chloramine solution		120
			0.2% Sulphochlorantin or Sulphochlorantin M solution	90 m		
			0.5% DP-2 solution	120 m		
			Anolyte produced on EKHA-30 unit containing 0.05% AC	90 m		
			Anolyte produced on STEL-MT-1 unit containing 0.09% AC	60 m		
			3% solution of hydrogen peroxide with 0.5% detergent at solution temperature of 50 °C	180 m		
			0.1% solution of desoxon-1 or desoxon-4	120 m		
8% Lysol solution	90 m					
5.	gloves	decontamination in steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 1.1 kgf/cm <sup>2</sup> (MPa 0.11), 120 ± 2 °C	45 m		
			aqueous saturated steam at a pressure of 1.1 kgf/cm <sup>2</sup> (MPa 0.11), 120 ± 2 °C	45 m		
		boiling	temperature of boiling water	30A		
		submersion in solution	3% chloramine solution	60		

				6% hydrogen peroxide solution	60	
				6% hydrogen peroxide solution with 0.5% detergent	60	
				0.2% Sulphochlorantin or Sulphochlorantin M solution	90	
				0.5% DP-2 solution	60	
				0.5% desoxon-1 or desoxon-4 solution	60	
				8% Lysol A solution	60	
6.	protective glasses, phonendoscope	double wiping at 15 min interval with subsequent washing with water		6% hydrogen peroxide solution	15	
		submersion		70 ° ethyl alcohol	30	
7.	rubber, fake leather boots, leather shoes	double wiping at 15 min interval		disinfectants and modes of use indicated in para. 2		
9. [sic]	caps, leather and fur footwear, shoes	chamber decontamination		steam mixture at temperature of 57-59 °C	45 m	formalin 75 ml/m <sup>3</sup> norm for 5 sets
		boiling		2% soda solution	30A	
		decontamination in steam sterilizer		aqueous saturated steam at a pressure of 1.5 kgf/cm <sup>2</sup> (126 ± 2 ° C), MPa 0.15	60 m	
		submersion in solution		3% chloramine solution	60 m	
				3% clarified lime or bleaching powder solution	60 m	
				0.5% Sulphochlorantin or Sulphochlorantin M solution	60 m	
				0.5% DP-2 solution	60 m	
10.	laboratory vessels (Petrie dishes, test tubes, pipettes, smear slides etc.					

			Anolyte produced in EKHA-30 unit with concentration of 0.05% AC	30 m
			Anolyte produced on STEL-MT-1 unit with concentration of 0.05% AC	30 m
			Anolyte produced on STEL unit with concentration of 0.05% AC	30 m
			6% hydrogen peroxide solution	60 m
			6% hydrogen peroxide solution with 0.5% detergent	60 m
			6% PFK-1 solution	60 m
			0.5% desoxon-1 or desoxon-4 solution	60 m
			5% Lysol A solution	60 m
11.	patient's dishes		2% soda solution	30A
		boiling together with food remnants	3% chloramine solution	60 m
		submersion in disinfectant solution, subsequent washing in hot soapy water, then in potable water	0.5% activated chloramine solution	60 m
			3% clarified lime or bleaching powder solution	60 m
			1.5% NGK solution	60m
			1% GKT solution	120 M
			3% DSGK solution	30 M
			0.2% Sulphochlorantin or Sulphochlorantin M solution	90 m
			0.5% DP-2 solution	120 m

			Analyte produced on EKHA-30 unit with concentration of 0.05% AC	120 m	
			0.1% solution of desoxon-1 or desoxon-4	1120 m	
			4% PFK-1 solution	60 m	
12.	virus-containing fluid, suspension of contaminated cell culture	decontamination in steam sterilizer	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (0.2 MPa) 132 ± 2 °C	45 m	
If it is not possible to decontaminate in a steam sterilizer:					
		boiling	boiling temperature of water	30A	
		wet with solution	disinfectant in concentrations of solutions indicated in para. 4	24 h	
13.	rubber plugs, hoses, bulbs for pipetting infected material, culture drying racks	decontamination in steam sterilizer	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (0.2 MPa) 132 ± 2 °C	20 m	
		boiling	boiling temperature of water	30 m	
14.	metal instruments after animal dissection	decontamination in steam sterilizer	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (0.2 MPa) 132 ± 2 °C	20 m	
		boiling	distilled water with 2% bicarbonate of soda	15 m	
		boiling	boiling temperature of water	30 m	
		submersion in solution	3% chloramine solution	60 m	
15.	hands in rubber gloves	washing in disinfectant solution	disinfectant in concentration of solutions indicated in para. 5	2 m	

			1% solution of chloramine	2 m	
			70 ° ethyl alcohol	2 m	
16.	hands, unprotected areas of skin when exposed to infective material by accident	Carefully wash with disinfectant solution or wipe with swab or cloth dipped in sA, then wash in warm water with individual toilet soap, dry with individual towel.	1% chloramine solution	10 m	
			70 ° ethyl alcohol	2 times in 3 m	
17.	bottles and barrels for animals	fill with solution to edges, wipe on outside with rag dipped in solution	3% lime solution	24 h	
			3% bleaching power dA	24 h	
			1.5% DTSGK, DSGK solution	24 h	
			1.5% NGK, GKT solution	24 h	
			8% Lysol A solution	24 h	
			3% chloramine solution	24 h	
18.	metal boxes, cages, rodent trapping equipment	decontamination in steam sterilizer	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (0.2 MPa) 132 ± 2 °C	20 m	
		dry heat decontamination	temperature 180 °C	60 m	
		submersion in solution	3% chloramine solution	120 m	
			5% Lysol a solution	120 m	

19.	carcasses of laboratory animals	decontamination in steam sterilizer	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (0.2 MPa) 132 ± 2 °C	60 m	
20.	air filters	incineration			
		irrigation - extracted, placed in polyethylene bag, tied and incinerated	systems and regimes indicated in para. 2 "Indoor surfaces etc." are used for irrigation.		
		submersion	systems indicated in para. 2 are used	48 h	
21.	fluid wastes, waste water	decontamination in steam sterilizer	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (0.2 MPa) 132 ± 2 °C	60 m	
		decontamination in steam sterilizer	aqueous saturated steam at a pressure of 1.5 kgf/cm <sup>2</sup> (0.15 MPa) 126 ± 2 °C	60 m	
		boiling		30 m	
		sprinkle with preparation and mix	lime	60 m	200 g/l
			bleaching powder	60 m	200 g/l
22.	patient excretions: excrement, sputum, vomitus, food remnants		DTSGK, DSGK; NGK, GKT	120 m	100 g/l
		sprinkle with preparation and mix	lime	120 m	200 g/kg
			dry lime	120 m	400 g/kg
			DTSGK, DSGK; NGK, GKT	120 m	200 g/kg
23.	vessels for excretions (chamber pots, vessels, buckets, tanks etc), swabs	submersion in one of disinfectant solutions with subsequent washing in water	3% chloramine solution	60 m	

				0.5% activated chloramine solution	60 m	
				3% clarified lime or bleaching powder solution	60 m	
				1.5% clarified or non-clarified DTSGK, DSGK; NGK, or GKT solution	60 m	
24.	urine, patient's mouth rinse	sprinkle and mix		dry lime or bleaching powder, DTSGK, DSGK; NGK, GKT	60 m	70 g/l 35 g/l
25.	sanitation technical equipment (tubs, toilet bowls, basins etc.)	double wiping with rag dipped in one of the disinfectant solutions		disinfectants and concentrations of solutions indicated in para. 2	120 m	
26.	clean-up material (rags, mops etc.)	boiling		2% soap-and-soda solution or solution of any detergent	30 m	
		submersion in one of disinfectant solutions with subsequent rinsing in water		disinfectants and concentrations of solution indicated in para. 4		
27.	outhouses	irrigate inside surfaces with one of disinfectant solutions		10% solution of lime or bleaching power		
				5% solution of DTSGK, DSGK; NGK, GKT		
28.	trash	wet with solution in ratio of 2 parts solution to 1 part trash		10% clarified lime or bleaching powder solution	120 m	
				5% NGK solution	120 m	
				7% GKT, DSGK solution	60 m	

			20% lime milk		60 m	
29.	vehicles	irrigate or wipe twice with rag dipped in solution at 15 m interval, then wipe with rag wet with water	3% chloramine solution		60 m	300 ml/m <sup>2</sup>
			0.2% Sulphochlorantin solution		90 m	300 ml/m <sup>2</sup>
			0.5% DP-2 solution		60 m	300 ml/m <sup>2</sup>
			8% Lysol A solution		60 m	300 ml/m <sup>2</sup>
30.	animal bedding, excretions, food remnants	decontamination in steam sterilizer	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (0.2 MPa) 132 ± 2 °C		60 m	
31.	bags for transporting wild rodents	boiling	2% soda solution		30A	
			water		30A	
<b>IV. Rickettsia and Burnet's Coxiella</b>						
1.	limited areas of soil (road)	irrigation	20% clarified or non-clarified solution of lime or bleaching powder, content no less than 5% active chlorine		120	2 l/m <sup>2</sup>
			15% solution of DTSGK, NGK containing no less than 5% active chlorine			
			8% solution of Lysol A		120	2 l/m <sup>2</sup>
			10% solution of caustic soda		120	2 l/m <sup>2</sup>



2.	indoor walls, floors, doors, equipment and other furniture	double irrigation at 30 min interval or double wiping at 15 min interval	3% solution of chloramine B or KHB.	120	500 ml/m <sup>2</sup> for each irrigation
			3% clarified lime or bleaching powder solution	120	
			1.5% solution of DTS GK or NGK	120	
			5% solution of caustic soda	120	
			6% solution of hydrogen peroxide	120	
			8% Lysol A solution	120	
			*In case of accident, wet infected surfaces with one of the above disinfectants.		
3.	protective clothing: smocks, kerchiefs, gauze masks, patient linen (underwear, bedding), handkerchiefs etc.	boiling	2% soda solution	30A	5 l/kg
			3% solution of chloramine B or KHB.	120	
			8% Lysol A solution	120	
			3% hydrogen peroxide with 0.5% detergent at temperature of 60 °C	120	
			0.5% activated chloramine solution	120	
			aqueous saturated steam at a pressure of 1.1 kgf/cm <sup>2</sup> (MPa 0.11), 120 ± 2 °C	60	
			aqueous saturated steam at a pressure of 1.1 kgf/cm <sup>2</sup> (MPa 0.11), 120 ± 2 °C	60	
4.	gloves	boiling	2% soda solution	30	
			submersion	60	
			3% solution of chloramine B or KHB.	60	

				6% hydrogen peroxide solution with 0.5% detergent	60	
				5% caustic soda solution	60	
				85 Lysol A solution	30	
5.	rubber or fake leather boots	double wiping at 15 min interval		3% solution of chloramine B or KHB.	30	
				3% solution of caustic soda	30	
				8% Lysol A solution	30	
				10% solution of caustic soda (for Burnet's Coxiella)	30	
6.	caps, hats, leather and fur footwear, shoes	chamber disinfection		steam mixture at temperature of 57-59 °C	45 <sup>R</sup> 210 <sup>K</sup>	
7.	protective glasses, phonendoscope etc.	double wiping at 15 min interval and subsequent rinsing in water		6% solution of hydrogen peroxide	30	
				70 ° alcohol	30	
8.	cotton jackets and trousers	chamber disinfection		vapor-formalin mixture at temperature of 80-90 °C	45	
9.	laboratory vessels (Petrie dishes, retorts, test tubes, pipettes etc.)	steam sterilizer (autoclave)		aqueous saturated steam at a pressure of 1.5 kgf/cm <sup>2</sup> (126 ± 2 °C), MPa 0.15, 126 ± 2 °C	60	
				boiling	60	
		submersion (for Rickettsia)		3% solution of chloramine B or KHB.	24 h	
				3% solution of clarified lime or bleaching powder	24 h	
				1.5% solution of DTSGK, NGK or GKT	24 h	

			3% solution of caustic soda	24 h	
			6% solution of hydrogen peroxide with detergent	24 h	
		submersion (for Burnet's Coxiella)	10% solution of caustic soda	48 h	
			8% solution of Lysol A	48 h	
10.	patient's dishes	boiling with food remnants	2% soda solution	30	
		submersion	6% s( of hydrogen peroxide with 0.5% detergent	120	2 l per disk ser
			3% solution of chloramine	120	
			0.5% activated chloramine solution	120	
			3% solution of clarified lime or bleaching powder solution	120	
			1.5% solution of DTS GK, NKG or PKT	120	
11.	cultures	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 1.5 kgf/cm <sup>2</sup> (126 ± 2 ° C), MPa 0.15, 126 ± 2 ° C	90	
		boiling	2% soda solution	60	
		submersion (for Rickettsia)	3% solution of chloramine B or KHB.	24 h	
		submersion (for Burnet's Coxiella)	10% solution of caustic soda	24 h	
12.	rubber plugs, hoses, bulbs for pipetting infected material, racks for culture drying	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 1.5 kgf/cm <sup>2</sup> (126 ± 2 ° C), MPa 0.15, 126 ± 2 ° C	45	
		boiling	2% soda solution	60	

13.	instruments after animal dissections	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 1.5 kgf/cm <sup>2</sup> (126 ± 2 ° C), MPa 0.15, 126 ± 2 ° C	60	
		boiling	2% soda solution	60	
14.	hands in rubber gloves	wiping or washing	70 ° ethyl alcohol	60	
			5% Lysol A solution	2	
			3% solution of chloramine B or KHB.	2	
			3% clarified lime or bleaching powder solution	2	
			1.5% solution of DTSGK, NGK, or GKT	2	
15.	hands, exposed areas of body infected in accident	wash thoroughly or wipe	1% chloramine solution 70 ° ethyl alcohol	at least twice in 5 min	
16.	bottles for animals, barrels for dissected animals	fill to edges and wipe on outside	3% solution of chloramine B or KHB.	24 h	
			3% solution of clarified lime or bleaching powder	24 h	
			1.5% solution of DTSGK, NGK or GKT	24 h	
			10% solution of caustic soda	24 h	
			6% solution of hydrogen peroxide	24 h	
			8% solution of Lysol A (for Burnet's Coxiella)	48 h	
17.	metal boxes, cages, rodent trapping equipment	treat with dry heat	temperature 180 ° C	60	
		steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 1.5 kgf/cm <sup>2</sup> (126 ± 2 ° C), MPa 0.15, 126 ± 2 ° C	45	
		submersion	disinfectants indicated in para. 16		

18.	carcasses of laboratory animals, bedding, food remnants, excretions	steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (0.2 MPa) 132 ± 2 °C	60	
		incineration			
19.	vivarium area	double irrigation	disinfectants indicated in para. 16	2 h	500 ml/m <sup>2</sup>
		steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (0.2 MPa) 132 ± 2 °C	60	
20.	liquid wastes, waste water	boiling	boiling temperature	30	
		sprinkle with one of disinfectants and mix	lime or bleaching powder	60	200 g/l
			DTSGK, NGK, GKT	120	200 g/l
			dry lime or bleaching powder	120	200 g/l
21.	patient excretions: sputum, excrement	sprinkle with one of disinfectants and mix	DTSGK, NGK, GKT	120	500 g/kg
			dry lime or bleaching powder	60	70 g/kg
22.	patient's urine or mouth rinse	sprinkle with one of disinfectants and mix	DTSGK or NGK	60	100 g/l
			GKT	90	100 g/l
		boiling	boiling temperature 100 °C	30A	
23.	food remnants	sprinkle with one of disinfectants and mix	dry lime or bleaching powder	120	400 g/kg
			DTSGK, NGK, GKT	120	500 g/kg
24.	bedding	chamber disinfection	vapor-air mixture at temperature of 80-90 °C	45	60 kg/m <sup>2</sup>
		decontamination in steam sterilizer (autoclave)	aqueous saturated steam at a pressure of 2.0 kgf/cm <sup>2</sup> (0.2 MPa) 132 ± 2 °C	45	

		boiling	boiling temperature of water	30A	
26.	chick embryos infected with Rickettsia	submersion	3% solution of caustic soda	2 days <sup>P</sup>	
27.	chick embryos infected with Burnet's Coxiella	submersion	3% solution of caustic soda	5 days <sup>P</sup>	
28.	sanitation technical equipment (tubes, toilet bowls, basins etc.)	double wipe with rag dipped in one of the disinfectants	disinfectants and solution concentrations indicated in para. 16	120	
29.	clean-up material (rags, mops, etc.)	boiling	2% soap-and-soda solution or any disinfectant solution	30A	
		submersion	disinfectants and solution concentrations indicated in para. 3	120	
30.	outhouses	irrigate internal surfaces with one of disinfectants	20% solution of lime or bleaching powder		
		incinerated	5% sA of DTSGK, NGK, GKT		
31.	trash	wet with disinfectant in ratio of 2:1	20% solution of lime or bleaching powder	120	
			5% DTSGK or NGK solution	120	
			7% GKT solution	120	
32.	vehicles	irrigated or double wiped with rag dipped in solution at 15 min interval, then wiped with rag dipped in water	3% chloramine solution	60	
			8% Lysol A solution	60	
			5% caustic soda solution	60	
<b>V. Pathogens of deep mycoses (coccidiosis, histoplasmosis, blastomycosis)</b>					

1.	indoor surfaces, equipment, walls, window sills, floors, tables, racks in areas where infected animals are kept, individual lockers, bed tables, other furniture	wiping	2% solution of DTSGK	60 m	double wipe with 30 min interval	
		washing		60 m		
				60 m		
				60 m		
				180 m		
2.	heat chamber surface	irrigation and wiping	6% hydrogen peroxide solution + 0.5% detergent	60 m	500 ml/m <sup>2</sup> , double wipe at 30 min interval	
3.	protective clothing, line:	treatment in steam sterilizer (autoclave)	1.1 kgf/cm <sup>2</sup> (120 ± 2 °C) 1.5 kgf/cm <sup>2</sup> (126 ± 2 °C) 2.0 kgf/cm <sup>2</sup> (132 ± 2 °C)	60 30 20		
		smocks, kerchiefs	2% soda solution	30		
		cotton gauze masks	submersion	35 Sulphochlorantin or Sulphochlorantin solution	60	
				2.5% benzylphenol solution	60	
				5% Lysol solution	60	
gloves		5% chloramine solution	120			
	protective glasses, hoes	boiling	2% soda solution	15		
		wiping	one of solution listed above		double wipe at 30 min interval	
		cotton jackets	vapor-air mixture at 80-90 °C	1.5-20	8-10 sets (60 kg/m <sup>2</sup> )	

	hats, leather footwear	chamber disinfection	vapor-formalin mixture at 57-59 °C	30	5 sets (30 kg/m <sup>2</sup> ), formalin 75 ml/m <sup>2</sup>
4.	laboratory vessels (Petrie dishes, test tubes, retorts, hoses, bulbs)	treatment in steam sterilizer (autoclave)	1.1 kgf/cm <sup>2</sup> (120 ± 2 °C) 1.5 kgf/cm <sup>2</sup> (126 ± 2 °C) 2.0 kgf/cm <sup>2</sup> (132 ± 2 °C)	60 30 20	
		boiling	2% soda solution	30	
		submersion	2% DTSGK solution	120	full submersion
			3% solution of Sulphochlorantin or Sulphochlorantin M	120	
5.	fungal cultures in dense nourishment media. Experimental test surfaces	treatment in steam sterilizer (autoclave)	2.5% solution of benzylphenol	120	
			5% Lysol solution	120	
			5% chloramine solution	120	
			1.1 kgf/cm <sup>2</sup> (120 ± 2 °C) 1.5 kgf/cm <sup>2</sup> (126 ± 2 °C) 2.0 kgf/cm <sup>2</sup> (132 ± 2 °C)	60 m 30 m 20 m	
6.	hands, infected parts of body	washing with gauze wipe	1% iodonate solution	1 <sup>h</sup>	
			2% DTSGK solution		
7.	rodent organs for histological examination	submersion	10% formalin solution	24 h	full submersion
8.	laboratory animal carcasses	incineration			



		treatment with steam sterilizer (autoclave)	1.5 kgf/cm <sup>2</sup> (126 ± 2 °C)	60 m	
		surface disinfection by submerston	10% Lysol solution	48 h	
9.	bottles for animals	fill to edges and wipe	2% solution of DTSGK	48 h	
			3% solution of Sulphochlorantin or Sulphochlorantin M	48 h	
			2% solution of benzyphenol	48 h	
			5% of solution of Lysol	48 h	
			5% solution of chloramine	48 h	
10.	instruments after animal dissections	boiling	2% soda solution	30 m	
11.	animal bedding, food remnants, excrement	treatment with steam sterilizer (autoclave)	1.5 kgf/cm <sup>2</sup> (126 ± 2 °C)	60 m	
		boiling	2% soda solution	30 m	
12.	rages, clean-up material	submerston	2% DTSGK solution	120 m	
			3% solution of Sulphochlorantin or Sulphochlorantin M	120 m	
			2.5% benzyphenol solution	120 m	
			5% Lysol solution	120 m	
			5% chloramine solution	120 m	
13.	metal barrels and boxes for dissected animals	treatment with steam sterilizer (autoclave)	1.1 kgf/cm <sup>2</sup> (120 ± 2 °C)	60 m	

14.	aerosol method of disinfection using pneumatic (PVAN) or turbulent (TAN) aerosol nozzles		2.0 kgf/cm <sup>2</sup> (132 ± 2 °C)	30 m	(1500 ml/m <sup>3</sup> )
<p>A - from moment of boiling start  R - for Rickettsia  K - for Burnet's Coxiella  P - with subsequent autoclaving  B - increase to 4 min when there is contact with infective material;  D - double treatment with 30 min interval; contact duration 2 hours.</p>		6% of hydrogen peroxide solution with 0.5% detergent			

Chemical tests for monitoring temperature parameters of steam sterilizer operating mode  
(Procedure Instruction on Monitoring Operation of Steam and Air Sterilizers No. 15/6-5 of  
2/28/91).

No.	Name of components	Color and shape of crystals, odor	Normative documents	Quantity of components	Temperature parameters to be monitored, °C			
					110+	120+	126+	132+
1	2	3	4	5	6	7	8	9
1	antipyrine (1) magenta acid or phenyl red dye (4) or bromothymol blue or gentian violet	colorless crystals or white powder without odor	GF X(2), art. 65 TU 6-09-3803-82 TU 6-09-5170-84 TU 6-09-2082-77	99.9 ± 0.01 0.1 ± 0.01	+(3)	-	-	-
2	resorcin dye	white or weakly yellowish shade, crystal powder with weak characteristic odor	GF X, art. 577	99.9 ± 0.01 0.1 ± 0.01	+	-	-	-
3.	elemental sulphur	yellow crystals (5)	TU 6-09-2546-77	100.0	-	+	-	-
4.	benzoic acid dye	colorless needle-like crystals or white powder	GF X, art. 9	95.24 ± 0.01 4.76 ± 0.01	-	+	-	-
5.	benzamide	colorless crystals	TU 6-09-14-21-04-81	100.0	-	-	+	-
6.	succinimide	colorless crystals in the form of lamellar needles	TU 6-09-08-889-83	100.0	-	-	+	-
7.	benzoic acid dye	colorless needle-like crystals or white powder	GOST 6413-77 GOST 10521-78	99.9 ± 0.01 0.1 ± 0.01	-	+	-	-

8.	D(+) mannose dye	colorless crystals in the form of rhombic prisms	TU 6-09- 07-666-76	99.9 ± 0.01  0.1 ± 0.01	-	-	-	+
9.	nicotinamide dye	white fine crystalline powder with weak odor	GF X, art. 452 TU 6-09- 08-852-82	99.9 ± 0.01  0.1 ± 0.01	-	-	+	-
10.	urea dye	colorless crystals	GOST 6691-77	95.24 ± 0.01  4.76 ± 0.01	-	-	-	+

*Notes:*

1 - refers to powerful medicinal drugs which must be used and stored with caution: storage in closed lockers, under dry conditions.

2 - GF X is the USSR State Pharmacopeia, publication X.

3 - '+' is the temperature parameter monitored by chemical compound.

4 - any of the dyes listed in prescription 1 is used.

5 - for use of sulphur as a chemical test, it is not advisable to add the dye, since when the compound is melted, it does not mix with the dye.

**Chemical tests to monitor temperature parameters  
of air sterilizer operating regimes (1)**

No.	Chem. compound name	Crystal color and shape, odor	Normative document	Component quantity, g	Temperature parameter to be monitored, °C	
					160-10 °C 160+2 °C	180-10 °C 180+2 °C
1	levomycitine (2)	white or with and weak yellow-green shade, crystal powder, odorless	GF X (3), art. 371	100.0	+ (4)	-
2	tartaric acid	white powder or transparent colorless crystals	GOST 5817-77 GOST 21205-83	100.0	-	+
3	hydroquinone	colorless or light gray silvery crystals	GOST 19627-74	100.0	-	--
4	thymourea	glittering colorless crystals	GOST 6344-73	100.0	-	+

*Notes:*

*1 - Dye is not used in the chemical tests to monitor operation of the air sterilizers, since these chemical compounds change their color when they reach the melting point.*

*2 - Refers to powerful medicinal drugs used and stored with caution, in closed lockers under dry conditions.*

*3 - GF X - State Pharmacopeia, publication X.*

*4 - "+" temperature regime monitored by chemical compound.*

The chemical indicators in the steam sterilizer are placed in each decontaminated container and two in the chamber itself; in air sterilizers from 5 to 15 depending on the chamber capacity.

Instead of the chemical tests, thermometric indicators may be used to monitor the temperature and sterilization time (TVI IS-160 or IS-180) in air sterilization, and in steam sterilization (IS-120 and IS-132), and test for the presence of steam. The indicators are strips

LN069-99

2-3 cm long detachable from paper tape with an indicator layer whose color changes irreversibly only when the sterilization regimes approved by GOST 22649-83 and OST 42-21-2-85 are observed.

**Bacteriological method of testing the effectiveness of steam sterilizer operation (Excerpt from the "Procedural Instructions for Monitoring Operation of Steam and Air Sterilizers" 1991 (No 15/6-5, USSR Health Ministry)**

1. Bacteriological monitoring of sterilizer operation is carried out after installation and repair of hardware, as well as in the process of operation (scheduled twice a year, or when unsatisfactory test results are obtained).

The effectiveness of sterilizer operation is tested by the bacteriological method using biotests based on the death of test-culture spores. Biotests are flasks of glass tube for drugs FI/1-5 ns 1, TU 64-0709-10-88 (insulin flasks) or dishes of aluminum foil (14 mm disk with depression about the size of an unsharpened pencil end), containing dried test culture spores *Bac. stearothermophilus* VKM V-718, placed in packages of packing paper (OST 42-21-2-85). The packaged tests are numbered and arranged at test points in the steam sterilizers (5-10 tests). At the end of sterilization, the biotests undergo bacteriological examination.

2. The strain *Bac. stearothermophilus* VKM V-718 is a motile thermophilic Gram-positive bacillus. It is cultured at a temperature of  $55 \pm 1$  °C, precluding the development of other widely disseminated microorganisms. The spores are oval, and arranged centrally. In beef extract bouillon (pH  $7.3 \pm 0.1$ ), after 24 hours they form turbidity of the medium, in a beef-extract agar (pH  $7.3 \pm 0.1$ ) weakly convex colonies 2-4 mm in diameter with an even edge. The strain is non-pathological to man and animals. The strain was produced at the All-Union Collection of Microorganisms of the Institute of Biochemistry and Physiology of Microorganisms and stored in the Cultures Museum of the Scientific Research Institute for Prophylactic Toxicology and Disinfection (117246 Moscow, Nauchnyy proyezd 18).

To an ampule of freeze-dried culture is added 0.2 ml of sterile tap water, after which it is left at room temperature for 30 minutes.

One or two droplets of culture are added to 2 test tubes of bouillon (MPB, Hottinger, nutritive dry) with 0.5% glucose. A daily culture is sown in test tubes of slant agar (Hottinger, beef-extract, dry nutritive). To obtain the spores, the culture grown on a hard nutritive medium is washed off with 5 ml of sterile tap water and transferred to flasks with a potato-peptone agar. The suspension is uniformly distributed along the surface of the medium by shaking the flask, and incubated at 55 ° for 10-12 days in a tilted position with the agar up. To create sufficient humidity open containers of water are placed in the incubator. On days 7, 10 and 12 the culture is checked for intensity of spore formation. A total of 80-90% spores in the field of view is considered adequate. The culture is washed off with sterile distilled water.

In order to free it of vegetative cells, the suspension is heated in a water bath at a temperature of 65-70 ° for 30 minutes, then centrifuged three times at 33.33 revolutions per second (2000 rpm) for 15 minutes each, washing out the deposit with sterile distilled water after each centrifuging. The washed spores are suspended in sterile distilled water in a ratio of 1:1 by volume. The spore suspension is stored in the refrigerator at a temperature of 4 °C in sterile test tubes closed by gauze plugs with rubber caps (storage life 2 years).

The purity of the culture at all stages of cultivation is monitored by sowing on agar slides.

To determine the titer of viable spores, 0.1 ml of initial suspension is diluted by a factor of ten to  $10^{-7}$  with sterile distilled water, and sown on 3 agar slides with 0.1 ml each roughly from  $10^{-5}$  to  $10^{-7}$  (the dilution limit depends on the titer of the obtained spores). The plantings are incubated for 48 hours, after which the colonies are counted. The titer of viable spores in the initial suspension is determined as the average number of colonies with allowance for dilution of the initial suspension and the volume of the sowing sample.

Example of calculations. Let us assume that for sowing on three Petrie dishes with a suspension agar in a dilution of 1:100,000 ( $10^{-5}$ ), 140, 110 and 134 colonies are counted. Similar plantations from dilutions of  $10^{-6}$  led to formation of 12, 14 and 16 colonies, from  $10^{-7}$ , 5, 3 and 7 colonies. Let us calculate the total number of colonies, and then the average number of colonies for each dilution: 128, 14 and 5.

From calculation of the plantation dose (0.1 ml per dish), we calculate the titer of viable spores in 1 ml of starting suspension with allowance for dilution, and then find the average number of colonies:

$$128 \times 10 \times 10^5 = 12 \times 8 \times 10^7;$$

$$14 \times 10 \times 10^6 = 14.0 \times 10^7;$$

$$5 \times 10 \times 10^7 = 50.0 \times 10^7;$$

Thus the titer of the initial suspension is:

$$(12 \times 8 + 14.0 + 50.0) \times 10^7 : 3 = 2.5 \times 10^8 \text{ spores per ml.}$$

The starting suspension must contain no fewer than  $2.5 \times 10^7$  -  $2.5 \times 10^8$  spores per ml. Spores in a quantity of  $5 \times 10^5$  -  $5 \times 10^6$  are introduced from the initial suspension using the pipette doser (TU 64-1-3329-81) in 0.02 ml to the carriers (sterile insulin flasks with gauze



plug or dishes of aluminum foil placed in Petrie dishes), dried in the incubator at 37 ° or a desiccator over a drying agent (silica gel, potassium chloride) at room temperature for 24 hours. To determine the actual population size, no fewer than 3 biotests from each group are investigated. A total of 1.0 ml of sterile distilled water is added to the flasks (dishes of aluminum foil, washed in wide-necked test tubes with beads in 10 ml) and shaken for 10 minutes on shaking apparatus, then sown on 3 agar slides in the amount of 0.1 ml suspension from three successive ten-fold dilutions.

3. The resistance of the test culture spores to the action of the water saturated steam at a positive pressure is tested at a temperature of  $120 \pm 2$  °C.

The biotests in their packing paper are placed in a sterilization box in the steam sterilizer chamber. After the pressure in the steam chamber reaches  $0.11 \pm 0.01$  MPa ( $1.1 \pm 0.1$  kgf/cm<sup>2</sup>), the steam sterilizer is purged (air is forced from the steam sterilizer chamber by steam) for 10 minutes with an open release nozzle and the pressure in the sterilization chamber is from 0.1 to 0.2 MPa (from 0.1 to 0.2 kgf/cm<sup>2</sup>). After purging, the steam pressure in the sterilization chamber is brought to  $0.11 \pm 0.01$  MPa ( $1.1 \pm 0.1$  kgf/cm<sup>2</sup>) at a temperature of  $120 \pm 2$  °C and 5 minutes after pressure is established (survival time of the test-culture spores) the steam is released. To reduce the time of steam affects before and after exposure, the pressure elevation takes 8 minutes to reach maximum, and the drop takes 3 minutes.

A similar investigation is conducted for 15 minutes (time of death of test-culture spores). The temper is checked with a maximal thermometer. At the end of the exposure time the biotests are removed from the sterilizer and the bacteriological examination carried out.

Biotest lots are considered useable if the resistance indices of the test-culture spores correspond to the above requirements.

4. To determine the effectiveness of sterilizer operation, 5 ml of nourishment medium is added by sterile procedures to the decontaminated biotests and the control (without sterilization). They are incubated at 55 °C for 7 days with daily inspection of the plantations, which are placed on agar slides from overgrown containers. When a polysynthetic medium is used with phenyl red indicator, the growth of the test culture is determined from the change in red color of the medium (pH  $7.7 \pm 0.1$ ) to yellow-orange (pH  $6.7 \pm 0.1$ ) owing to breakdown of glucose with formation of acid.

In order to preclude a false negative (with test-culture growth present, there is no change in color of the nutritive medium), the flasks (test tubes) must be tightly closed with sterile rubber plugs (No. 7.5; 12.5).

The absence of growth of the test-culture points to effective sterilizer operation. Growth of other microorganism cultures is due to secondary contamination.

When there is growth of the test strains, a repeat test is performed on twice as many biotests. If in the repeat test the test cultures are not inactivated, a careful examination of the technical status of the hardware and monitors/meters is carried out. If there is no test-culture grown in the control biotest (not subjected to sterilization), the reason is identified (non-viability of test-culture, failure to follow procedure for preparing the biotests, nutritive media, or cultivation conditions).

#### 5. Used for spore formation:

- potato-peptone agar (peptone - 5.0, chalk - 1.0, agar - 25.0, potato water - 1000 ml, pH  $7.1 \pm 0.1$ ). Raw potato (200 g cleaned potato per 1 l of tap water) is thoroughly washed, cleaned of skin and eyes, cut up into small pieces, placed in tap water and boiled for 30 minutes after water begins to boil (do not use new potatoes). The water is allowed to sit and is filtered through a gauze filter when cooled. The filtrate is brought to the initial volume. A pH of  $7.1 \pm 0.1$  is established. Peptone and agar are added. Heat, stirring until the agar completely dissolves, then filter through a gauze filter, then add chalk. Pour into flasks, sterilize at  $120^\circ\text{C}$  for 30 minutes. After sterilization, cut the medium in the flasks: wheat agar ("Artek" or "Poltavskaya" wheat) - 500.0, agar 25.0, distilled water 1000 ml, pH  $7.3 \pm 0.1$ .

The "Artek" ("Poltavskaya") wheat is placed in distilled water. After 12 hours the extract is carefully drained without the residue, then topped off to the initial volume. The agar is added and heated in a water bath or in an autoclave (steamed for 1 hour). The cooled agar is placed in a pan and the residue is cut up. The agar is heated in a water bath, with constant stirring. A pH of  $7.3 \pm 0.1$  is established. It is poured into flasks. Sterilized by team 1 hour a day for 3 days. After sterilization the medium is cut.

6. Used for the test: Hottinger's bouillon, pH 7.3; Hottinger's agar Ph 7.3; dry nutritive bouillon pH 7.1 (Dagestan Scientific Research Institute for Nutritive Media); dry nutritive agar pH 7.3 (Dagestan Scientific Research Institute for Nutritive Media); dry nutritive media for sterility testing (Central NIIVS enterprise im. I.M. Mechinkov) pH 7.0; bouillon from a mixture of blood clots, polysynthetic media with phenyl red indicator Ph 7.7 (ammonium monophosphate  $\text{NH}_4\text{OH}_2\text{PO}_4$ , 1.0 g; magnesium sulphate  $\text{MgSO}_4$ , 0.2 g; potassium chloride KCl, 0.2 g; glucose 5.0 g, phenol red, 0.02 g, Hottinger's bouillon with nitrosamine, 140-160 mg%, 200 ml, distilled water, 800 ml, pH  $7.7 \pm 0.1$ ). The components are mixed and dissolved during heating in a water bath. The pH is brought to  $7.7 \pm 0.1$ . It is poured into flasks and sterilized at  $110^\circ\text{C}$  for 30 minutes).

**Procedure for replacing fine filters of the ventilation exhaust system and determining their efficacy**

1. Fine filters (D-13, D-15, D-23 FTO etc.) of ventilation exhaust systems are replaced per schedule. When there is an increase in dynamic resistance of the filter by a factor of 2 or when it is damaged, it may be replaced earlier.
2. Before removal, the filter and the main air duct undergo preliminary disinfection by formalin vapors or by the aerosol method.
3. The disinfectant is atomized through a connector pipe in the air duct with the ventilation running. At the end of atomization, the ventilation is turned on and at the end of the exposure time the filter may be removed.
4. The work of filter removal is carried out in a type IV suit and rubber gloves (under work sleeves) and a respirator.
5. The removed filter is placed in a kraft-bag or other container and transferred for autoclaving or incineration as authorized.
6. Before installation, a filter must be checked for leakage (from an oil fog, biological aerosol or other method). In the operating process, the filter is checked for leakage at least once a quarter.
7. The tasks of replacing the filter are carried out by technical personnel under the supervision a division associate responsible for compliance with biological safety requirements.
8. To evaluate the protective efficacy of the filters, a culture of *B. prodigiosum* (*ser. marcescens*, *chromobacterium prodigiosum*) or *E. coli* is used as the model to create the aerosol. Special devices, atomizers, are used to create the aerosol. A special nozzle is used to ensure minimal dispersion of the bacterial aerosol into the environment and to direct the jet of the aerosol into the opening of the air duct up stream of the filter. To determine the concentration and the fractional makeup of the biological aerosol, the BP-50 impactor is used. It was developed by the Scientific Research Institute for Biological Instrument Building.
9. The aerosol is sampled by two impactors simultaneously before it passes through the filter (control) and after it passes through (experiment). From the results of growth of the test

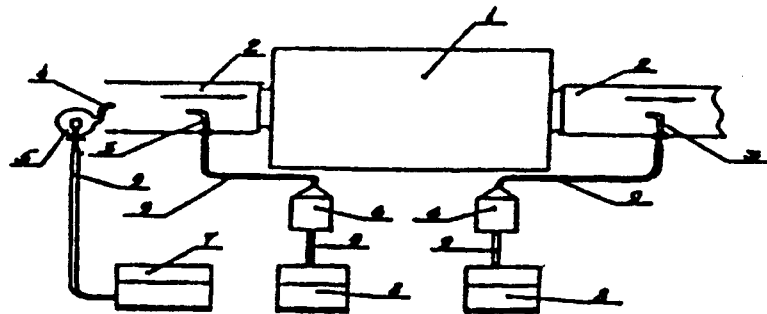
strain on agar slides before and after passage of the filter, the protective efficacy may be ascertained. A one-day culture of the test strain in concentrations of  $5 \times 10^8$  to  $1 \times 10^9$  m.k. per ml is used. To conduct the experiment, the instruments are installed as follows (see diagram): the nozzle (4) is placed on the air duct opening upstream of the filter (2) by bolts; the compressor hoses (9) are placed on the end of the atomizer (5) injector. Two microbiological impactors BP-50(6) are connected to the inlet and outlet openings of the air duct down stream from the filter (2); the compressor (7) and two aspirations (8) (normal vacuum cleaners) are connected to the system. Before the start of the experiment, the operation of the compressor and the speed of air movement through the impactor are tested. The experiment is conducted with ventilation running.

10. The prepared suspension of the test strain is added to the atomizer tank, after which the injector is inserted. The atomizer is set at the level of the air duct opening, the compressor and both impactors are turned on. The following conditions are maintained: the rate of fluid atomization  $Q_{zh} = 1$  ml/min, rate of air atomization  $V = 50$  l/min, time of atomization 10 minutes, average diameter of aerosol particles  $d_{sr} = 2.4$  micrometers ( $lgd = 0.389$ ), maximal diameter of particles  $d_{max} = 7$  micrometers with logarithmic normal distribution (standard deviation  $lgd = 0.229$ ); rate of aerosol sampling by impactor BP-50  $V = 50$  l/min, time of aerosol sampling 10 minutes.

After this time, first the compressor is turned off, and then the impactors. The Petrie dishes are removed from the impactors and incubated at  $37^\circ\text{C}$  for 2 days. After the experiment the unit is disinfected.

11. The results are recorded after 24 and 48 hours. In the *B. prodigiosum* population, along with the typically colored colonies, other different-colored variants may also appear: pink, weakly pink, or with a pink center.

The effectiveness of aerosol particle entrapment by the filter may be judged from the number of aerosol particles settled before and after it. The filter effectiveness is expressed in a percentage. If the filters are functional, there must be no growth of test-culture colonies on dishes downstream from the filter, while upstream from the filter (to assure test reliability), there must be no fewer than 200 colonies on the BP-50 impactor dishes.



## ОБОЗНАЧЕНИЕ:

1. Фильтр Д-13(Д-15)
  2. Воздуховод вентиляционной системы
  3. Втулка для отбора пробы
  4. Носитель распылителя
  5. Распылитель
  6. Импактор ДП-50
  7. Компрессор
  8. Аспиратор отбора пробы
  9. Соединительные втулки
- Направление воздушного потока

DIAGRAM OF FILTER TEST  
with ventilation system running

Designations: 1. D-13 (D-15) filter; 2. ventilation system air duct; 3. connector pipe for sampling; 4. atomizer nozzle; 5. atomizer; 6. BP-50 impactor; 7. compressor; 8. sample aspirator; 9. connector hoses; (arrow) direction of air flow

Requirements for disinfection of laboratory vessels with infected nutritive media in portable disinfection chambers

### **1. General**

1.1. Decontamination of laboratory vessels in mobile disinfection chambers is performed only when there are epidemic signs in a mass examination of the populace for cholera.

1.2. Laboratory vessels are disinfected in mobile disinfection chambers by the vapor-air method.

1.3. The active agent in this method is the vapor-air mixture (hot wet air) at a temperature of 97-98 °.

1.4. Before the start of operation of the disinfection chamber, the disinfector is obliged to check its technical condition (it is not permitted to disinfect laboratory vessels in a faulty chamber).

### **2. Mobile Disinfection Chamber Work Procedure**

2.1. Before laboratory vessels are loaded, the chamber is cleared of all objects transported in it, except for wooden rags placed on the floor. Work in the disinfection chamber is not permitted if the opening for condensate drainage is closed.

2.2. Before the chamber is loaded with the first lot of laboratory vessels, it is heated to 80 ° and kept at this temperature for 15 minutes. After heating and subsequent ventilation through the open door, the work of loading the chamber with laboratory vessels begins.

The loading is from the side opposite the disinfection chamber control front (the dirty zone).

2.3. Laboratory vessels with a capacity of no more than 1 l are placed in tanks or other containers with covers after use. These tanks must have small openings on the sides at a distance of 10 cm from the bottom. The tanks are numbered and are installed in the chamber at two levels: at the bottom on the wooden floor racks, without touching, and at the top, suspended at a height of 0.9-1 from floor level using 2 or 3 arms, which are part of the disinfection unit set, for each tank (container) depending on the weight. The tanks must be

open.

2.4. The loaded chamber is heated for 20 minutes to 98 °C. At this point exposure time count begins. The disinfection exposure is 120 minutes. In the exposure process, steam enters the chamber steadily. This regime assures disinfection of laboratory vessels with infected nutritive media. The contents inside the tanks are uniformly heated. The thermometer readings and exposure are noted in a special log.

2.5. At the end of the exposure the door is opened on the control console side (clean side), the chamber is ventilated for 5 minutes, and then the tanks of laboratory vessels are unloaded. Canvas mittens are used in this process to protect the hands from burns.

### **3. Thermal and Bacteriological Monitoring**

3.1. The effectiveness of disinfection of the laboratory vessels with spent nutritive media is determined by two methods: thermal and bacteriological.

3.2. The thermal test is carried out for every chamber loading, using the maximal thermometers by placing them in the center of each tank in special bags, which are numbered the same as the tanks.

3.3. At the end of vessel disinfection in the chamber, the bags are taken out of the tanks, and the readings of the maximal thermometers are entered in a special log.

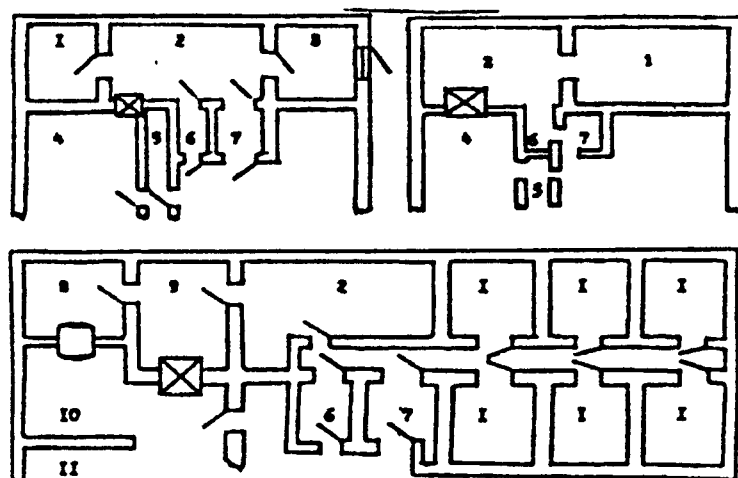
3.4. If there is uniform temperature distribution in the disinfected objects in the chamber, the difference in the thermometer readings in the steam-air method will be 1-2 °C.

3.5. The bacteriological test of disinfection is carried out periodically once a month using plantings of disinfected liquid from the tanks of maximal volume. After thorough mixing, a pipette is used to remove 0.5 ml of suspension from the flasks and it is sown in a test tube with a beef-extract bouillon, and the water from the tank is filtered in a volume of no less than 100 ml. The bacteriological plantings are placed in an incubator at a temperature of 37 °. The results of the plantings are read every 24-48 hours. If there is not culture growth, the laboratory vessels are considered disinfected.

**Requirements for examination of waste water for pathogenic microflora.**

1. Enterprises (institutions) which work with level I and II pathogenicity microorganisms must examine the waste water for the microorganisms which they use in their work.
2. The sampling of waste water must be carried out from all wells of the sewer system of the enterprise (institution) before it enters the general interception ditch.
3. The number of samples is determined by the enterprise (institution) director depending on the type of pathogen, the nature of volume of the work, by agreement with the territorial State Sanitation and Epidemiological Oversight agencies.
4. If there are local purification structures at the enterprise (institution), it is necessary to determine the residual concentration of active materials and used disinfectants in the waste waters before they enter the general interception ditch.
5. Waste water sampling and laboratory investigation are carried out per the normative and procedural documents in force, in compliance with the requirements of biological safety.
6. Each enterprise (institution) must develop "Procedural Recommendations for Waste Water Inspection" with allowance for local conditions and specifics.
7. The results of the investigations are recorded in a special log and signed by the persons who perform them.





- 1 - Биологическая  
 2 - Лаборатория  
 3 - Полевая комната  
 4 - Бактериологическая  
 5 - Предбанник  
 6 - Комната для снятия противоэпидемиологического костюма  
 7 - Комната для одевания противоэпидемиологического костюма  
 8 - Комната для загрузки материала в автоклав  
 9 - Комната обеззараживания инвентаря для содержания б/в животных  
 10 - Комната для разгрузки автоклава  
 11 - Комната для разборки обеззараженного материала (мочалки)
- 12 - окно (дверь) для приема полевой материал  
 13 - окно (передающее окно)  
 14 - проходной автоклав

### Schematic diagrams of rooms in block for work with infected animals

1. biological testing room; 2. dissection lab; 3. field room; 4. bacteriological lab; 5. pre-cabinet; 6. room for removing anti-plague suit; 7. room for donning anti-plague suit; 8. room for placing material in autoclave; 9. room for disinfecting implements for holding biotest animal; 10. room for unloading autoclave; 11. room for sorting disinfected material (washroom); 12. window (door) for reception of field material; 13. airlock (transfer window); 14. walk-through autoclave



**Biological Hazard!**