ANNEX

to Recommendation of
the Eurasian Economic Commission's Board

No. _____ dated _____ ___, 20__

GUIDELINES
for Organizing and Conducting Microbiological Monitoring of the Production Environment for Manufacturers of Sterile and Non-Sterile Medicinal Products

1. Scope

1. These Guidelines contain provisions for the organization and conduct of microbiological monitoring of the production environment for manufacturers of sterile and non-sterile medicinal products of the Member States of the Eurasian Economic Union (hereinafter, the Member States, the Union, respectively).

2. These Guidelines are based on the generalization of scientific data and practical recommendations of international documents and acts of the Union's bodies regulating the circulation of medicines, as well as the generalization of accumulated experience of pharmaceutical manufacturers of the Member States.

3. These Guidelines contain provisions for minimizing the risks of contamination of medicinal products with microorganisms (mold and yeast fungi, spore-forming and non-spore-forming bacteria), allow for the best way to ensure the right level of microbiological monitoring as one of the tools for comprehensive protection of medicinal products.

4. The provisions of this Guidelines and the monitoring parameters for microbiological assessment should be applied to clean rooms (areas), equipment and personnel working in clean rooms (areas).

5. This Guidelines do not address microbiological monitoring of clean media creating conditions for and/or are involved in the production process, such as purified water, water for injection, compressed gas, clean steam, etc.

2. Terms and Definitions

6. For the purposes hereof, the terms below shall have the following meanings:

"biocontamination": contamination of materials, products, people, surfaces, liquids, gases or air with viable particles;

"local isolate": microorganisms found in the production environment;

"monitoring of the production environment": observation of the state of objects of the production environment (rooms, equipment, air of the working area, technological environment, personnel) to determine and (or) predict the moment of transition to the limit state on the basis of comparison of measured parameters with specified values;

"adverse trend": an increase in the frequency of deviations from the alert level or action level, or repeated detection of low concentrations of microorganisms below the alert level during microbiological control, or noncompliance of a pharmaceutical ingredient or finished product, indicating a loss of process control;

"equipped state (idle state)": a state in which the mounting of all installations is complete, including a working HVAC system, along with the installed major production equipment, ready for operation, but not in operation and with no personnel present in the room;

"sampling plan": a documented plan that describes sampling procedures and methods under controlled environmental conditions; it specifies the locations, frequency, and quantities of samples to be taken;

"production environment monitoring program": a documented program that describes the rules for routine production environment control for all monitored

parameters and includes an action plan used if the control results exceed the action level;

"contamination control strategy (CCS)": a planned set of microbial, pyrogen, and particle control activities derived from current product and process understanding that ensures process efficiency and product quality. Monitoring activities may include parameters and characteristics related to APIs, reference materials and product components, operating conditions of production facilities and equipment, in-process control activities, finished product specifications, associated methodologies, and monitoring and control frequencies;

"control point": a point in a clean room at which biocontamination is controlled and at which the hazard can be prevented, eliminated, or reduced to an acceptable level;

"action level": an established relevant value (e.g., microbial limit, airborne particulate limit) that, if exceeded, should trigger an appropriate investigation and corrective actions in accordance with the investigation;

"alarm level": an established relevant value (e.g., microbial concentration, airborne particulate concentration) that provides an early warning of a potential deviation from normal operating conditions and a validated condition that does not necessarily form a basis for a corrective action, but does initiate careful consideration and follow-up activities necessary to identify a potential problem. Alarm levels are set on the basis of historical and trend information and are subject to periodic review (analysis). The alarm level can be based on a number of parameters including atypical trends, individual deviations above a set limit, and recurring events;

"clean area": an area with defined cleanliness standards for particles and microorganisms, usually containing several combined clean rooms;

"clean room": a room designed, maintained, and controlled to prevent contamination of medicines by particles and microorganisms. "class A": a class A area;

"operational (functioning) state": a state in which the cleanroom installation is complete, the HVAC systems are fully operational, and the equipment is installed and operating in the manufacturer's specified operating mode with the maximum number of personnel present, performing or simulating routine production activities.

3. General Provisions

1. Contamination Control Strategy

7. A contamination control strategy (CCS) should be applied at the manufacturing site to identify all critical control points and to evaluate the efficiency of all control (design, procedural, technical and organizational) activities and monitoring activities undertaken to manage risks to the quality and safety of medicines. The combined contamination control strategy should form a sustainable guarantee to prevent contamination. The contamination control strategy should be actively reviewed and updated as appropriate, and should promote continuous improvement in production and control methods. Its efficiency should be part of a periodic management review.

8. Requirements for the production of sterile medicinal products specified in Annex No. 1 to the Rules of Good Manufacturing Practice of the Eurasian Economic Union approved by Decision No. 77 dated November 3, 2016 of the Council of the Eurasian Economic Commission (hereinafter, Annex No. 1 to the Rules of Good Manufacturing Practice) (e.g., contamination control strategy, room design, clean room classification, qualification, validation, monitoring, process clothing and its use) may be used in the production of other types of products, as well as in the production of sterile medicinal products. If a manufacturer chooses to use the requirements specified in Annex 1 to the Rules of Manufacturing Practice for Nonsterile Products, the manufacturer must clearly document which principles have been used and demonstrate its compliance with those principles.

9. The process for implementing a contamination control strategy at a facility is described in Annex No. 1 to these Guidelines.

2. Microbiological Monitoring of the Production Environment

10. Microbiological monitoring of the production environment (hereinafter, monitoring) provides the manufacturer with key information on compliance of production conditions with the established levels of microbial contamination and allows to prevent the release of potentially contaminated medicinal products, as well as to prevent the possibility of microbial contamination in the future by analyzing the obtained data and identifying adverse trends.

11. The principles of quality risk management should be applied to the design of the monitoring system.

12. Monitoring should be sufficient, reasonable, and regular to provide the necessary information to demonstrate the stability of the production environment within established parameters, to evaluate the efficiency of cleaning and disinfection procedures, and to detect changes in the types of microbial flora (e.g., the emergence of organisms resistant to disinfection regimes).

13. For aseptic processes, monitoring should be carried out in a way that covers all interventions, temporary episodes or any episodes of deterioration (spoilage) of the system, and avoids any risks due to interventions when monitoring actions are carried out.

14. If deviations from the established parameters are detected, investigations should be carried out in accordance with the manufacturer's approved procedure.

15. The investigation must be sufficiently complete to determine the root cause and develop the necessary corrective and preventive actions (CAPAs). The investigation should at least include:

review of room maintenance, cleaning and disinfection (areas, equipment) documentation and records;

assessment of the occurrence of extraordinary events;

assessment of the effects of physical or operational parameters (e.g., changes in ambient temperature and/or relative humidity);

personnel training status evaluation;

assessment of the efficiency of the used disinfectants, etc.

16. The depth of the investigation depends on the classification of the deviation (there may be a different approach in case of a deviation from a trend or exceeding the maximum action limits). The investigation must necessarily include an assessment of the impact on the product manufactured during the deviation identification period (or the period of its possible occurrence). Following the investigation, actions should be taken to correct or eliminate the most likely causes of microbial contamination, and actions should be taken to prevent the occurrence of similar abnormalities in the future (CAPAs). The manufacturer should have procedures to verify the efficiency of corrective and preventive actions (CAPAs) taken (timelines, criteria).

17. In addition to changes in the level of microbial contamination of work environment objects, changes in controlled conditions may be indicated by how often the achievement of the alarm and action levels is detected.

18. The total number and types of microorganisms (bacteria and fungi) released from the work environment depend on many factors, including geographical and climatic conditions.

19. The pathways for microorganisms to enter clean rooms (clean areas) are manifold:

personnel (which is the most frequent source of microbial contamination);

air of production facilities;

water and gases used in production;

raw materials (the nature of contamination depends on the nature, storage and processing conditions of raw materials);

packaging materials;

tools, technical equipment, communication devices, containers, carts, cleaning and disinfection equipment, etc.

20. Employees performing sampling should be trained in the sampling rules and requirements for the organization and conduct of microbiological control in the drug manufacturing facilities.

21. The following aspects should be taken into account when organizing microbiological monitoring:

Microorganisms detected under the production environment control activities may be in a damaged state caused by stressful conditions and, therefore, may be difficult to recover;

Due to the specificity of microbiological samples, the collection, transportation and storage issues should be very carefully considered to prevent the risk of microbial death (suppression) or multiplication;

Many factors can affect the detection rate of microorganisms. Identical sample volumes collected using different methods and nutrient media may demonstrate different levels of microbial contamination;

The study results indicate that microbial recovery rates can be < 50 % for surface monitoring, even when relatively high levels of inoculum are used on standardized plates. In real-world production environments where microorganisms are subjected to varying levels of stress exposure, the degree of extraction may be lower;

Monitoring is not able to prove the absence of microbial contamination. A false sense of security should not arise from infrequent detection of microbial contamination. The absence of colony growth in a sample test may indicate that no growth was detected because the result obtained is below the detection limit of the analytical system used. But this does not mean that the production environment is completely free of microbial contaminants.

3. Production Environment Microbiological Monitoring Program

22. To organize monitoring, a manufacturer should develop a production environment microbiological monitoring program, defining the control objects and establishing requirements for the procedure of microbiological control of production facilities (hereinafter referred to as the monitoring program).

23. The monitoring program is developed in accordance with the principles of quality risk management on the basis of the clean room (area) qualification and equipment cleaning validation results (hereinafter referred to as qualification (validation)).

The monitoring program includes:

a routine microbiological control procedure;

control objects and points;

sampling frequency;

maximum action limits, as well as alarm and action levels;

control methods;

trend evaluation frequency and procedure;

a procedure to be followed when adverse trends or deviations are identified;

a procedure for maintaining monitoring records;

a procedure for confirming the monitoring program efficiency and an obtained data usage and transfer procedure.

24. The scope of monitoring determined by the qualification (validation) results and the conducted risk analysis results and included in the monitoring program shall be maintained for a specified period of time (not more than one year), unless any adverse trends or deviations are revealed.

25. After a set period, trends in monitoring results are reviewed and the monitoring program is analyzed.

26. If no changes are necessary, monitoring activities continue at the same level. In this case, the need to revise the monitoring program is determined by the results of subsequent trend reviews for the established period, identified deviation investigation results, as well as when implementing changes.

27. If during a monitoring result trend review and monitoring program analysis a need for any change (control point number (place), sampling frequency, etc.) is revealed, a risk analysis is conducted, based on which changes are made to the monitoring program and a new monitoring scope is established for the next

period (not more than one year). Trends in microbiological control results are reviewed after a specified period and the monitoring program is analyzed.

28. All actions to change the monitoring program are conducted through the change control system.

4. Selection of Control Points

29. During the commissioning of production rooms (areas) and equipment, the manufacturer qualifies the clean rooms (areas) and validates the equipment cleaning processes. For qualification (validation) activities, the control points at which samples will be collected for microbiological testing must be identified. Control points are established based on the risk analysis results. A quality risk level is determined based on the peculiarities of production activities and requirements to the level of microbiological purity.

1. Selection of Microbiological Control Points for Clean Room (Area) Qualification

30. To determine the place and number of microbiological control points for clean room (area) qualification, it is allowed to use the grid method described in Annex No. 2 hereto.

31. Factors to be considered (but not limited to) during a risk analysis performed to select microbiological control points for clean room (area) qualification:

applicability of the grid method for selecting control points;

criticality of the process, which is conducted in a clean room (area);

an acceptable level of microbial contamination of the product;

the level of microbial contamination of the initial raw materials and the possibility of its impact on the room (area) cleanliness;

areas of contact between the production environment and the exposed product;

duration of production processes in a given room (area);

the areas of the room where the staff do the most work;

number of employees, time spent in the room, and a level of activities when performing work;

objects that are difficult to clean and disinfect;

frequency of cleaning (disinfection);

the stage (operation) of the production process that is the most critical in terms of the risk of microbial contamination of the product;

personnel, materials and waste flows;

an air flow (supply-exhaust) profile, ventilation and air conditioning system design (filter classification, HEPA filters in each room or only at the air inlet to the production area, pressure drop profile);

clean room layout (floor area, design (presence of dust and other contamination areas), number of doors, adjacent rooms, through openings from one room to another, etc.);

surface materials used (stainless steel, glass, PVC panels, etc.) in terms of the risk of microcracking;

whether sampling activities at this section can disrupt the production process (risk of erroneous data collection or product contamination);

whether sampling activities should be done during production or at the end of a shift or after critical process operations.

2. Selection of Microbiological Control Points for Equipment Cleaning Validation

32. Factors to be considered (but not limited to) during a risk analysis performed to select microbiological control points for equipment cleaning validation:

product contact;

equipment design: joints, hard-to-clean areas, tight spaces, long hoses, etc.;

application of CIP or manual cleaning;

cleaning and disinfection frequency;

duration of production campaigns.

33. A grid method may be used to select control points for equipment cleaning validation if the equipment design allows that and the equipment area is known.

3. Determination of Microbiological Control Points for Production Environment Monitoring

34. Microbiological control points for routine monitoring of the production environment are determined in accordance with the principles of quality risk management on the basis of the qualification (validation) results. The number of control points during qualification (validation) is, as a rule, extended. Qualification results may indicate the need to optimize the monitoring scope and the number of control points for routine monitoring may be reduced.

35. During production, the control points (e.g. surfaces), may change.

36. The selected control points may be categorized by their inherent risk into critical, medium, and low risk control points. This division is further used in developing a sampling plan, establishing the monitoring frequency, and providing a rationale for microbiological contamination alarm levels and actions at these points.

37. In order to avoid confusion of sampling points, especially in the case of surface inspection, it is recommended to indicate them on diagrams with marked technical equipment, transfer windows and doors. Photographs of control objects with control points can be used.

Such diagrams can be useful in developing sampling plans and determining personnel flows involved in sampling activities in clean rooms (areas).

38. It is allowed to use specially manufactured labels, which can be guaranteed to be subjected to repeated treatment with disinfectants, to identify the control points at sampling points on the technical equipment and in the rooms. An example of the identification is shown in Figure 1.

Figure 1. Identification of control points by means of labels

5. Control Methods

39. For microbiological monitoring, qualitative, semi-quantitative and quantitative methods are used to determine microbiological contamination in the production environment. For routine control, the same methods are used as for the initial qualification (validation) of control objects.

40. A manufacturer should validate the methods used. Validation should include at least the standardization of sampling activities (sampling procedure, sampling device type) and incubation time, determination of recovery from different surface types with acceptable repeatability of results. Revalidation criteria should be established (new sampling methods, types of surfaces, types of sampling devices, etc.).

41. The adoption of appropriate alternative monitoring systems (e.g., rapid method systems) is considered by manufacturers to accelerate the detection of microbiological contamination problems and reduce product risks. Such rapid or automated microbiological monitoring methods may be applied after validation has demonstrated their equivalence to or superiority over the established methodology.

42. If other or new technologies are used, which results are expressed differently (not in CFU), the manufacturer should justify the limits used on the basis

of scientific data and (if possible) indicate their relationship to the limits expressed in CFU.

43. Sampling methods should not pose a risk of microbial contamination to the manufacturing operations.

44. If aseptic operations are performed, the sampling method used should be justified as part of the contamination control strategy. It shall be demonstrated that it does not adversely affect air flows in class A and B areas. Clean room (area) surfaces and equipment should be monitored after a critical operation has been completed.

45. The selected time of sample incubation should be optimal based on the need for rapid results of microbiological tests and the necessary time for recovery of damaged microorganisms and microorganisms with depressed physiological functions.

46. Universal sample incubation conditions necessary for the growth and colony formation of microorganisms present in the production environment, suitable for most cases, are:

for aerobic bacteria: temperature (32.5 ± 2.5) °C and incubation time of 48 h to 72 h;

for yeast and mold fungi: temperature (22.5 ± 2.5) °C and incubation time of 5 to 7 days.

Other shorter incubation times may be used and should be justified by the manufacturer through validation of the results.

At the same time, it should be taken into account that if the incubation time is shortened, there is a risk of obtaining underestimated test results (e.g. for slow-growing microorganisms with suppressed physiological functions or in case of mutual suppression of microorganism growth).

47. A universal nutrient medium for the control of aerobic microorganisms (bacteria and fungi) (e.g., soy-casein/trypticase-soy agar or soy-casein/trypticase-soy broth) may be used. The incubation regime of the universal nutrient medium

should be selected based on the risk analysis results, depending on the types of microorganisms to be isolated from the production environment, and validated.

48. Examples of incubation regimes:

Incubation for 48 – 72 h at (22.5 ± 2.5) °C, followed by transfer to the regime of 48 – 72 h at (32.5 ± 2.5) °C ensures growth of damaged and (or) slow-growing microorganisms. If the temperature coherence is reversed, detection of damaged or slow-growing microorganisms may be difficult;

Incubation for 48 – 72 h at (32.5 ± 2.5) °C, followed by transfer to 48 – 72 h at (22.5 ± 2.5) °C ensures rapid growth of coccal flora.

1. Storage and Transportation Times for Collected Samples

49. The microbiological condition of a sample shall not change between the sampling and testing times. Sample transportation and/or storage times should be kept to a minimum and determined by validation.

2. Methods of Air Control

50. Microbiological air monitoring is conducted using a combination of sedimentation (passive) and aspiration (active) sampling methods. The frequency of the combination is set based on a risk analysis.

51. If aseptic operations are carried out that require continuous microbiological monitoring, the aspiration method may use equipment that provides air sampling during the entire production process. If no such equipment is available, a sampling frequency established on the basis of a risk analysis should be used, e.g.:

sampling at short time intervals (beginning, middle and end of the aseptic filling process);

sampling during critical interventions, aseptic assembly of equipment, etc.

Sedimentation method

52. The sedimentation method is based on settling of aerosol particles with microorganisms on them from the air of the production environment on the horizontal surface of the nutrient medium during a certain period of exposure time.

53. Sedimentation plates (a nutrient medium in Petri dishes) should be exposed to the production environment for no more than 4 hours. For classes A and B, during the entire duration of the operations performed (including equipment set-up), and it should be replaced in accordance with the requirements after no more than 4 hours. Exposure times should be based on validation, including recovery studies; they should not adversely affect the suitability of the media used.

54. Individual sedimentation plates may be exposed for less than 4 hours. In this case, if there is any growth of microbial colonies, recalculation to 4 hours is not necessary, as the maximum action limits specified in Annex No. 1 to the Good Manufacturing Practice should be interpreted as limits for exposure up to 4 hours.

55. The advantages of the sedimentation method are in its ease of use. The main disadvantages of the method are the detection of only large fast-sedimenting live particles, uncertainty in the volume of the sample taken, and the strong influence of the velocity and direction of the airflow relative to the surface of the medium on the test result. In fact, this method is semi-quantitative. This method is appropriate only in combination with an active control method of microbial contamination of the air.

Aspiration method

56. The aspiration method allows determining the number of microorganisms in 1 m^3 using special equipment (aspirators) subject to periodic calibration or verification.

57. Since different sampling devices vary from one to another, a manufacturer should evaluate the overall suitability of the device for sampling under specific operating conditions prior to commissioning (speed (volume flow rate), sampling duration and time, volume of air sample collected, etc.).

58. Depending on the sampling device type, a statistical correction to determine the most likely number of microorganisms in the sample (a Feller correction) may be required when processing the results. Usually, information on the permissibility of such a correction can be found in the operating manual of the device.

59. The device should be selected based on the characteristics of the area where it will be used, taking into account the factors set out in GOST ISO 14698-1, Clean rooms and related controlled environments. Biocontamination control. Part 1. General Principles and Methods.

60. At each control point, samples are taken into two Petri dishes, one with a nutrient medium for bacterial growth and another with a nutrient medium for fungal growth.

61. If a universal nutrient medium is used, it is acceptable to perform sampling at a control point per plate.

62. The volume of the air sample taken in class A and B rooms shall not be less than 1 m^3; in cleanliness classes C and D, it is allowed to reduce the sampling volume and the corresponding recalculations of the results obtained in accordance with the volume of 1 m^3.

3. Methods of Surface Control

63. In microbiological monitoring of surfaces, sampling is performed:

by the contact plate method (replica plating or imprint) using 55 mm diameter Petri dishes (Rodac)/contact plates of known area;

by the flushing method using sterile swabs.

The method used for sampling should be justified.

64. For microbiological monitoring of personnel gloves, the imprint method (5 fingers, from both hands) or the rinsing method using sterile swabs should be used. The method used for sampling should be justified.

65. During control of microbial contamination of surfaces that may contain residual amounts of disinfectants, antimicrobials (e.g. antibiotics), it is recommended to use media containing neutralizers to prevent the risk of suppression of microbial growth caused by various chemicals. If substances not specified in the Pharmacopoeia of the Eurasian Economic Union, approved by Decision No. 100 dated August 11, 2020 of the Board of the Eurasian Economic Commission (hereinafter, the Union's Pharmacopoeia), or the concentration of used pharmacopoeial neutralizers differs from the concentration specified in the Union's Pharmacopoeia, the applicability of such neutralizers and the efficiency of chemical substance neutralization (in the concentration that is actually present on controlled surfaces) should be confirmed during validation.

Contact plate method

66. The essence of the contact plate method is as follows: contact plates with an agar-base nutrient medium are applied to the surface under study and kept for 3 – 5 seconds (contact time) at the same pressure on the surface under study; in this case, contact plates used on flat surfaces are not allowed to rotate. In practical training of personnel, the pressure force can be demonstrated using a scale (the scale reading should tentatively be about 400 g). Specialized equipment (e.g., specialized contact plate applicators that allow standardization of force) may be used to standardize sampling by the contact method.

67. After sampling is performed, the control surface should be cleaned of residues of the nutrient medium and disinfected.

Swab sampling method

68. The essence of the method is rinsing a controlled surface section (hereinafter referred to as the swab area) with a swab moistened with a sterile medium.

69. The swab area can vary from 25 to 100 cm^2. The swab area depends on the criticality of the control object, the room (area) cleanliness class, the controlled object area, the expected level of microbial contamination, etc. The manufacturer should choose the swab area value between 25 and 100 cm^2 based on the principles of quality risk management and taking into account the recovery percent established in the validation of the swabbing method.

70. A phosphate buffer solution with sodium chloride and peptone (pH 7.0) or 0.9 % sodium chloride solution (physiological solution) or another acceptable and industrially manufactured medium, supplied in test tubes with sterile swabs, should be used as a medium for moistening the swab.

71. Volume of a sterile medium in the test tube

When inoculating a swabbing sample on a nutrient medium by the surface (streaking) method, the volume of the sterile medium in the tube should be sufficient only to moisten the swab (1 – 2 ml). It is not allowed to immerse the swab into the sterile medium contained in the test tube after sampling.

When inoculating the swabbing sample by the pour plate method, the volume of the sterile medium in the test tube should be sufficient to rinse the swab (2 – 10 ml). When interpreting the results, the volume taken for inoculation and the total volume of the medium in the test tube must be considered. For example, in case of inoculation of 1 ml of the rinsing fluid obtained by rinsing a swab in 10 ml of the sterile medium, a recalculation is required by multiplying the result obtained by 10.

72. Swab sampling technique

At the sampling place, the swab is moistened by tilting the test tube to allow contact with the sterile medium inside. Swabbing is carried out with a swab located at an inclination to the controlled surface, as shown in Figure 2, carefully wiping the surface with accurate movements of closely spaced lines/strokes, excluding gaps between the strokes. The swabbing is then repeated using the same motion, but in a direction perpendicular to the previous strokes (as illustrated in Figure 3).

Figure 2. Swab positioning during sampling.

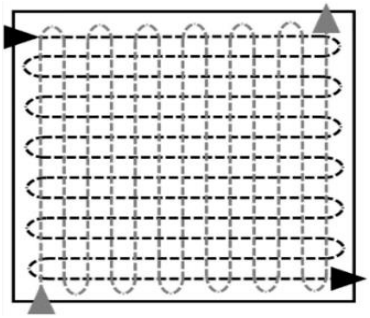


Figure 3. Direction of swab movements when swabbing.

73. Surface inoculation (streaking) method.

The swab must remain moist until inoculation begins. Inoculation should be carried out with a wet swab over the entire agar surface in a Petri dish in parallel closely spaced lines in one direction, without changing the degree of pressure, but rotating the swab along the axis. Each new movement should not overlap the previous one, as shown in Figure 4.

It should be taken into account that the result strongly depends on the technique of execution: the angle of swab inclination, pressure on the swab, the number of strokes made, etc.

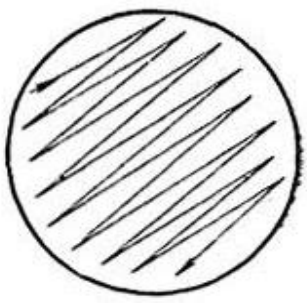


Figure 4. Direction of swab movements when performing inoculation.

74. Pour plate method.

The swab is rinsed thoroughly before inoculation in order to extract microorganisms into the rinsing liquid. Inoculation is done by the pour plate method according to the requirements of the Union's Pharmacopoeia.

In the case of validated inhibitory effects of a residual antimicrobial agent in the rinsing fluid during sampling, that could not be eliminated by other methods, the membrane filtration method is recommended.

75. Imprint method.

Sampling should be performed from both hands. One contact plate or Petri dish is used for each hand. For microbiologic monitoring of gloved hands, five imprints should be made alternately on the agar surface (contact plate or Petri dish). To make the touching complete, alternately tap for at least 5 seconds with each finger on the agar surface so that the imprints do not overlap (as shown in Figure 5).

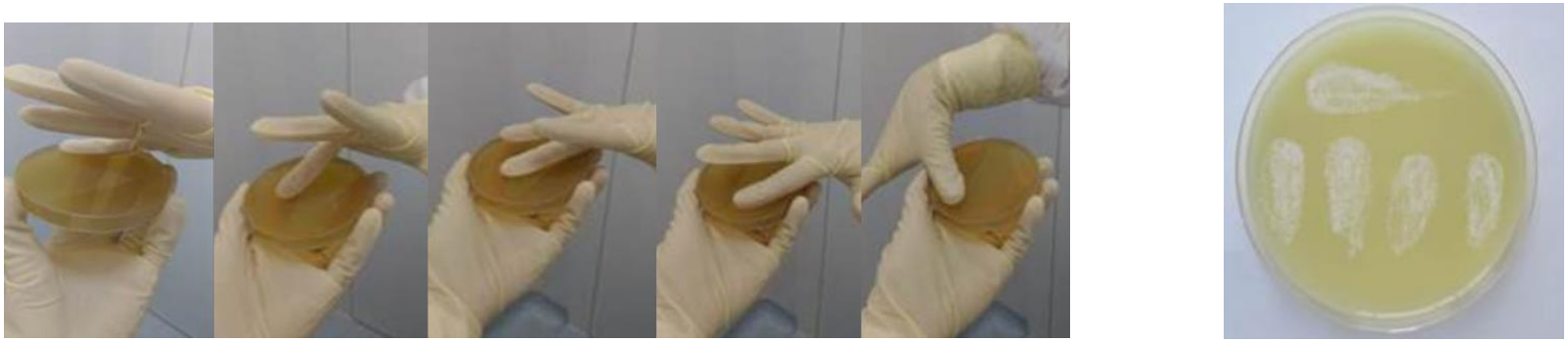


Figure 5. Sampling by the imprint method.

6. Frequency of Control

76. The objective of microbiological monitoring is the timely detection of deviations and the likely path of contamination of controlled production environment objects, while providing the possibility to implement timely and efficient measures to prevent product contamination. The sampling frequency established by the monitoring program shall ensure that this objective is met.

77. The frequency of monitoring in class A and B rooms of sterile drug product manufacturing facilities is set out in Annex No. 1 to Good Manufacturing Practice. A manufacturer should adhere to the established monitoring frequency. The frequency may be changed only to comply with stricter requirements.

78. In the case of aseptic operations, monitoring should also be carried out inside clean rooms (areas) when normal production operations are not performed (e.g. before the production activities start, at the end of a batch production process, and after a shutdown period).

79. For objects with a lower degree of criticality, the manufacturer independently determines the frequency of microbiological control in accordance with the principles of quality risk management. The frequency established should be documented.

80. When determining the sampling frequency, the production environment microbiological control results for the past period should be taken into account.

81. The frequency of microbiological monitoring can vary considerably depending on (but not limited to) the following factors:

the type of product being manufactured;

the length of production campaigns;

planning and technological decisions;

the degree of human intervention in the process (a technological process involving manual operations has an increased risk of product contamination; in such cases, the frequency of routine control should be increased);

the use of final sterilization;

the intensity of manufacturing activities;

the age of the company;

level of maintenance;

the personnel qualification level;

the level of personnel workload;

the control data of the preceding period.

82. In addition, changes in sampling frequency, whether temporary or permanent, may be required on the basis of changes in regulatory requirements, advances in microbiological knowledge, acquisition of new equipment or construction of new facilities, etc.

83. Microbiological control of production rooms (areas) is carried out both in the operated and equipped state. Control of rooms (areas) in the equipped state allows to control the quality of general cleaning and to evaluate the operation of the air treatment system without load. Frequency of control of rooms (areas) in the equipped state is established on the basis of risk assessment taking into account the purpose of rooms (areas), criticality of operations performed in them, etc.

The rooms (areas) and equipment are not monitored:

during cleaning operations;

if the room or equipment is labeled "Cleaning Required", "Repair";

during maintenance operations.

2. Control Frequency and Methods for Manufacturers of Sterile Medicinal Products

Table 1

Air control frequency and methods

Class	Control frequency and methods
A	Continuous microbiological monitoring in class A using a combination of sedimentation and aspiration methods should be carried out throughout the duration of a critical process, including the equipment (aseptic unit) assembly, and the critical
B	

	process itself. For facilities with terminal sterilization, the frequency is set based on a risk analysis. A similar approach should be considered for class B clean rooms based on the risk of exposure to aseptic process. The frequency of combining sedimentation and aspiration methods is established based on a risk analysis.
C	Daily to monthly sampling (depending on the criticality). Sedimentation and aspiration methods in combination with a frequency established based on a risk analysis.
D	Weekly to quarterly (depending on the criticality). Sedimentation and aspiration methods in combination with a frequency established based on a risk analysis.

Table 2

Surface control frequency and methods

Control object Control objects (points) are set by the manufacturer on the basis of the risk analysis results.	Control frequency and methods
Class A	
Equipment surfaces. Working surfaces	For aseptic processes, after the end of a critical process operation. For facilities with terminal sterilization, the frequency is set based on a risk analysis. Contact plate method and/or swabbing method.
Room surfaces	After completion of a critical process operation. For facilities with terminal sterilization, the frequency is set based on a risk analysis. Contact plate method and/or swabbing method.
Floors	Daily to monthly (depending on the criticality) Contact plate method and/or swabbing method.
Class B	
Equipment surfaces. Working surfaces	After and/or during a critical process operation if the risk caused by sampling is minimized. For facilities with terminal sterilization, the sampling frequency shall be established based on a risk analysis. Contact plate method and/or swabbing method.
Room surfaces	After or during a critical process operation if the risk caused by sampling is minimized.

Control object Control objects (points) are set by the manufacturer on the basis of the risk analysis results.	Control frequency and methods
	For facilities with terminal sterilization, the sampling frequency shall be established based on a risk analysis. Contact plate method and/or swabbing method.
Floors	Weekly to monthly (depending on the criticality) Contact plate method and/or swabbing method.
Class C	
Equipment surfaces. Working surfaces	Weekly to monthly (depending on the criticality) in the operational state Contact plate method and/or swabbing method.
Room surfaces	Weekly to monthly (depending on the criticality) Contact plate method and/or swabbing method.
Floors	Weekly to monthly (depending on the criticality) in the operational state Contact plate method and/or swabbing method.
Class D	
Equipment surfaces. Working surfaces	Weekly to quarterly (depending on the criticality) in the operational state Contact plate method and/or swabbing method.
Room surfaces	Monthly to quarterly (depending on the criticality) in the operational state Contact plate method and/or swabbing method.
Floors	Monthly to quarterly (depending on the contact plate method and/or swabbing method).

Table 3

Control frequency and methods of personnel gloves and cleanroom apparel

Control object (control points are set by the manufacturer based on the risk analysis results)	Control frequency and methods
Class A	
Gloves	After performing a critical process operation. Each time when exiting a class A clean area

Control object (control points are set by the manufacturer based on the risk analysis results)	Control frequency and methods
	Glove imprint method (5 fingers, from both hands). For sampling from hard-to-reach places (between fingers), it is acceptable to use the swab method.
Forearms	Each time when exiting a class A clean area. If the operations are manual, each employee after a critical process operation. Contact plate method and/or swabbing method.
Hood (around the mask), forehead, upper front of overalls	
Class B	
Gloves	In each series, after critical process operations have been performed Each time when exiting a class B clean room Glove imprint method (5 fingers, from both hands). For sampling from hard-to-reach places (between fingers), it is acceptable to use the swab method.
Forearms	Each time when exiting a class B clean room (gloves and apparel). Contact plate method and/or swabbing method.
Hood (around the mask), forehead, upper front of overalls	
Class C	
Forearms	At least once every six months. Contact plate method and/or swabbing method.
Upper front of the overalls, forehead	
Class D	
Forearms	At least once every six months. Contact plate method and/or swabbing method.
Upper front of the overalls, forehead	

84. The risk assessment should evaluate the points and frequency of personnel monitoring based on the operations being performed, as well as the proximity to critical areas. Monitoring should include periodic sampling from personnel during the process.

85. If glove monitoring is performed after critical interventions; the top pair of gloves should be changed before the resumption (continuation) of an operation or activity.

86. If cleanroom apparel monitoring is required after critical interventions, the apparel should be changed before continuing the operation or activity in a clean room.

87. If operations are manual (e.g. aseptic preparation or filling), the increased risk should lead to a clear focus on microbiological monitoring of the cleanroom apparel. This should be justified in the contamination control strategy.

88. If several successive interventions are required in a class A area during equipment assembly or repair work, monitoring of personnel cleanroom apparel and gloves should be performed after all successive interventions have been completed.

89. For the manufacture of aseptic medicines, the need for the following monitoring activities should be assessed.

After significant interventions in a class A area (e.g., arm and upper torso penetration). Gloves and at least 4 points of the upper part of the cleanroom apparel (mask/hood, upper front of overalls, sleeves (forearms)) should be monitored.

After minor interventions in a class A area (sleeves (forearms) and gloves only). It is allowed to control only gloves.

Control Frequency and Methods
for Manufacturers of Non-Sterile Medicinal Products

Table 4

Air control frequency and methods

Class	Control frequency and methods
C	Weekly to monthly sampling (depending on the criticality). Sedimentation and aspiration methods in combination with a frequency established based on a risk analysis.
D	Weekly to quarterly (depending on the criticality) Sedimentation and aspiration methods in combination with frequency established based on a risk analysis.

Table 5

Surface control frequency and methods

<table>
<tr><td colspan="2">Control object
Control objects (points) are set by the manufacturer on the basis of the risk analysis results.</td><td>Control frequency and methods</td></tr>
<tr><td colspan="3">Class C</td></tr>
<tr><td rowspan="2">Equipment surfaces</td><td>direct product contact</td><td rowspan="4">Weekly to monthly (depending on the criticality).
Contact plate method and/or swabbing method.</td></tr>
<tr><td>no product contact</td></tr>
<tr><td colspan="2">Working surfaces</td></tr>
<tr><td colspan="2">Room surfaces</td></tr>
<tr><td colspan="3">Class D</td></tr>
<tr><td rowspan="2">Equipment surfaces</td><td>direct product contact</td><td>Weekly to monthly (depending on the criticality).
Contact plate method and/or swabbing method.</td></tr>
<tr><td>no product contact</td><td rowspan="3">Weekly to quarterly (depending on the criticality).
Contact plate method and/or swabbing method.</td></tr>
<tr><td colspan="2">Working surfaces</td></tr>
<tr><td colspan="2">Room surfaces</td></tr>
</table>

Table 6

Control frequency and methods for personnel gloves and cleanroom apparel

Control object (control points are set by the manufacturer based on the risk analysis results)	Control frequency and methods
Class C	
Gloves	The need for and frequency of control are established on the basis of a risk analysis. Imprint method (5 fingers). For sampling from hard-to-reach places, it is allowed to use the method of rinsing with a swab.
Cleanroom apparel (forearms, upper front of overalls at the level of production operations)	At least quarterly. Contact plate method and/or swabbing method.
Class D	
Gloves	The need for and frequency of control are established on the basis of a risk analysis. Imprint method (5 fingers).

Control object (control points are set by the manufacturer based on the risk analysis results)	Control frequency and methods
	For sampling from hard-to-reach places, it is allowed to use the method of rinsing with a swab.
Cleanroom apparel (forearms, upper front of overalls at the level of production operations)	At least once every 6 months. Contact plate method and/or swabbing method.

7. Maximum Action Limits

90. The maximum action limits for microbial contamination of objects in the production environment have been established.

91. To determine the maximum action limits for objects (control methods) not specified in Annex No. 1 to the rules of Manufacturing Practice, the statistical methods of calculation based on historical data should be used (Annex No. 3 to these Guidelines).

92. A conversion factor determined as a result of the swabbing method validation against the contact plate method may be used to determine the maximum action limits for the swabbing method. The conversion factor is only valid for validated conditions (used nutrient media, swab types, etc.)

93. The maximum action limits for the equipped state are set independently by the manufacturer on the basis of a risk analysis and historical data.

8. Alarm and Action Levels

94. Alarm and action levels for monitoring objects shall be set individually, based on the qualification (validation) results and analysis of available historical data of routine monitoring, taking into account the maximum limits of action levels for microbial contamination established in Annex No. 1 to the rules of Manufacturing Practice.

95. Correctly established alarm and action levels can ensure early detection of unfavorable trends.

96. There are different ways to integrate alarm and action levels into a monitoring program. In one case, the maximum action limit is supplemented by an alarm level set based on historical data. In another case, the maximum action limit can be supplemented by two control levels: an alarm level and an action level.

97. For critical production rooms (areas) of the aseptic process, it is expedient to use a single reference level value that is both an alarm level and an action level at the same time.

98. The alarm and action levels should be applied in accordance with a written procedure. To ensure consistency in analyzing alarm and/or action levels, the logical steps of investigation and/or corrective actions should be defined in advance. Records should demonstrate that any exceedance has been assessed and that appropriate follow-up activities have taken place.

8.1. Determining Numerical Values of the Alarm and Action Levels in the Absence of Historical Data

99. Initially, in the absence of historical data, the alarm and action levels can be defined as a percent of the maximum action limit set based on a risk analysis taking into account the qualification (validation) results.

Examples of possible alarm and action level options are shown in Tables 7 and 8.

Table 7

Example of alarm and action level values (option 1)

Cleanliness class	Normative values	Action level (75 % of the maximum action limit)	Alarm level (50 % of the maximum action limit)
Air (sedimentation, CFU/4 hours)			
B	5	4	3
C	50	38	25
D	100	75	50
Air (aspiration, CFU/m^3)			
B	10	8	5
C	100	75	50
D	200	150	100

Cleanliness class	Normative values	Action level (75 % of the maximum action limit)	Alarm level (50 % of the maximum action limit)
Surfaces (contact plates (diameter: 55 mm), CFU/plate)			
B	5	4	3
C	25	19	13
D	50	38	25

Table 8

Example of alarm and action level values (option 2)

Cleanliness class	Normative values	Action level (50 % of the maximum action limit)	Alarm level (25 % of the maximum action limit)
Air (sedimentation, CFU/4 hours)			
B	5	3	1
C	50	25	6
D	100	50	13
Air (aspiration, CFU/m^3)			
B	10	5	1
C	100	50	13
D	200	100	25
Surfaces (contact plates (diameter: 55 mm), CFU/plate)			
B	5	3	1
C	25	13	3
D	50	25	6

2. Determination of Numerical Values of the Alarm and Action Levels Based on Accumulated Historical Data

100. Alarm and/or action levels can be derived statistically from historical data. With this approach, one should expect random deviations from these levels at frequencies characteristic of the particular mathematical model used in their calculation.

101. In the pharmaceutical industry, a wide variety of statistical tools exist for monitoring processes and determining reference levels, but the difficulty in using these tools in relation to microbiological data should be considered, as they:

usually do not have a normal distribution;

often contain many null values;

are highly variable;

are highly dispersed;

in some cases, only a small amount of data is available (e.g., when spot control is applied).

102. Acceptable options of tools for statistical processing of monitoring results to determine numerical values of the alarm and action levels are given in Annex No. 3 to these Guidelines.

103. In order to avoid too high values of the alarm and action levels, data exceeding the maximum action limits should be excluded when calculating by statistical methods.

104. It is recommended that the calculated reference levels be reviewed at least annually or at other frequencies established by the monitoring program to ensure that sufficient additional monitoring results are included in the calculation.

9. Trend Assessment (Trend Analysis)

105. Monitoring procedures should define the approach to assessing trends. Trends include, but are not limited to, the following indicators:

an increasing number of out-of-limit values for the action or alarm levels;

consecutive out-of-limit values for the alarm levels;

regular but isolated violations of the action level limits that may have a common cause (e.g. individual (single) deviations always occurring after scheduled preventive maintenance);

changes in the type of microbial flora and in the number and prevalence of certain microorganisms. Particular attention should be paid to microorganisms detected that may indicate loss of control, deterioration of cleanliness, or the presence of microorganisms that are difficult to control, such as spore-forming microorganisms and mold fungi.

106. The evaluation of trends allows to analyze long-term changes in microbiological monitoring data, identify the points of process exit from the stable state and to determine the direction and intensity of changes in the microbiological status of the production environment. It is recommended to use result distribution graphs for visualization.

107. Statistical evaluation of microbiological monitoring data should be carried out by the manufacturer at least once a year. An important point is that the statistical processing does not take into account results outside the established limits.

108. The main trend assessment steps are:

data collection;

selection of analytical methods;

statistical analysis of data: sample definition, sample analysis, comparative analysis of samples;

interpretation of the results of the completed analysis.

109. The trend assessment results are the basis for the development of corrective and preventive actions aimed at reducing the risk of contamination and maintaining an acceptable level of the microbial load in the production environment.

110. On the basis of the trend assessment, alarm and action levels are calculated for the next reporting period.

1. Recommended Methods for Trend Evaluation:

Control point analysis

111. This method graphically describes the microbial load at a particular control point at a specific time point, and visually demonstrates the point's exceedance of specified levels. For visualization, results distribution graphs are used, showing (using lines) the alarm and action levels, as well as the maximum allowable limit of microbial contamination.

Detection frequency

112. For aseptic production, the method of detection of random fluctuations from stable trends can be used, which is described in the U.S. Pharmacopoeia as one of the main pharmacopoeias in accordance with the Concept of Harmonization of the Pharmacopoeias of the Eurasian Economic Union Member States, approved by Decision No. 119 dated September 22, 2015 of the Council of the Eurasian Economic Commission. The method estimates data by growth by determining the percentage of samples with any sprouts to the total number of samples taken.

For example, a detection rate of 1 % would mean that only 1 % of the samples taken have any contamination, regardless of the number of colonies (99 % of the samples taken are completely free of contamination).

The frequency of detection indicator is calculated according to the formula:

$$\frac{\text{Number of nonzero results}}{\text{Total number of results}} \times 100\%$$

To assess the trend, the indicator is compared to the previous period.

Manufacturers of non-sterile medicinal products can also use the principle of this method to establish detection frequency criteria (e.g. certain types of microorganisms in the production environment, etc.).

Limit value approach (percentile)

113. The limit value approach (percentile) is described in Annex No. 3 to these Guidelines.

Normal distribution model approach
(3σ Rule)

114. The normal distribution model approach is described in Annex No. 3 to these Guidelines.

Shewhart charts

115. The method of constructing a Shewhart control chart is described in Annex No. 3 to these Guidelines.

Review of species diversity

116. The method allows to identify the causes of contamination in production.

10. Identification of Microorganisms

117. Identification of microorganisms is the determination of the belonging of individual populations of microorganisms to a species, genus, family on the basis of the study of cultural, morphological, tinctorial, biochemical and other properties.

118. Identification plays an important role in assessing the potential impact of microorganisms identified during monitoring on the stability of the relevant production environment conditions and product quality in accordance with the standards.

119. The required detailing of the properties of microorganisms depends on the cleanliness class of the room (area) from which the microorganisms are isolated and is presented in Table 9:

Table 9

Degree of identification of microorganisms isolated from the production environment

Cleanliness class	Strain identification				
	at a value up to the alarm level	if the alarm level is exceeded	if the action level/ maximum microbial contamination limit is exceeded	if spore-forming microorganisms are isolated	if mold fungi are isolated
A, B*	species identification				
C, D**	–	by culture-specific, morphologic characteristics	genus (species) identification	by culture-specific, morphological, tinctorial characteristics	by culture-specific, morphologic characteristics

*Microorganisms found in class A and B areas should be identified to their biological species. The potential impact of such microorganisms on product quality (for each batch affected) as well as on the overall control condition should be assessed.

**Care should be taken to identify microorganisms found in cleanliness class C and D rooms (e.g., when action limits or alarm levels are exceeded) or following the isolation of microorganisms that may indicate loss of control, deterioration of cleanliness, and when microorganisms that are difficult to control (e.g., spore-forming microorganisms or mold fungi) are isolated. Such work should be carried out with a sufficient frequency in accordance with an appropriate understanding of the typical flora present in these areas. The frequency of microbial identification should be justified and prescribed in internal procedures.

120. Representative local isolates (hereinafter referred to as local isolates) from the production environment can be used in the control of nutrient media used for monitoring of the production environment, validation tests, control of antimicrobial efficiency of disinfectants, etc.

121. It is recommended to supplement the working collection of pathogens deposited in the collections and depositories of microorganisms specified in the Union's Pharmacopoeia with local isolates.

122. For each local isolate, a data sheet (specification) should be prepared, which should contain information on the date of isolation and species properties, and may also contain its visualization (e.g., a photograph of the isolated microorganism).

123. Local isolates should belong to those species of pathogens, the possibility to perform activities with which is confirmed by the relevant license (if applicable).

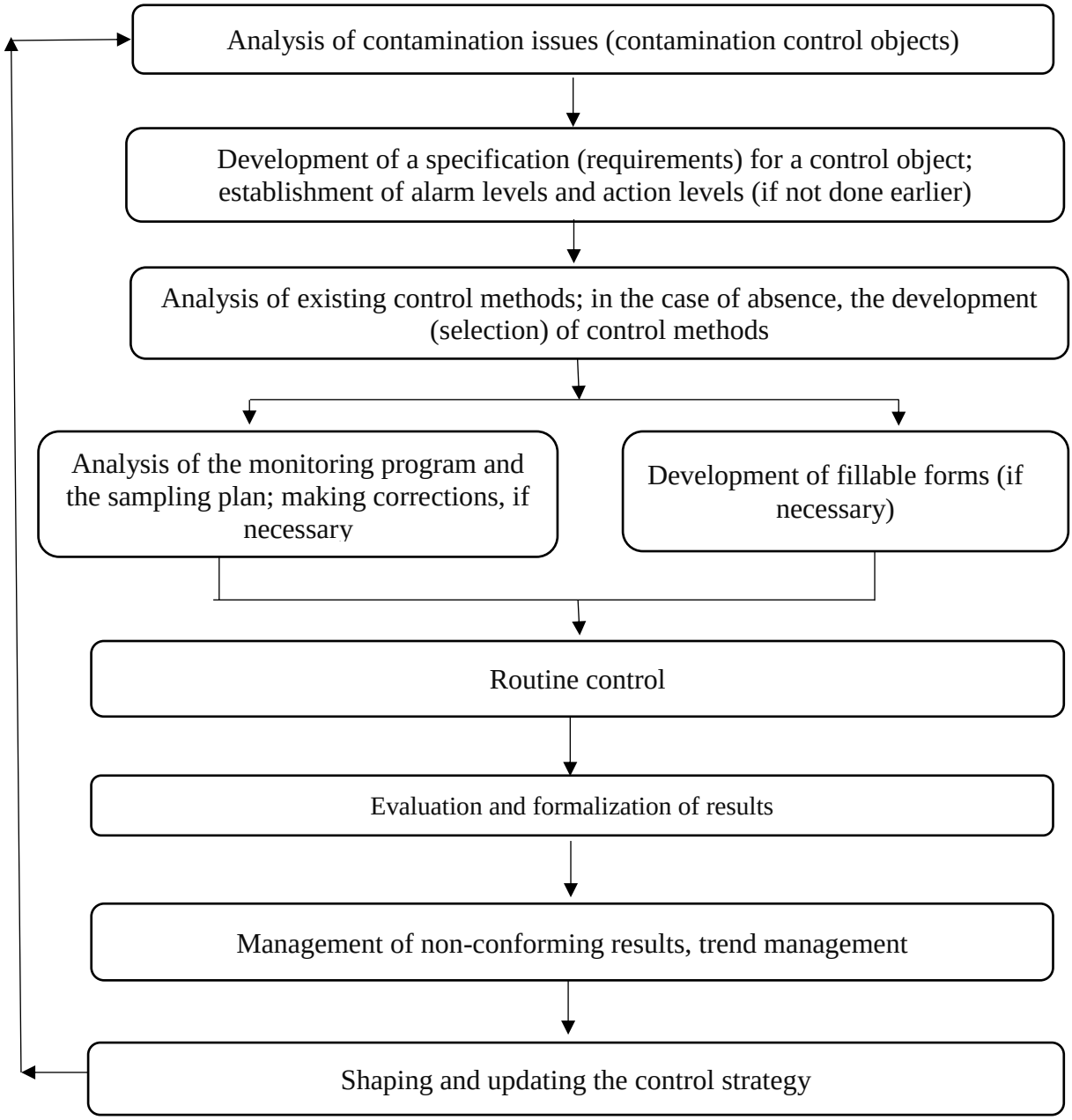
124. Local isolates should be maintained and used in accordance with established procedures for the maintenance and use of a working collection of pathogens.

125. In order to determine seasonal trends, emergence of new microorganism species, etc., the frequency of review of detected microorganisms should be established.

Annex No. 1

to the Guidelines for Organizing and Conducting Microbiological Monitoring of the Production Environment for Manufacturers of Sterile and Non-Sterile Medicinal Products

FLOW CHART
of Implementation of a Contamination Control Strategy at a Company



Annex No. 2

to the Guidelines for Organizing and Conducting Microbiological Monitoring of the Production Environment for Manufacturers of Sterile and Non-Sterile Medicinal Products

GRID METHOD

1. Divide the clean room (clean area) into an equal number of sections with equal areas. The number of sections depends on the floor area of the clean room (clean area) and is defined as the minimum number of sampling points.

If no other criteria are defined in the risk analysis, the number of sampling points should be determined based on the following conditions:

the number of air sampling points:

N/3, but not less than one (according to Table 1);

the number of surface sampling points:

3 + N/3 in each work area,

where

N is the minimum number of sampling points in accordance with GOST R ISO 14644-1, Cleanrooms and associated controlled environments. Part 1. Classification of air cleanliness by particle concentration.

Table 1

Recommended minimum number of air sampling points

Floor area of the clean room (clean area), m^2	Minimum number of sampling points
≤ 8	1
$> 8 \leq 28$	2
$> 28 \leq 52$	3
$> 52 \leq 68$	4
$> 68 \leq 104$	5
$> 104 \leq 148$	6

Floor area of the clean room (clean area), m^2	Minimum number of sampling points
> 148 ≤ 232	7
> 232 ≤ 436	8
> 436 ≤ 1000	9
> 1000	calculated according to the formula*

* The minimum number of sampling points is calculated by the formula:

$$N_L = \frac{(27 \times \left(\frac{A}{1000}\right))}{3},$$

where:

N_L is the minimum number of sampling points to be evaluated, rounded to the next whole number;

A is the area of a clean room (clean area), m^2.

2. Prepare information (in a tabular or other form) on the operations conducted within each section. At this stage, it may be necessary to group sections into functional blocks, as defining control points in each section for some facilities may not be practical and may not take into account the interdependencies of the steps in the production process. It is therefore recommended that the individual sections are grouped into functional blocks that cover different similar production processes (e.g. the filling block of a filling line, or the process control block).

3. Determine the location of control points on the basis of the risk analysis.

The provisions of subsections 1 and 2, Section 4 of these Guidelines should be considered during a risk analysis.

During the risk analysis, it may be necessary to identify additional control points within the perimeter of assigned sections at locations that are

recognized as critical. Additional sections and control points can be obtained by dividing a section into equal areas.

Annex No. 3

to the Guidelines for Organizing and Conducting Microbiological Monitoring of the Production Environment for Manufacturers of Sterile and Non-Sterile Medicinal Products

METHODS FOR CALCULATING MICROBIAL CONTAMINATION LIMITS

1. Limit value approach (percentile)

1. A percentile is a measure used in statistics indicating the value below which a given percentage of the results obtained from a sample fall. That is, a percentile is a defined portion of the sample that includes values below or equal to a specified maximum action limit. For example, the 95th percentile is the score below which 95% of the results obtained do not exceed a specified maximum action limit indicator.

2. The percentile directly affects the acceptance threshold below which a monitoring result is considered part of the expected variability. For example, if 100 tests per year are performed as part of monitoring, the 99th or 95th percent would indicate that 1 or 5 values are expected to exceed the reference level, respectively, even if the monitored environment is completely stable.

3. The alarm and action levels should be calculated based on historical data for the period established by the monitoring program.

When calculating percentiles, data exceeding the maximum action limits should be excluded to avoid alarm and action levels that are too high.

The percentile is calculated using the formula:

$$P\,(x) = \frac{n}{N} \times 100\,,$$

where:

P(x) is the percentile; x is the estimated indicator;

n is the number of values below "x";

N is the total number of values.

Based on the results of previously conducted microbiological monitoring studies, the following percentile values are optimal (without additional justification):

for alarm levels:

k = 0.975 (97.5th percentile) or k = 0.95 (95th percentile);

for action levels:

k = 0.9985 (99.85 percentile) or k = 0.99 (99th percentile).

The choice of a different percentile value used in the calculations should be justified in the monitoring program.

4. If levels calculated through percentile fall below the values set by the monitoring program (e.g., those specified in Tables 7 and 8 of these Guidelines), the tabulated alarm (action) levels should be used to estimate trends for the next period. Comparing levels calculated through percentiles to tabulated values prevents setting levels that are too low to be of value for trend assessment.

2. Normal distribution model approach (3σ Rule)

5. The 3σ Rule, or rule of three standard deviations, is a statistical tool used to determine the value of deviation of an observed variable from the mean.

This approach is recommended only when there is a large amount of data (more than 100 points) and the distribution model is in accordance with a normal distribution. Alarm and action levels are set based on the calculation of the average value.

6. The 3σ Rule can be applied to any data that has a normal distribution.

7. The 3σ Rule allows us to identify values that are far from the mean and may be outliers. This helps identify data anomalies and potential problems.

8. The 3σ Rule has restrictions:

a) the assumption of normal distribution. The 3σ Rule is based on the assumption that the data has a normal distribution. If the data do not meet this assumption, the rule may produce inaccurate results;

b) the restrictions of interpretation. The 3σ Rule allows us to determine the probability of finding values within a certain range, but does not give exact numerical values. This limits its application in some cases where accurate probability estimation is required;

c) dependence on a sample size. The 3σ Rule may produce different results depending on the sample size. The larger the sample, the more accurate results can be obtained using the 3σ Rule.

d) it does not take other factors into account. The 3σ Rule does not take into account other factors that may affect the right data. It considers only the spread of values relative to the mean, without considering possible systematic errors or the influence of other variables.

The normal distribution is calculated using the formula:

$$\varphi(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{(x-m)^2}{2\sigma^2}},$$

where:

π is 3.142;

e is the basis of the natural logarithm 2.718;

m is mathematical expectation (arithmetic mean of the sample);

σ^2 is the variance;

σ is the value of standard deviation;

x is the value for which the probability density function is calculated.

The plotted normal distribution will take the form of a bulb-shaped curve (as shown in Figure 1) with the ranges of + σ, + 2σ, + 3σ labeled.

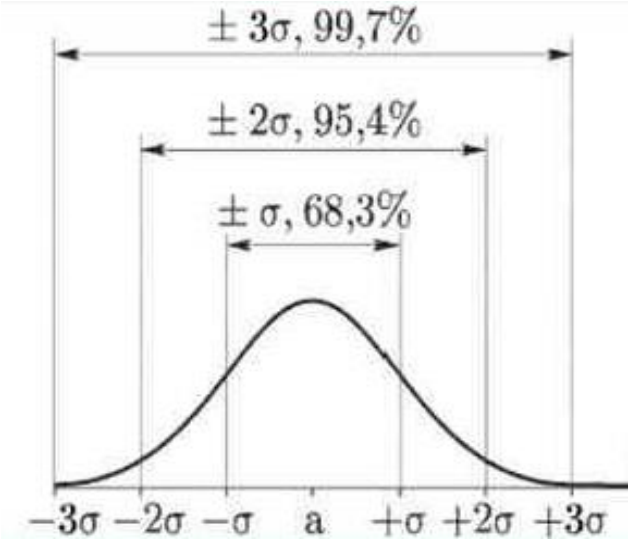


Figure 1. Normal distribution graph

Action level = M ± 3σ. Alarm level = M ± 2σ

By superimposing the experimental results on the microbiological monitoring value density plot, the distribution of the results obtained can be evaluated. Under a normal distribution, approximately 99.7% of all values fall within 3σ of the mathematical expectation (mean), about 95% fall within 2σ, and about 68% of values fall within only σ.

The 2σ values are, as a rule, the action level.

3. Shewhart charts

9. The purpose of constructing a Shewhart control chart (hereinafter referred to as a Shewhart chart) is to identify the points of process deviation from the stable state for subsequent identification of the causes of the deviation and their elimination. The objectives of constructing a Shewhart chart are to determine the boundaries of a systematic process variability and to predict the process behavior in the near future based on past data about the process.

10. The Shewhart chart has a center line in accordance with the mean value of the characteristic, two statistically defined control limits relative to the center line, which are called the upper control limit and the lower control limit. The control

limits on the Shewhart chart are + 3σ away from the center line, where σ is the general standard deviation of the statistic used.

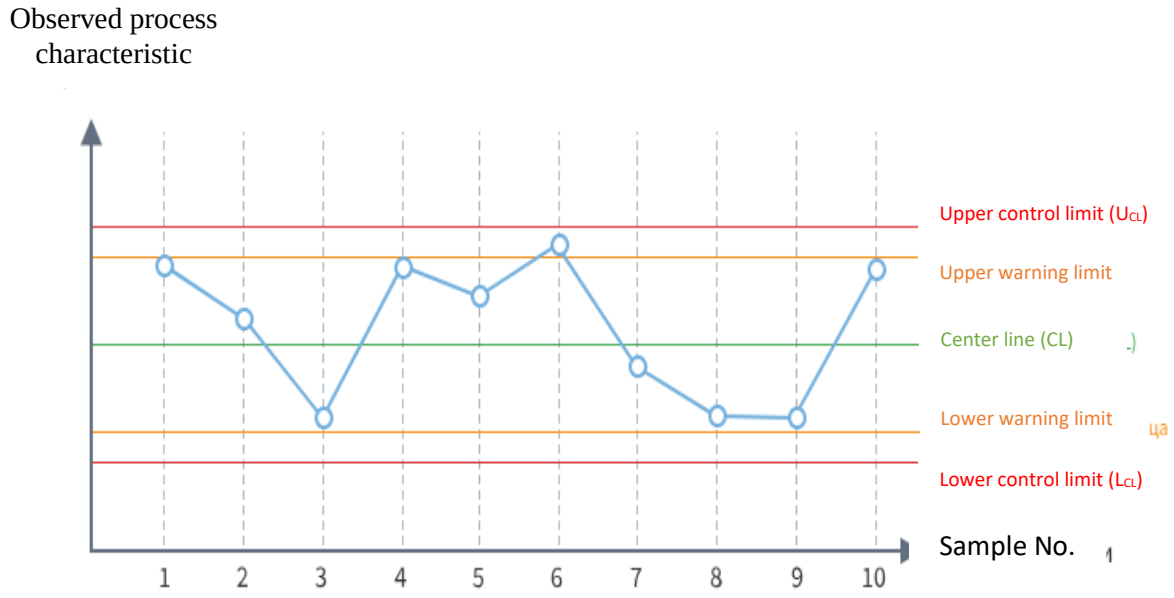


Figure 2. Shewhart control chart

11. Shewhart charts allow us to assess, in addition to the distribution of values and their symmetry, the presence of out-of-trend (OOT) values.

The OOT attributes are:

a) nine consecutive points in the + σ zone or on the same side of the center line;

b) six increasing or decreasing points in a row;

c) fourteen alternately increasing or decreasing points;

d) two out of three consecutive points in the + 3σ zone;

e) four out of five consecutive points in or outside the + 2σ zone;

f) fifteen consecutive points in the + σ zone above and below the center line;

g) eight consecutive points on both sides of the center line and none in the + σ zone.

The manufacturer may not apply all of the attributes to assess OOT values, but may choose some combinations of them.

12. The results of microbiological monitoring, according to the accumulated experience, have a distribution different from normal, which is characterized by the following:

the histogram of the data is not bell-shaped, but has a more beveled or pronounced asymmetric shape;

the asymmetry coefficient is greater or less than 0;

high excess ratio;

The p-value of the Shapiro-Wilk test (a statistical test of data normality) is less than the chosen level of significance (usually 0.05).

With a distribution other than normal, only 88% instead of 99.7% of all values can be in the + 3σ range. In this case, it is necessary to carry out a risk assessment taking into account the impact of the production environment on the quality of manufactured products, to assess the correlation between microbial contamination of the production environment and microbiological purity of manufactured medicines.

4. Approach based on an assessment of historical microbial contamination data

13. For all control objects in cleanliness class B, C and D:

the alarm level is determined by the formula:

$$C_{mean} + 3\sqrt{C_{mean}},$$

where:

C_{mean} is the mean CFU value for the maximum possible time interval (at least 100 values);

the action level is determined by the formula:

$$C_{mean} + 6\sqrt{C_{mean}},$$

where:

C_{mean} is the mean CFU value for the maximum possible time interval (at least 100 values).