MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION

GENERAL PHARMACOPOEIA MONOGRAPH

Immunobiological medicinal products	**GPM. 1.8.1.0002.15** First Edition

The present General Pharmacopoeia Monograph applies to immunobiological medicinal products. Immunobiological medicinal products (immunobiologicals) are medicinal products of biological origin designed for the immunological diagnostics, prevention, and treatment of various diseases. Administration of immunobiologicals to a human results in the acquisition of active (vaccines, toxoids) or passive (normal and specific human immunoglobulins, including hyperimmune plasmas, heterologous immunoglobulins and sera, and monoclonal antibodies) immunity, Immunobiologicals also include some other medicinal products of biological origin (bacteriophages, probiotics, cytokines, including interferons, allergens and allergoids, microbial enzymes), as well as medicinal products obtained by biotechnological processes, including genetic engineering techniques.

All immunobiologicals can be divided into therapeutic and therapeutic-prophylactic, prophylactic proper, and diagnostic agents based on their application.

Immunobiologicals can contain live or inactivated microorganisms (bacteria, viruses), their antigens, including proteins, peptides, or derivatives of proteins and peptides, glycoproteins; antibodies to them and other active substances of biological origin (such as cytokines, monoclonal antibodies, cell receptors, recombinant proteins similar to plasma coagulation factors, vaccines containing recombinant proteins, etc.).

Immunobiologicals are manufactured both as monodrugs and as complex (combined or associated) drugs.

Immunobiologicals may contain excipients that have different functions (ad-

juvants, sorbents, preservatives, stabilizers, fillers, etc.) and should be approved for the manufacture of immunobiologicals.

MANUFACTURE

The manufacture of immunobiologicals involves a complex and diversified technological process (e. g., culture of microbial strains and eukaryotic cells, extraction of substances from human and animal biological tissues and blood, use of recombinant DNA technology, hybridoma technology, etc.). The manufacture of immunobiologicals should be in compliance with applicable manufacture organization and drug quality control requirements. Changes in the manufacturing process, adoption of new regulations or manufacturing method, which can affect the quality of immunobiologicals and / or the reproducibility of the process, necessitate that evidence be available of their suitability for batch manufacture, as well as validation materials.

Quality of immunobiologicals is ensured by the following main conditions:

- use in the manufacturing process of only well-studied, genetically stable industrial microbial strains comprehensively characterized and stored in official collections, which should be checked annually for all biological properties in accordance with regulatory requirements; genetic stability of an industrial strain is the criterion that limits the microbial passage number;
- adequate culture media should be used, which should be capable of good growth (raw materials, reactants and reagents used in the manufacture of culture media should have a Certificate of Quality);
- cell cultures stored in official collections and approved for use in manufacture should be used, as recommended by the WHO (native human serum and penicillin antibiotics must not be used for cell cultures);
- chicken embryos used in the manufacture of immunobiologicals should be obtained only from healthy poultry in poultry farms free from chicken infections; the quality of supplied embryos should be demonstrated by veterinary certificates and certificates issued by the veterinary laboratory

concerning the sanitary status of the livestock, including microbiological and biochemical controls;

- animals and birds used in the manufacture of immunobiologicals should be obtained from farms free from bacterial, viral, prion, and other infections pathogenic for humans, which should be confirmed by veterinary certificates and certificates issued by the veterinary laboratory concerning the sanitary status of the livestock, including microbiological and biochemical controls;
- all requirements established for the donor's health are met during the manufacture of immunobiologicals from human blood plasma and blood cells and human organ cells;
- immunobiologicals should meet all requirements of the Normative Document on all quality parameters.

Drug substances used in the manufacture of dosage forms, if manufactured by a different enterprise, must be registered in accordance with the established procedure.

In the event the manufacture and / or testing of the medicinal product involves use of microorganisms belonging to pathogenicity (hazard) groups I – II or III – IV, all handling should be done in accordance with applicable sanitary-epidemiological requirements.

Some tests that serve as critical steps / points in the manufacturing technology must be determined for intermediate manufacturing stages; such tests, for instance, include air-tightness and vacuum testing.

Air-tightness and vacuum testing.

1. Ampoule and vial air-tightness should be determined by physical testing. Vials and ampoules must be air-tight; all vial and ampoules belonging to the respective batch of the immunobiological should be controlled.

Vials and ampoules containing the medicinal product should be placed in a cassette and immersed in a container filled with purified water stained with methylene blue solution (the samples should be completely under water). The container

is then covered with its lid, and excessive pressure is achieved (0.7 ± 0.05 MPa) and maintained for 1 – 3 minutes. After that, atmospheric pressure is achieved, the container is opened, the cassette with the samples is taken out and examined for the presence of methylene blue solution-stained water in the vials. Samples containing stained water should be discarded.

2**.** The presence of vacuum in ampoules are determined by visual examination, by the colour of the luminescence produced by the gas (determination of the colour of the luminescence produced by the gas in ampoules containing immunobiologicals following its stimulation with a high-frequency electric field by means of d'Arsonval or Tesla apparatuses). Air pressure inside the ampoule is the decisive parameter for ampoule air-tightness and vacuum control. The range of measurement is from 10 Pa to 100 kPa. Acceptable pressure values are from 10 Pa — 1 kPa. The frequency of electromagnetic waves varies from 20 kHz to 50 kHz, and the electric potential from 15 kV to 20 kV. The colour of the luminescence will differ depending on the pressure (vacuum depth).

All ampoules of the batch should be controlled for the presence of vacuum. If ampoules or vials containing a medicinal product are stored at low temperature, they should be kept at room temperature for some time before the test.

The pressure is determined in accordance with Table 1.

Table 1. Correspondence between luminescence colour and pressure.

Pressure	Luminescence colour
10 — 100 Pa	pale blue
100 — 1000 Pa	pink blue
1 — 5 kPa	violet
5 — 100 kPa	no luminescence

Air-tightness quality control for ampoules and vials with immunobiologicals that are pressurized after being filled with protective gas at atmospheric pressure should be performed on all ampoules of the batch. Contact of the high-frequency electrode with the ampoule soldering point should be avoided during the test.

Requirements for pressure (vacuum depth) are established by Pharmacopoe-

ia Monographs or Normative Documents.

Vials are controlled at random, the sample size is $0.5\sqrt{N}$, where N is the number of vials in the batch. If at least one unpressurized vial is found in the sample, all vials of the batch should be tested.

Immunobiologicals are available in different pharmaceutical forms: lyophilized powder, powder, solution, suspension, tablets, capsules, granules, suppositories, ointment. Lyophilized powder may be supplied along with a diluent approved for medical application that should be present at the dose appropriate for the respective method of administration. The diluent should have no effect on the quality of the medicinal product.

TESTS

Methods used for testing should be described in maximum detail; the properties of the reagents, reactants, laboratory equipment, devices, as well as the requirements established for experimental animals, microbial and cell cultures, etc., should be specified.

Sensitivity requirements established for methods used to test immunobiologicals and for test results should conform to the WHO recommendations.

General Pharmacopoeia Monographs “Tablets,” “Suppositories,” “Powders,” and “Capsules” regulate the quality parameters of immunobiologicals supplied in respective pharmaceutical forms.

Description. The test involves description of the properties of the respective dosage form of the tested medicinal product.

Identification. Identity should be established by laboratory methods that should permit specific identification of the medicinal product: biological, immunobiological, molecular, chemical, or physicochemical methods.

Transparency. The test is carried out in accordance with the General Pharmacopoeia Monograph “Transparency and turbidity of liquids.”

The “Transparency” or “Transparency of reconstituted solution” section should include requirements for the liquid or reconstituted medicinal product, its transparency, opalescence, suspension or sedimentation. If the medicinal product

has to be dissolved, the composition and volume of the solvent should be specified.

Colour intensity. The test is carried out in accordance with the General Pharmacopoeia Monograph "Colour intensity of liquids."

The "Colour intensity" or "Colour intensity of reconstituted solution" section should include requirements for the colour intensity of the solution of the medicinal product obtained with the diluent specified in the Instructions for Medical Use, as well as methods used for the determination and assessment of these parameters. Colour of liquids is determined by visual examination versus an appropriate reference standard.

Particulate matter. The test is carried out in accordance with the General Pharmacopoeia Monograph "Visible particulate matter in parenteral pharmaceutical forms and ophthalmic pharmaceutical forms" and the General Pharmacopoeia Monograph "Invisible particulate matter in parenteral pharmaceutical forms"; the absence of visible particulate matter and a content limit for invisible particles should be specified, along with the control methods used.

Control of adsorbed medicinal products and corpuscular bacterial vaccines should be done by means of visual examination.

Reconstitution time (for lyophilized powders and powders). The reconstitution time of the medicinal product should be specified in the Pharmacopoeia Monograph. The required amount of the solvent is carefully transferred into the receptacle with the medicinal product using a pipette or a syringe for injection. Within the regulated period of time, the contents of the receptacle should completely dissolve or disperse producing a solution or suspension. The Pharmacopoeia Monograph should specify the analytical conditions (the solvent used, its volume, and, if necessary, solvent temperature and the need for shaking).

Disintegration time (for tablets and capsules). The solvent used, its volume, and, if necessary, the dissolution conditions (solvent temperature, stirring, shaking) should be specified.

The test is carried out in accordance with the General Pharmacopoeia Monograph "Disintegration of tablets and capsules."

Melting temperature and time or time to complete loss of shape (for suppositories). For suppositories with a lipophilic basis, time to complete loss of shape should be determined in accordance with the General Pharmacopoeia Monograph "Determination of time to complete loss of shape for lipophilic suppositories".

pH value. The acceptable pH range should be indicated. The preparation procedure for the tested sample should be specified, as well as the dilution (if necessary) and dissolution – along with the volume and name of the solvent. The test is carried out by potentiometry, in accordance with the General Pharmacopoeia Monograph "Ionometry."

Extractable volume. The extractable volume must meet the requirements established by the Pharmacopoeia Monographs and be not less than the nominal volume. The test is carried out in accordance with the General Pharmacopoeia Monograph "Extractable volume for parenteral pharmaceutical forms."

Weight loss on drying or Water content. The test is carried out in accordance with the General Pharmacopoeia Monographs "Weight loss on drying" or "Determination of water."

Mean weight and deviation from the mean weight (for tablets, suppositories, capsule contents, fixed-dose powders and lyophilized powders). Requirements for the mean weight and maximum acceptable deviations from the mean weight should be included in accordance with the General Pharmacopoeia Monograph "Weight uniformity of fixed-dose pharmaceutical forms."

Sterility. Immunobiologicals supplied as injectable pharmaceutical forms or eye drops must be sterile. The sterility test should be carried out by direct culture or membrane filtration in accordance with the General Pharmacopoeia Monograph "Sterility."

Mycoplasmas. If necessary, the Pharmacopoeia Monograph should include tests for the absence of Mycoplasmas. The test should be carried out in accordance with the General Pharmacopoeia Monograph "Mycoplasma presence test."

Microbial contamination. Acceptable quantities of non-pathogenic micro-

organisms should be specified in the Pharmacopoeia Monographs. The microbial contamination test performed for should be carried out in accordance with the General Pharmacopoeia Monograph ”Microbial contamination.” Non-injectable pharmaceutical forms of immunobiologicals are subject to this test.

Pyrogenicity. The pyrogenicity test should be carried out for those pharmaceutical forms of immunobiologicals that are designed for intravenous, intramuscular, or subcutaneous administration. The test should be carried out in accordance with the General Pharmacopoeia Monograph “Pyrogenicity.” Requirements established for sample preparation and the test dose (weight, volume, or other measurements) should be specified in the Pharmacopoeia Monograph.

Bacterial endotoxins. The bacterial endotoxins test should be carried out for those pharmaceutical forms of immunobiologicals that are designed for intravenous, intramuscular, or subcutaneous administration. The test should be carried out in accordance with the General Pharmacopoeia Monograph “Bacterial endotoxins.” The exception is medicinal products that contain bacterial endotoxins as an active component. Requirements established for sample preparation and the test dose (weight, volume, or other measurements) should be specified in the Pharmacopoeia Monograph.

Abnormal toxicity. The abnormal toxicity test for medicinal products designed for intravenous, intramuscular, or subcutaneous administration should be carried out in accordance with the General Pharmacopoeia Monograph “Abnormal toxicity”. The sample preparation procedure and the doses and method of administration should be specified in the Pharmacopoeia Monograph. Abnormal toxicity tests for other pharmaceutical forms should be described in the respective Pharmacopoeia Monograph.

Specific safety. The specific safety test should include a method (or methods) performed *in vitro* and / or *in vivo* and producing results that can demonstrate that the tested medicinal product possesses no virulent (toxic) properties characteristic of the stock microbial strains used to prepare the active component(s) of the medicinal product.

Such parameters include the absence of viable microorganisms of the industrial strain for non-live inactivated or subunit (such as chemical vaccines) bacterial and viral medicinal products, the absence of any traces of the active toxin for toxoids, the absence of bacteria belonging to the industrial strains for bacteriophages, the absence of the inducing virus for natural interferon, etc. Respective tests should be performed using methods with the best possible sensitivity. The specific safety test should be described in the Pharmacopoeia Monograph.

Specific innocuity. The test should be carried out for non-injectable medicinal products containing live microorganisms. The specific innocuity test for probiotics should be performed in accordance with the General Pharmacopoeia Monograph "Safety of probiotics *in vivo*." Specific innocuity criteria should be specified, as well as requirements for experimental animals used as controls and their amount, the doses, dilution conditions, and methods of administration used for the medicinal product, the duration of observation and controlled parameters.

Specific activity. The specific activity test should be carried out for immunobiologicals using quantification methods for the antigen or other active component. The choice of *in vitro* and / or *in vivo* methods of specific activity testing depends on the type of the medicinal product, and they are the most reliable tool to characterize its efficacy in practical application. Specific activity testing should be performed using appropriate standard samples (reference products) calibrated versus International Standard Samples (if such are available).

If mathematical methods are employed for calculation of test results, the Pharmacopoeia Monograph should contain the respective equation and an explanation of the symbols, along with an example of the calculation if necessary. If the calculation is done with an application software, an appropriate reference should be provided.

Chemical parameters. Immunobiologicals should be tested for the content of protein, nucleic acids, polysaccharides, sugars, phosphorus, etc., provided that determination of these parameters is not included in the sections "Identification," "Specific safety," and "Specific activity." Each parameter to be tested should be

described in an individual section.

Substances included in the medicinal product. This test determines the amount of substances used for inactivation of bacteria or viruses, and it is also used for medicinal products containing a sorbent, preservative, stabilizer, etc. Each parameter to be tested should be described in an individual section.

Impurities. An acceptable content of substances, including substances of biological origin that may contaminate the medicinal product or be produced during storage, should be specified for immunobiologicals (per volume or weight unit). For instance, immunoglobulins should be controlled for the content of other serum proteins, the presence of viruses contaminating biological substrates (cell cultures, sera, etc.), the content of ovalbumin in medicinal products prepared with bird embryos, the content of ammonium ions in serum preparations, the content of protein and DNA of producing cells in immunobiologicals obtained by genetic engineering technology. The content of protein and DNA of producing cells in immunobiologicals should not exceed limits specified by applicable international requirements. Impurities may be determined during the manufacturing process prior to the inclusion of excipients.

Virologic safety. Immunobiologicals obtained from human blood, plasma, organs and tissues must be demonstrated to be virologically safe with regard to the following markers: **Hepatitis B virus surface antigen (HBsAg); antibodies to human immunodeficiency viruses (HIV-1, 2), HIV p24 antigen**; **and antibodies to hepatitis C virus.**

Industrial microbial strains and reference strains. If industrial microbial strains and reference strains are used, their Latin names should be specified, along with the storage location, catalogue number, storage conditions, and acceptable passage number (if necessary) with the passage conditions and culture substrate. If necessary, any requirements established for strain characteristics and not included in the Certificate should be specified (e. g., LD_{50}).

Diluents supplied with lyophilized medicinal products. Solvents approved for medical application for the respective route of administration may be used as

diluents for lyophilized medicinal products, provided that they do not affect the quality of the medicinal product. Requirements for the quality of the diluent should be specified in the Pharmacopoeia Monograph, which should include all quality parameters for the control of the diluent.

Packaging. Primary packaging of an immunobiological preparation should ensure preservation of the claimed properties of the medicinal product throughout the regulated shelf-life and be approved for packaging of medicinal products and the respective method of administration. The capacity of packages used for lyophilized medicinal products should in most cases allow addition of the specified diluent volume with subsequent due mixing of the contents. Ampoules are not recommended as containers for injectable pharmaceutical forms undergoing multi-dose pre-packaging.

Labeling. The primary package should include the name of the medicinal product, the name or logotype of the manufacturer, the batch number, the date of manufacture, the shelf-life ("expiry date: "), the dose or concentration, or the activity.

The consumer (outer) package should include the name of the medicinal product, the name and address of the manufacturer of the medicinal product, the pharmaceutical form, the batch number, the date of manufacture, the shelf-life ("expiry date: "), the method of administration, the dose or concentration, or the activity, information concerning the composition and amount of the medicinal product per pack, the storage conditions, the pharmacy dispensing conditions, the Marketing Authorization Number, a bar code, and precautionary information.

If the consumer (outer) package contains additional components (a solvent for a lyophilized medicinal product, reference test liquid, diluted serum for skin tests, etc.), the name of the additional component, its concentration, composition information, volume, and batch number should be included as well. If the pack also contains dosing devices, medical appliances, etc., the consumer (outer) package should additionally include information about their presence.

The consumer (outer) package of medicinal products obtained from human

blood, plasma, organs or tissues should include mentioning “Contains no antibodies to HIV-1, HIV-2, hepatitis C virus, or hepatitis B virus surface antigen.”

Storage. The storage conditions for an immunobiological should ensure preservation of all properties of the medicinal product throughout the regulated shelf-life. The storage temperature should in most cases lie within the range of 2 to 8 ^{0}C, unless otherwise specified in the Pharmacopoeia Monograph. Adjuvant-adsorbed medicinal products should not undergo freezing.

Transportation. The temperature and other conditions for transportation should in most cases be the same as the storage conditions. Any other proposed transportation temperature should be demonstrated by appropriate factual material. In this case, the respective section of the Normative Document should include time-keeping requirements for such transportation conditions.