MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION

GENERAL PHARMACOPOEIA MONOGRAPH

Gel immunodiffusion **GPM**.1.8.2.0001.15

First Edition

The present General Pharmacopoeia Monograph applies to the method used for the identification (species specificity) testing of medicinal products.

The principle of the gel immunodiffusion method is that a precipitate (appearing as precipitation lines) forms as a result of the interaction between an antigen and an antibody taken in an equivalent ratio.

Identity (species specificity) is demonstrated by the emergence of a precipitation line with the antigens of a serum containing only species specific proteins (for instance, when demonstrating the species specificity of a human blood preparation with a serum precipitating human blood proteins).

Procedure description

An alcohol-processed glass plate with dimensions 26 x 75 mm or 90 × 120 mm or a 100 mm glass Petri dish (as specified in the Normative Document) is placed strictly horizontally on an object table. Melted agar (temperature, (50 ± 5) °C) is applied on the glass plate (or glass Petri dish) in a quantity sufficient to form a layer with a thickness of 2.4 to 2.6 mm (for instance, 5.5 to 6.0 mL should be applied on a 26 x 75 mm glass). The glass plate (or glass Petri dish) is placed in the moist chamber of an exsiccator with a small amount of water for 30 to 40 minutes, to allow the gel fasten.

Wells for reagents (Figure 1) are prepared in the set agar gel with a template, using punching tubes made of brass or stainless steel that have a pointed end on the

internal diameter side (2 to 3 mm), or standard stamps are used. The distance between the external borders of the wells should be 5 to 6 mm. The agarose stoppers are carefully removed from the wells using a cross-sectioned Pasteur pipette, avoiding separation of the gel from the glass.

The wells are filled with samples according to the schema (Figure 1), with the sample volume not exceeding the volume of the well (the sample volume to be introduced should be specified in the Normative Document).

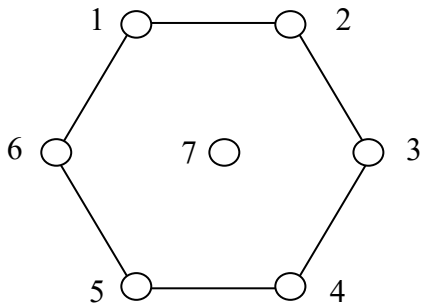

Figure 1 – Reagent application schema

1 – serum precipitating species specific proteins (for instance, human blood proteins when demonstrating the species specificity of human blood preparations);
2 – standard reagent (for instance, a control human serum when demonstrating the species specificity of human blood preparations);
3, 4, 6 – sera precipitating other proteins (for instance, horse, porcine, bovine proteins when demonstrating the species specificity of human blood preparations);
5 – negative control (0.9 % sodium chloride solution);
7 – tested medicinal product.

The glass plate (or glass Petri dish) with agarose gel is placed in the moist chamber and left to stand at room temperature for 24 hours or at (5 ± 3) °C for 48 hours.

Interpretation of obtained results is done by visual examination, analyzing precipitation lines. Immunodiffusion specificity is confirmed by the presence of a precipitation line of the standard reagent and the respective serum precipitating the species specific proteins. For instance, to demonstrate the species specificity of human blood preparations, precipitation lines should be

observed of a serum precipitating human blood proteins with the tested medicinal product and with the control human serum (Figure 2).

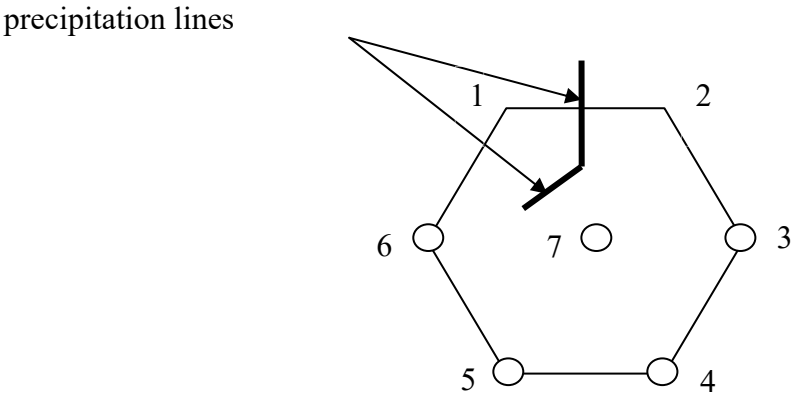

Figure 2 – Interpretation of obtained results in the species specificity test for human blood preparations

1 – serum precipitating human blood proteins;
2 – control human serum (standard reagent);
3 – serum precipitating horse blood proteins;
4 – serum precipitating porcine blood proteins;
5 – negative control (0.9 % sodium chloride solution);
6 – serum precipitating bovine blood proteins;
7 – tested medicinal product.

Note

1. Preparation of the agarose gel

Transfer 12.5 g of agar into a 1000 mL chemical glass, add 500 mL of purified water, and leave the gel for one hour at room temperature to swell. The glass with its contents is placed in a boiling water bath and left to stand until the agar has completely melted. The volume of the gel solution is brought to the mark with 0.9 % sodium chloride solution, and mixing follows. The agar solution is filtered through gauze (2 or 3 layers) and poured into vials. The melted agar should be transparent.

The shelf-life of the cooled agarose gel is 1 month if stored at (5 ± 3) °C.

Agarose gel of a different composition may be stored as specified in the Normative Document.