5.7 Tests for particulate contamination

Particulate contamination of injections and parenteral infusions consists of extraneous, mobile, undissolved particles, other than gas bubbles, unintentionally present in the solutions.

The types of preparations for which compliance with these tests are required are stated in respective general monographs.

5.7.1 Subvisible particles

This chapter is based on the internationally-harmonized texts developed by the Pharmacopoeial Discussion Group (PDG). Some editorial modifications have been made in order to be in line with the style used in The International Pharmacopoeia *. It should be noted, however, that acceptance criteria of parenteral preparations having a nominal volume of 100 mL were exempted from the PDG harmonization. For the purpose of* The International Pharmacopoeia*, 100 mL is classified as a small-volume parenteral preparation and the criteria are set accordingly.*

For the determination of particulate contamination two procedures, Method A (Light Obscuration Particle Count Test) and Method B (Microscopic Particle Count Test), are specified hereinafter. When examining injections and parenteral infusions for subvisible particles Method A is preferably applied. However, it may be necessary to test some preparations by the light obscuration particle count test followed by the microscopic particle count test to reach a conclusion on conformance to the requirements.

Not all parenteral preparations can be examined for subvisible particles by one or both of these methods. When Method A is not applicable, e.g. in the case of preparations having reduced clarity or increased viscosity, the test should be carried out according to Method B. Emulsions, colloids and liposomal preparations are examples. Similarly, products that produce air or gas bubbles when drawn into the sensor may also require microscopic particle count testing. If the viscosity of the preparation to be tested is sufficiently high so as to preclude its examination by either test method, a quantitative dilution with an appropriate diluent may be made to decrease viscosity, as necessary, to allow the analysis to be performed.

The results obtained in examining a discrete unit or group of units for particulate contamination cannot be extrapolated with certainty to other units that remain untested. Thus, statistically-sound sampling plans must be developed if valid inferences are to be drawn from observed data to characterize the level of particulate contamination in a large group of units.

METHOD A. LIGHT OBSCURATION PARTICLE COUNT TEST

Use a suitable apparatus based on the principle of light blockage which allows an automatic determination of the size of particles and the number of particles according to size.

The apparatus is calibrated using dispersions of spherical particles of known sizes between 10 µm and 25 µm. These standard particles are dispersed in particle-free water R. Care must be taken to avoid aggregation of particles during dispersion.

General precautions

The test is carried out under conditions limiting particulate contamination, preferably in a unidirectional airflow cabinet.

Very carefully wash the glassware and filtration equipment used, except for the membrane filters, with a warm detergent solution and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse the equipment from top to bottom, outside and then inside, with particle-free water R.

Take care not to introduce air bubbles into the preparation to be examined, especially when fractions of the preparation are being transferred to the container in which the determination is to be carried out.

In order to check that the environment is suitable for the test, that the glassware is properly cleaned and that the water to be used is particle-free, the following test is carried out: determine the particulate contamination of 5 samples of particle-free water R, each of 5 mL, according to the method described below. If the number of particles of 10 µm or greater size exceeds 25 for the combined 25 mL, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware and water are suitable for the test.

Method

Mix the contents of the sample by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of particle-free water R and remove the closure, avoiding any contamination of the contents. Eliminate gas bubbles by appropriate measures such as allowing to stand for 2 min or sonicating.

For large-volume parenterals, single units are tested. For small-volume parenterals less than 25 mL in volume, the contents of 10 or more units are combined in a cleaned container to obtain a volume of not less than 25 mL; where justified and authorized, the test solution may be prepared by mixing the contents of a suitable number of vials and diluting to 25 mL with particle-free water R or with an appropriate solvent without contamination of particles when particle-free water R is not suitable. Small-volume parenterals having a volume of 25 mL or more may be tested individually.

Powders for parenteral use are reconstituted with particle-free water R or with an appropriate solvent without contamination of particles when particle-free water R is not suitable.

The number of test specimens must be adequate to provide a statistically sound assessment. For large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, fewer than 10 units may be tested, based on an appropriate sampling plan.

Remove four portions, each of not less than 5 mL, and count the number of particles equal to or greater than 10 µm and 25 µm. Disregard the result obtained for the first portion and calculate the mean number of particles for the preparation to be examined.

Evaluation

For preparations supplied in containers with a nominal volume of more than 100 mL, apply the criteria of test A.1.

For preparations supplied in containers with a nominal volume of 100 mL or less, apply the criteria of test A.2.

If the average number of particles exceeds the limits, test the preparation by the Microscopic Particle Count Test.

Test A.1. Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of more than 100 mL.

The preparation complies with the test if the average number of particles present in the units tested does not exceed 25 per millilitre equal to or greater than 10 µm and does not exceed 3 per millilitre equal to or greater than 25 µm.

Test A.2. Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of 100 mL or less.

The preparation complies with the test if the average number of particles present in the units tested does not exceed 6000 per container equal to or greater than 10 µm and does not exceed 600 per container equal to or greater than 25 µm.

METHOD B. MICROSCOPIC PARTICLE COUNT TEST

Use a suitable binocular microscope, filter assembly for retaining particulate contamination and membrane filter for examination.

The microscope is equipped with an ocular micrometer calibrated with an objective micrometer, a mechanical stage capable of holding and traversing the entire filtration area of the membrane filter, two suitable illuminators to provide episcopic illumination in addition to oblique illumination and is adjusted to 100 ± 10 magnifications.

The ocular micrometer is a circular diameter graticule (see Figure 1) and consists of a large circle divided by crosshairs into quadrants, transparent and black reference circles 10 µm and 25 µm in diameter at 100 magnifications and a linear scale graduated in 10 µm increments. It is calibrated using a stage micrometer that is certified by either a national, regional or international standard institution. A relative error of the linear scale of the graticule within ± 2% is acceptable. The large circle is designated the graticule field of view (GFOV).

Two illuminators are required. One is an episcopic bright-field illuminator internal to the microscope, the other is an external, focusable auxiliary illuminator adjustable to give reflected oblique illumination at an angle of 10° to 20°.

The filter assembly for retaining particulate contamination consists of a filter holder made of glass or other suitable material and is equipped with a vacuum source and a suitable membrane filter.

The membrane filter is of suitable size, black or dark grey in colour, non-gridded or gridded and 1.0 µm or finer in nominal pore size.

General precautions

The test is carried out under conditions limiting particulate contamination, preferably in a laminar-flow cabinet.

Very carefully wash the glassware and filter assembly used, except for the membrane filter, with a warm detergent solution and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use rinse both sides of the membrane filter and the equipment from top to bottom, outside and then inside, with particle-free water R.

In order to check that the environment is suitable for the test, that the glassware and the membrane filter are properly cleaned and that the water to be used is particle-free, the following test is carried out: determine the particulate contamination of a 50 mL volume of particle-free water R according to the method described below. If more than 20 particles 10 µm or larger in size or if more than 5 particles 25 µm or larger in size are present within the filtration area, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware, membrane filter and water are suitable for the test.

Method

Mix the contents of the samples by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of particle-free water R and remove the closure, avoiding any contamination of the contents.

For large-volume parenterals, single units are tested. For small-volume parenterals less than 25 mL in volume, the contents of 10 or more units are combined in a cleaned container; where justified and authorized, the test solution may be prepared by mixing the contents of a suitable number of vials and diluting to 25 mL with particle-free water R or with an appropriate solvent without contamination of particles when particle-free water R is not suitable. Small-volume parenterals having a volume of 25 mL or more may be tested individually.

Powders for parenteral use are constituted with particle-free water R or with an appropriate solvent without contamination of particles when particle-free water R is not suitable.

The number of test specimens must be adequate to provide a statistically sound assessment. For large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, fewer than 10 units may be tested, based on an appropriate sampling plan.

Wet the inside of the filter holder fitted with the membrane filter with several millilitre of particle-free water R. Transfer to the filtration funnel the total volume of a solution pool or of a single unit and apply vacuum. If needed add stepwise a portion of the solution until the entire volume is filtered. After the last addition of solution, begin rinsing the inner walls of the filter holder by using a jet of particle-free water R. Maintain the vacuum until the surface of the membrane filter is free from liquid. Place the filter in a Petri dish and allow the filter to air-dry with the cover slightly ajar. After the filter has been dried, place the Petri dish on the stage of the microscope, scan the entire membrane filter under the reflected light from the illuminating device and count the number of particles that are equal to or greater than 10 µm and the number of particles that are equal to or greater than 25 µm. Alternatively, partial filter count and determination of the total filter count by calculation is allowed. Calculate the mean number of particles for the preparation to be examined.

The particle sizing process with the use of the circular diameter graticule is carried out by transforming mentally the image of each particle into a circle and then comparing it to the 10 µm and 25 µm graticule reference circles. Thereby the particles are not moved from their initial locations within the graticule field of view and are not superimposed on the reference circles for comparison. The inner diameter of the transparent graticule reference circles is used to size white and transparent particles, while dark particles are sized by using the outer diameter of the black opaque graticule reference circles.

In performing the microscopic particle count test do not attempt to size or enumerate amorphous, semiliquid or otherwise morphologically indistinct materials that have the appearance of a stain or discoloration on the membrane filter. These materials show little or no surface relief and present a gelatinous or film-like appearance. In such cases the interpretation of enumeration may be aided by testing a sample of the solution by the light obscuration particle count test.

Evaluation

For preparations supplied in containers with a nominal volume of more than 100 mL, apply the criteria of test B.1.

For preparations supplied in containers with a nominal volume of 100 mL or less, apply the criteria of test B.2.

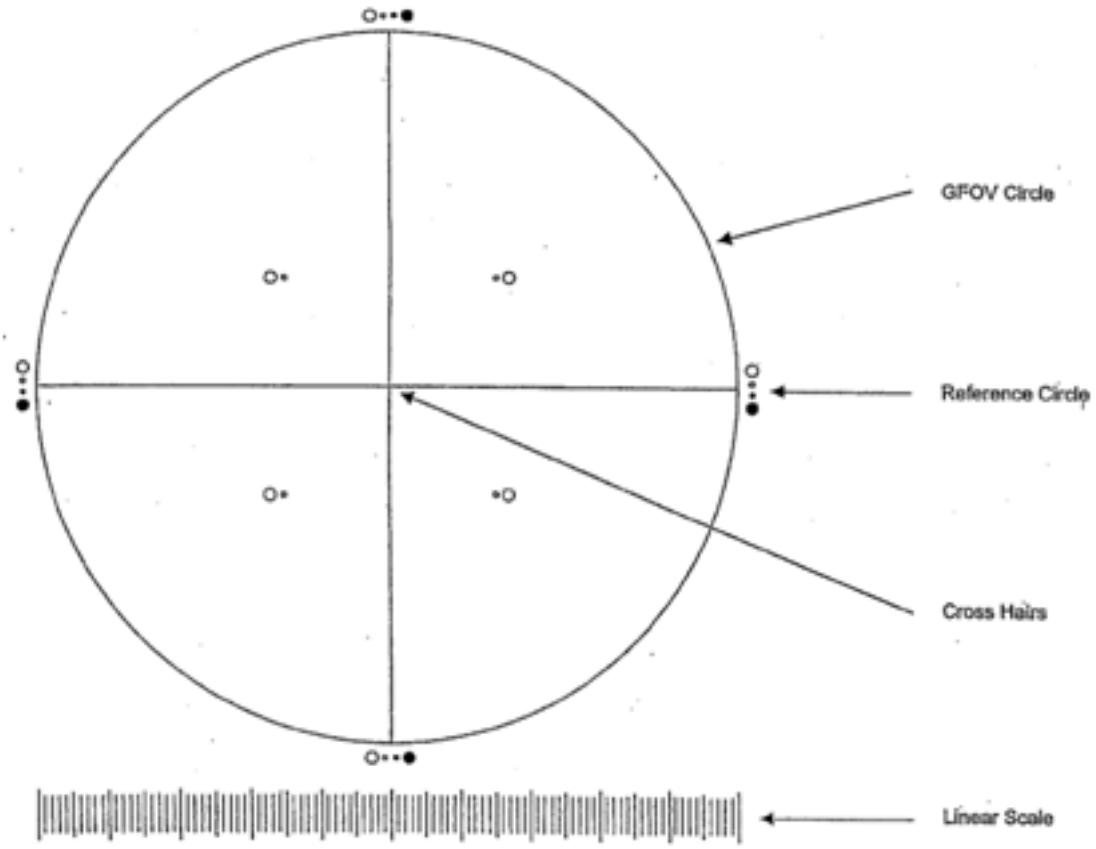
Test B.1. Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of more than 100 mL.

The preparation complies with the test if the average number of particles present in the units tested does not exceed 12 per millilitre equal to or greater than 10 µm and does not exceed 2 per millilitre equal to or greater than 25 µm.

Test B.2. Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of 100 mL or less.

The preparation complies with the test if the average number of particles present in the units tested does not exceed 3000 per container equal to or greater than 10 µm and does not exceed 300 per container equal to or greater than 25 µm.

Figure 1. Circular diameter graticule

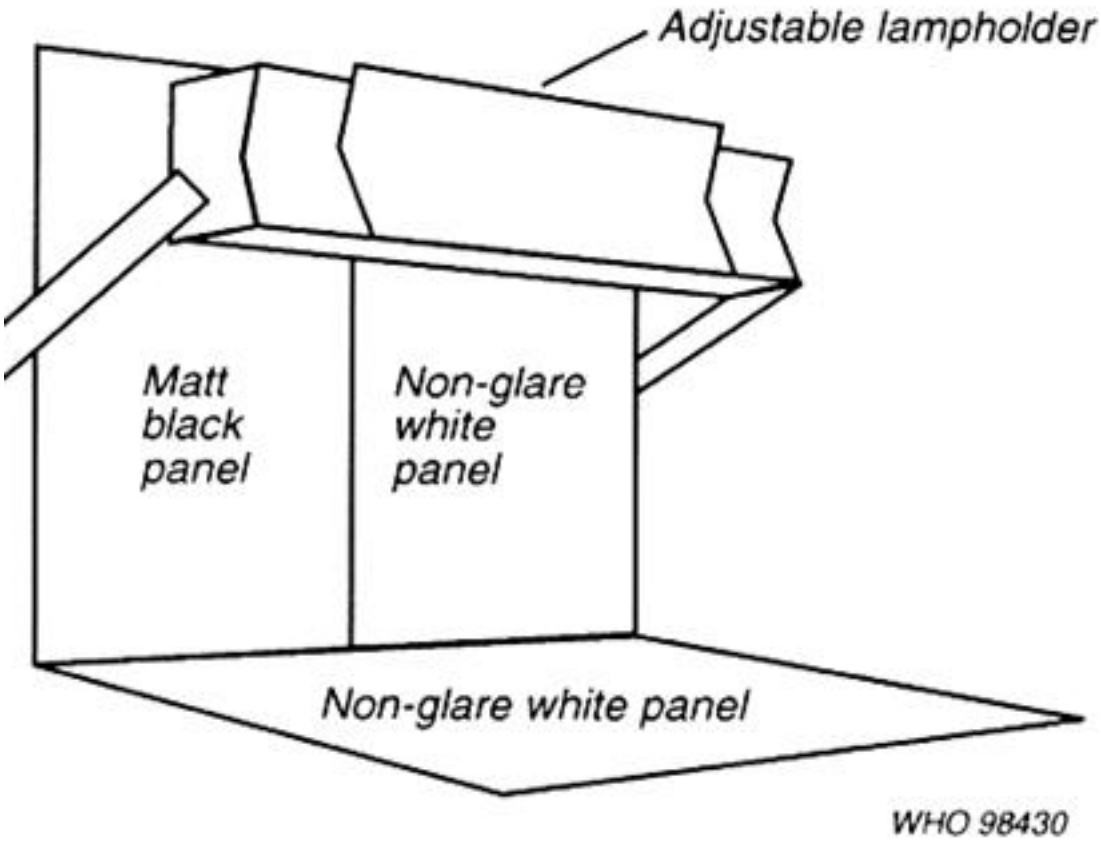


5.7.2 Visible particles

This test provides a simple method for the detection of visible particles. It is performed in accordance with the provisions of good manufacturing practices. The test is not intended for use by a manufacturer for batch release purposes. To ensure that a product will meet pharmacopoeial specifications with respect to visible particulate matter, if and when tested, manufacturers should carry out a 100% inspection and rejection of unsatisfactory items prior to release or use other appropriate means.

Subvisible particles and the nature of the particles are not identified by this method.

Figure 2. Apparatus for visible particles



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Apparatus[1]

The apparatus (Figure 2) consists of a viewing station comprising:

- -a matt black panel of appropriate size held in a vertical position;
- -a non-glare white panel of appropriate size held in a vertical position next to the black panel;
- -an adjustable lamp holder fitted with a shaded, white-light source and with a light diffuser (a viewing illuminator containing two 13-W fluorescent tubes, each 525 mm in length is suitable). The intensity of illumination at the viewing point is maintained between 2000 lux and 3750 lux for clear glass ampoules. Higher values are preferable for coloured glass and plastic containers.

Recommended procedure

Remove any adherent labels from the container and wash and dry the outside. Gently swirl or invert each individual container, making sure that no air bubbles are introduced and observe for about 5 seconds in front of the white panel. Repeat the procedure in front of the black panel.

Record the presence of any particles. Repeat the procedure for a further 19 containers.

The preparation fails the test if one or more particles are found in more than one container.

When the test is applied to reconstituted solutions from powder for injections, the test fails if particles are found in more than two containers.

[1] This method was developed by WHO in collaboration with Group 12 of the European Pharmacopoeia Commission.