INTERNATIONAL STANDARD

ISO 8536-4

Sixth edition
2019-09

Infusion equipment for medical use —

Part 4: Infusion sets for single use, gravity feed

Matériel de perfusion à usage médical —

Partie 4: Appareils de perfusion non réutilisables, à alimentation par gravité

Reference number
ISO 8536-4:2019(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 76, *Transfusion, infusion and injection, and blood processing equipment for medical and pharmaceutical use*.

This sixth edition cancels and replaces the fifth edition (ISO 8536-4:2010), which has been technically revised. It also incorporates the Amendment ISO 8536-4:2010/Amd.1:2013.

The main changes compared to the previous edition are as follows:

— Clause 5 'Designation' now refers to Clause 10 'Labelling';

— the physical requirements – especially regarding stand-alone air-inlet devices – have been further clarified;

— Clause 10 'Labelling' has been updated;

— test for leakage in A.3 has been updated;

— determination of flow rate in A.5 has been totally reviewed;

— normative references in Clause 2 and the Bibliography have been updated.

A list of all parts in the ISO 8536 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Infusion equipment for medical use —

Part 4:
Infusion sets for single use, gravity feed

1 Scope

This document specifies requirements for single use, gravity feed infusion sets for medical use in order to ensure their compatibility with containers for infusion solutions and intravenous equipment.

Secondary aims of this document are to provide guidance on specifications relating to the quality and performance of materials used in infusion sets and to present designations for infusion set components.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 7864, *Sterile hypodermic needles for single use — Requirements and test methods*

ISO 8536-13, *Infusion equipment for medical use — Part 13: Graduated flow regulators for single use with fluid contact*

ISO 8536-14, *Infusion equipment for medical use — Part 14: Clamps and flow regulators for transfusion and infusion equipment without fluid contact*

ISO 14644-1, *Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness by particle concentration*

ISO 15223-1, *Medical devices — Symbols to be used with medical device labels, labelling and information to be supplied — Part 1: General requirements*

ISO 80369-7, *Small-bore connectors for liquids and gases in healthcare applications — Part 7: Connectors for intravascular or hypodermic applications*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

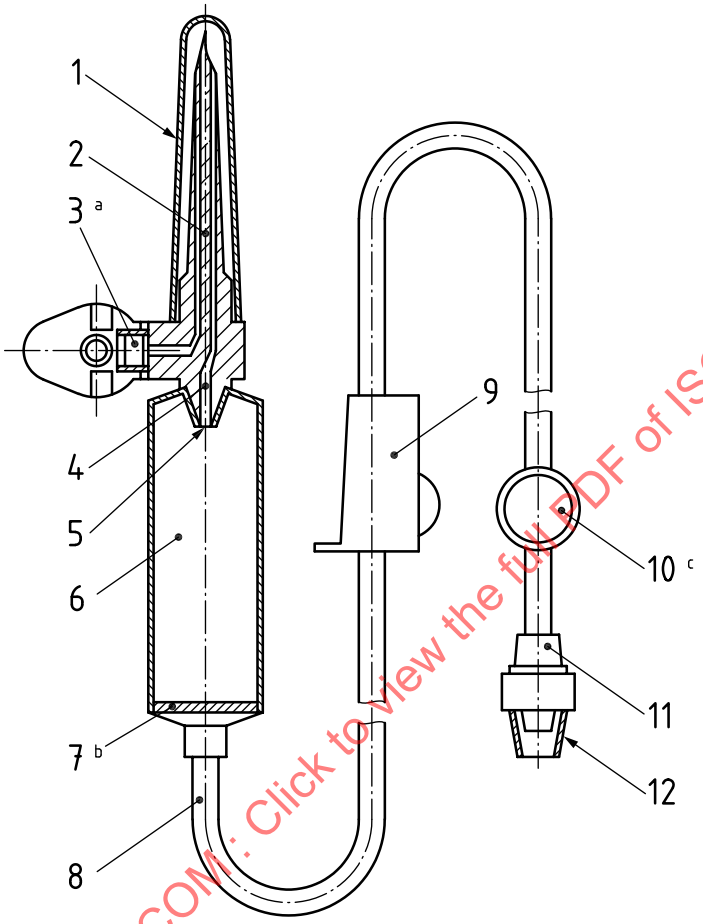
— IEC Electropedia: available at http://www.electropedia.org/

4 General requirements

4.1 The nomenclature to be used for components of infusion sets and of a stand-alone air-inlet device is given in Figures 1, 2 and 3. These figures illustrate examples of the configuration of infusion sets and air-inlet devices; other configurations may be used provided they lead to the same results. Infusion sets

as illustrated in Figure 2 should only be used for collapsible plastic containers. Infusion sets as illustrated in Figure 2 used with stand-alone air-inlet devices as illustrated in Figure 3, or infusion sets as illustrated in Figure 1, shall be used for rigid containers.

4.2 The infusion set shall be provided with protective caps. The air-inlet device shall be provided with a protective cap over the closure-piercing device or needle (see Figure 3).



Key

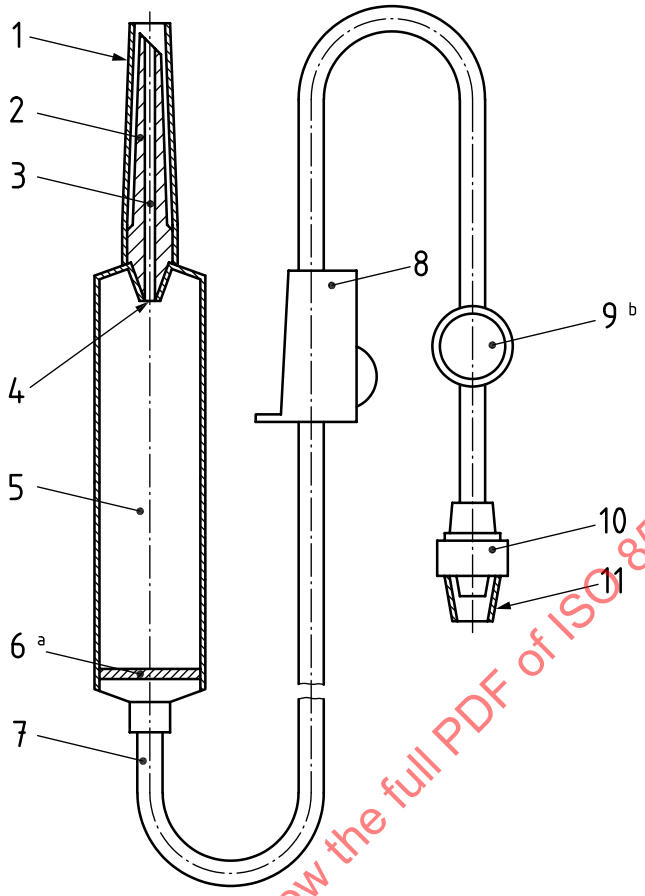
1 protective cap of closure-piercing device
2 closure-piercing device
3 integral air-inlet with air filter and closure
4 fluid channel
5 drip tube
6 drip chamber
7 fluid filter
8 tubing
9 flow regulator
10 injection site
11 male conical fitting
12 protective cap of male conical fitting

a Closure of the air-inlet is optional.

b The fluid filter may be positioned at other sites, preferably near the patient access.

c The injection site is optional.

Figure 1 — Example of a vented infusion set



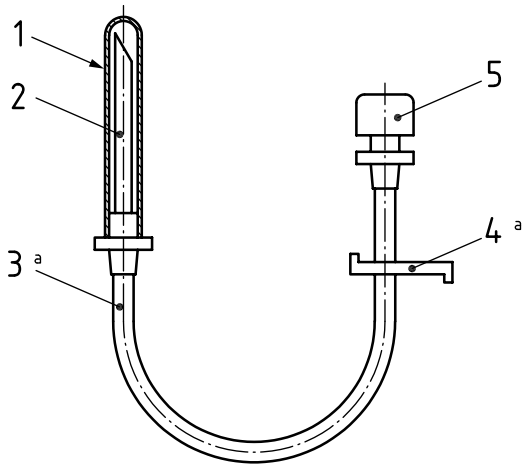
Key

1 protective cap of closure-piercing device
2 closure-piercing device
3 fluid channel
4 drip tube
5 drip chamber
6 fluid filter
7 tubing
8 flow regulator
9 injection site
10 male conical fitting
11 protective cap of the male conical fitting

[a] The fluid filter may be positioned at other sites, preferably near the patient access.

[b] The injection site is optional.

Figure 2 — Example of a non-vented infusion set



Key

1	protective cap	4	clamp
2	closure-piercing device or needle	5	air-inlet with air filter
3	tubing		
a	Other designs are acceptable if the same safety aspects are ensured.		

Figure 3 — Example of a stand-alone air-inlet device

5 Designation

Designation shall follow label requirements according to Clause 10.

6 Materials

The materials from which the infusion set, its components and the stand-alone air-inlet device are manufactured (as described in Clause 4) shall comply with the requirements specified in Clause 7. Where components of the infusion set come into contact with solutions, the materials shall also comply with the requirements specified in Clauses 8 and 9.

7 Physical requirements

7.1 Particulate contamination

The infusion set and stand-alone air-inlet device shall be manufactured under conditions that minimize particulate contamination. All parts shall be smooth and clean at the fluid pathway surfaces. When tested as specified in A.2, the number of particles shall not exceed the contamination index limit.

7.2 Leakage

The infusion set, when tested in accordance with A.3, shall show no signs of air leakage.

7.3 Tensile strength

When tested as specified in A.4, the infusion set, excluding protective caps, shall withstand a static tensile force of not less than 15 N for 15 s.

7.4 Closure-piercing device

The dimensions of the closure-piercing device shall conform to the dimensions shown in Figure 4. The cross-section of the closure-piercing device over the length of 15 mm shall be a circle.

NOTE The dimension of 15 mm in Figure 4 is a reference measurement.

The closure-piercing device shall be capable of piercing and penetrating the closure of a fluid container without pre-piercing. No coring should occur during this procedure.

Dimensions in millimetres

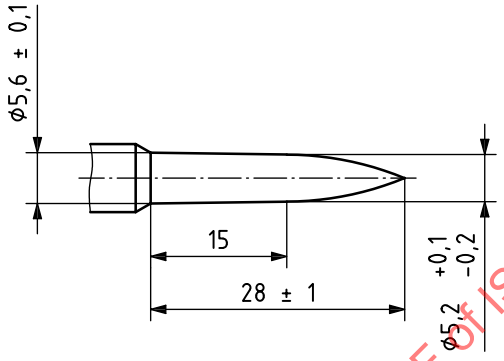


Figure 4 — Dimensions of the closure-piercing device

7.5 Air-inlet device

The air-inlet device can be an integral part of the infusion set (Figure 1) or a stand-alone device (Figure 3).

The air-inlet device shall be provided with an air filter to prevent the ingress of microorganisms into the container into which the device is to be inserted.

When the air-inlet device is in use the air admitted into the container shall not become entrained in the liquid-entry of the closure-piercing device.

The air filter and the design of the air-inlet device shall be such that all air entering the rigid container passes through it, and such that the flow of fluid is not reduced by more than 20 % of that from a freely ventilated container when tested in accordance with A.5.2 and A.5.3.

The closure-piercing device or needle of the stand-alone device shall be capable of piercing and penetrating the closure of a fluid container without pre-piercing. No coring should occur during this procedure.

7.6 Tubing

The tubing, made of flexible material, shall be transparent or sufficiently translucent that the interface of air and water during the passage of air bubbles can be observed with normal or corrected vision.

The tubing from the distal end to the drip chamber shall be not less than 1 500 mm in length, including the injection site, when provided, and the male conical fitting.

7.7 Fluid filter

The infusion set shall be provided with a fluid filter.

When tested in accordance with A.6, the retention of latex particles on the filter shall be not less than 80 %.

7.8 Drip chamber and drip tube

The drip chamber shall permit continuous observation of the fall of drops. The liquid shall enter the drip chamber through a tube that projects into the chamber. There shall be a distance of not less than 40 mm between the end of the drip tube and the outlet of the chamber and a distance of not less than 20 mm between the drip tube and the fluid filter. The wall of the drip chamber shall not be closer than 5 mm to the end of the drip tube. Depending on the design, the drip tube shall be such that 20 drops or 60 drops of distilled water at (23 ± 2) °C at a flow rate of (50 ± 10) drops/min deliver a volume of (1 ± 0,1) ml or a mass of (1 ± 0,1) g. The drip chamber should permit and facilitate the priming procedure.

7.9 Flow regulator

The flow regulator shall be in accordance with ISO 8536-13 or ISO 8536-14.

7.10 Flow rate of infusion set

The infusion set without the use of an air-inlet device shall deliver not less than 1 000 ml of a sodium chloride solution [concentration of NaCl = 9 g/l] in 10 min for a drip tube that delivers 1 ml with 20 drops. Testing shall be done in accordance with A.5.1.

7.11 Injection site

When provided, the self-sealing injection site shall reseal when tested in accordance with A.7, and there shall be no leakage of water. The injection site should be located near the male conical fitting.

7.12 Male conical fitting

The distal end of the tubing shall terminate in a male conical fitting in accordance with ISO 80369-7.

7.13 Protective caps

The protective caps shall cover the respective surfaces of the infusion equipment to prevent contamination from surrounding environment, to avoid stick injuries and packaging damages. Protective caps should be secure but easily removable.

8 Chemical requirements

8.1 Reducing (oxidizable) matter

When tested in accordance with B.2, the difference of volume of $Na_2S_2O_3$ solution [concentration of $Na_2S_2O_3$ = 0,005 mol/l] for the extract solution S_1 and of volume of $Na_2S_2O_3$ solution for blank solution S_0 shall not exceed 2,0 ml.

8.2 Metal ions

The extract shall not contain in total more than 1 µg/ml of barium, chromium, copper, lead and tin, and not more than 0,1 µg/ml of cadmium, when determined by atomic absorption spectroscopy (AAS) or an equivalent method.

When tested in accordance with B.3, the intensity of the colour produced in the test solution shall not exceed that of the standard matching solution with a concentration of Pb^{2+} = 1 µg/ml.

8.3 Titration acidity or alkalinity

When tested in accordance with B.4, not more than 1 ml of either standard volumetric solution shall be required for the indicator to change to the colour grey.

8.4 Residue on evaporation

When tested in accordance with B.5, the total amount of dry residue shall not exceed 5 mg.

8.5 UV absorption of extract solution

When tested in accordance with B.6, the extract solution S_1 shall be $S(\lambda) < 0{,}1$ with λ in the range from 250 nm to 320 nm.

9 Biological requirements

9.1 General

The infusion set and the stand-alone air-inlet device shall be assessed for biological compatibility according to the guidelines given in C.2.

9.2 Sterility

The infusion set and the stand-alone air-inlet device in its unit container shall have been subjected to a validated sterilization process, e.g. ISO 11135, ISO 11137-1, ISO 11137-2 and ISO 17665.

9.3 Pyrogenicity

The infusion set and the stand-alone air-inlet device shall be assessed for freedom from pyrogens by using a suitable test. The test result shall indicate that the infusion set and stand-alone air-inlet device are free from pyrogens. Guidance on testing for pyrogenicity is given in C.1.

9.4 Haemolysis

The infusion set shall be assessed for freedom from haemolytic constituents. The test result shall indicate that the infusion set is free from haemolytic reactions. Guidance on testing for haemolytic constituents is given in ISO 10993-4.

9.5 Toxicity

Materials shall be assessed for toxicity by carrying out suitable tests. The test results shall indicate freedom from toxicity. Guidance on testing for toxicity is given in ISO 10993-1.

10 Labelling

10.1 General

The labelling shall include the requirements as specified in 10.2 and 10.3. If graphical symbols are used, then refer to ISO 15223-1.

NOTE The presence of substances of interest can be indicated by using symbol 2725 of ISO 7000 by replacing the "XXX" by the abbreviation of the substance. The absence of substances of interest can be indicated by crossing the respective symbol.

10.2 Unit container

The unit container shall be labelled at least with the following information:

a) the name and address of the manufacturer;

b) a description of the contents;

c) indication that the infusion set is free from pyrogens, or that the infusion set is free from bacterial endotoxins;

d) indication that the infusion set is sterile, using the graphical symbol as given in ISO 15223-1;

e) the lot (batch) designation, prefixed by the word LOT, or using the graphical symbol according to ISO 15223-1;

f) year and month of expiry, accompanied by appropriate wording or the graphical symbol according to ISO 15223-1;

g) indication that the infusion set is for single use only, or equivalent wording, or using the graphical symbol according to ISO 15223-1;

h) the letter "G", which stands for gravity and whose type height shall stand out clearly from surrounding text;

i) a statement that depending on the drip tube design 20 drops or 60 drops of distilled water are equivalent to a volume of (1 ± 0,1) ml or a mass of (1 ± 0,1) g;

j) the nominal dimensions of the intravenous needle, if included.

If the available space is too small to give all this information in legible characters and/or symbols, the information may be reduced to e) and f). In this case the information as required in this subclause shall be given on the label of the next bigger shelf or multi-unit container.

10.3 Shelf or multi-unit container

The shelf or multi-unit container shall be labelled at least with the following information:

a) the name and address of the manufacturer;

b) a description of the contents;

c) the lot (batch) designation, prefixed by the word LOT, or using the graphical symbol according to ISO 15223-1;

d) year and month of expiry, accompanied by appropriate wording or the graphical symbol according to ISO 15223-1;

e) instructions for use, including warnings, e.g. about detached protective caps (instructions for use may also take the form of an insert);

f) the letter "G", which stands for gravity, and whose type height shall stand out clearly from surrounding text;

g) a storage note, if any.

11 Packaging

11.1 The infusion sets and the air-inlet devices shall be individually packed so that they remain sterile during storage. The unit container shall be sealed in a tamper-evident manner.

11.2 The infusion sets and the air-inlet devices shall be packed and sterilized in such a way that there are no flattened portions or kinks that limit the performance of the infusion sets when they are ready for use.

12 Disposal

Information for secure and environmentally sound disposal of single-use infusion sets should be given, e.g. “Always dispose of blood contaminated products in a manner consistent with established biohazard procedures.”

Annex A
(normative)

Physical tests

A.1 General

All physical tests shall be performed at a temperature of (23 ± 2) °C unless other temperatures are given in the test method.

A.2 Test for particulate contamination

A.2.1 Principle

The particles are rinsed from the inner fluid pathway surfaces of the infusion set and stand-alone air-inlet device, collected on a membrane filter and microscopically counted.

A.2.2 Reagents and materials

A.2.2.1 Distilled water, filtered through a membrane of pore size 0,2 µm or similar quality of water.

A.2.2.2 Non-powdered gloves.

A.2.2.3 Vacuum filter, single membrane filter of pore size 0,45 µm.

A.2.3 Procedure

The filter unit, filter and all other equipment shall be thoroughly cleaned before the test using distilled water (A.2.2.1).

Flush through 10 ready-to-use infusion appliances, under laminar flow conditions (clean-air work station class N5 in accordance with ISO 14644-1), with 500 ml of water according to A.2.2.1. The total volume is subsequently vacuum filtered according to A.2.2.3. Place the particles on the membrane screen filter under a microscope at a magnification of × 50 using diagonally incident illumination, and measure and count in accordance with the size categories given in Table A.1.

A.2.4 Determination of results

A.2.4.1 General

An appropriate number of single infusion sets (minimum of 10) are tested. The number of particles per 10 infusion sets tested in each of the three size categories is the assay result.

A.2.4.2 Particle counts

The values obtained from a blank control sample shall be recorded in a test report and taken into account when calculating the contamination index limit.

The blank control sample is the number and size of particles obtained from 10 equivalent 500 ml water samples classified in accordance with the three size categories set out in Table A.1, using the same test equipment but not passed through the appliances under test.

The number of particles in the blank, N_b, shall not exceed the value of 9. Otherwise, the test apparatus shall be disassembled, re-cleaned, and the background test performed again. Values of the blank determination shall be noted in the test report.

Table A.1 — Evaluation of contamination by particles

Particle parameters	Size category		
	1	2	3
Particle size in µm	25 to 50	51 to 100	over 100
Number of particles in 10 infusion appliances	n_{a1}	n_{a2}	n_{a3}
Number of particles in the blank control sample	n_{b1}	n_{b2}	n_{b3}
Evaluation coefficient	0,1	0,2	5

The contamination index limit is calculated as follows.

For each of the three size categories, multiply the number of particles in 10 infusion appliances by the evaluation coefficients, and add the results in order to obtain the number of particles in the infusion appliances (test pieces), N_a. Then, for each of the size categories, multiply the number of particles in the blank control sample by the evaluation coefficients and add the results to obtain the number of particles in the blank sample, N_b.

Subtract N_b from N_a to obtain the contamination index limit.

Number of particles in the infusion appliances (test pieces):

$$N_a = n_{a1} \times 0{,}1 + n_{a2} \times 0{,}2 + n_{a3} \times 5 \quad \text{(A.1)}$$

Number of particles in the blank sample:

$$N_b = n_{b1} \times 0{,}1 + n_{b2} \times 0{,}2 + n_{b3} \times 5 \quad \text{(A.2)}$$

Contamination index limit:

$$N = N_a - N_b \leq 90 \quad \text{(A.3)}$$

A.3 Test for leakage

A.3.1 At the beginning of the test, the whole system shall be tempered at the test temperature.

A.3.2 Connect the infusion set with air supply and close all remaining openings. Apply air with an internal positive pressure of 50 kPa to the infusion set for 15 s. Atmospheric pressure shall be the reference pressure. Inspect the infusion set for any leakage of air under water at (40 ± 1) °C.

A.3.3 Fill the infusion set with degassed and distilled water. Connect the infusion set to a vacuum device and close all remaining openings. Apply vacuum with an internal negative pressure of 20 kPa to the infusion set for 15 s. Atmospheric pressure shall be the reference pressure. Inspect if air enters into the infusion set.

A.4 Test for tensile strength

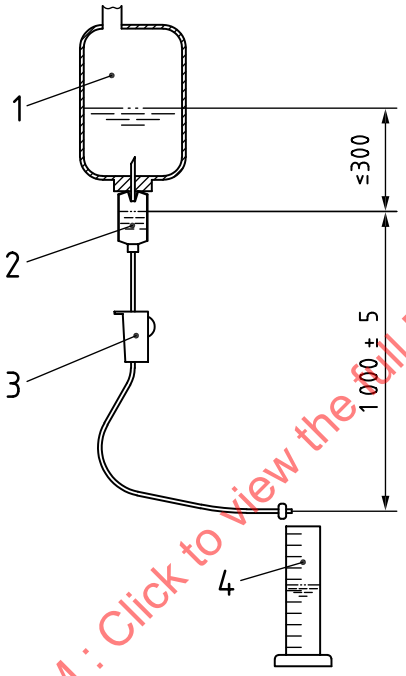
Expose the infusion set to be tested to a static tensile force of 15 N applied along the longitudinal axis for 15 s. Inspect whether the infusion set withstands the test force applied.

A.5 Determination of flow rate

A.5.1 Infusion set without air-inlet device

Use a container or bag filled with min 1 000 ml of sodium chloride solution [concentration of NaCl = 9 g/l]. If a rigid or semi-rigid container is used, the container shall be cut open at the top to provide a freely vented system. Insert the closure-piercing device of the infusion set into the container or bag port. Fill the drip chamber to be about ⅔ full. Open the flow regulator and fill the complete infusion set. Close the flow regulator. Arrange the test setup so that there is a height of (1 000 ± 5) mm between the liquid level of the drip chamber and the outlet of the infusion set at the beginning of the test. The liquid level between that in the drip chamber and the container or bag shall not exceed 300 mm (see Figure A.1).

Dimensions in millimetres



Key

1 container or bag
2 closure-piercing device
3 flow regulator
4 drip chamber

Figure A.1 — Example test setup to determine the flow rate

Open the flow regulator of the infusion and set it to maximum flow. Measure the time until 1 000 ml has been flowed through the infusion set or measure the volume delivered within 10 min.

If the time is measured, then the requirement for the time is equal or less than 10 min. If the volume is measured, then the requirement for the volume is equal or more than 1 000 ml.

A.5.2 Infusion set with integral air-inlet device

A.5.2.1 Close the air entry of the integral air-inlet device of the infusion set. Follow the instruction of A.5.1 for the infusion set and calculate the flow rate Q_0 = volume/time.

A.5.2.2 Use a rigid container. Follow the instruction of A.5.1 but do not cut an opening in the container. The container shall not be freely vented. Ensure that the closure of the integral air-inlet device is in open position. Calculate the flow rate Q_1 = volume/time. The flow rate Q_1 shall fulfill the requirement

$$Q_1 \geq Q_0 \times 0,8 \tag{A.4}$$

A.5.3 Infusion set with stand-alone air-inlet device

A.5.3.1 Follow the instruction of A.5.1 for the infusion set without using the stand-alone air-inlet device and calculate the flow rate Q_0 = volume/time.

A.5.3.2 Use a rigid container. Follow the instruction of A.5.1 but do not cut an opening in the container. The container shall not be freely vented. Insert the stand-alone air-inlet device after inserting the closure-piercing device of the infusion set into the container. Calculate the flow rate Q_1 = volume/time. The flow rate Q_1 shall fulfill the requirement as given in Formula (A.4).

A.6 Test for efficiency of the fluid filter

A.6.1 Preparation of the test fluid

Use an aqueous suspension of latex particles with a diameter of (20 ± 1) µm and a concentration of approximately 1 000 particles per 100 ml as a test liquid.

A.6.2 Procedure

Assemble the fluid filter and position it so that it is equivalent to that of actual use in a suitable test apparatus in accordance with Figure A.2. Cut the tubing of the infusion set approximately 100 mm below the fluid filter.

Flush the fluid filter with 5 ml of the test fluid from the storage bottle and discard the filtrate. Pass 100 ml of the test fluid through the fluid filter and collect the effluent under vacuum after passing it through a black gridded membrane filter with a pore size of 5 µm to 8 µm and 47 mm diameter. Mount the membrane with any retained latex particles on a suitable microscope slide or holder and count the latex particles in a minimum of 50 % of the grid squares under a magnification of × 50 to × 100. Disregard any particles which are obviously non-latex.

All procedures involved in this test should be conducted in a clean environment, if possible under laminar flow.

A.6.3 Expression of results

The retention rate, R, of the filter, expressed as a percentage, is given by

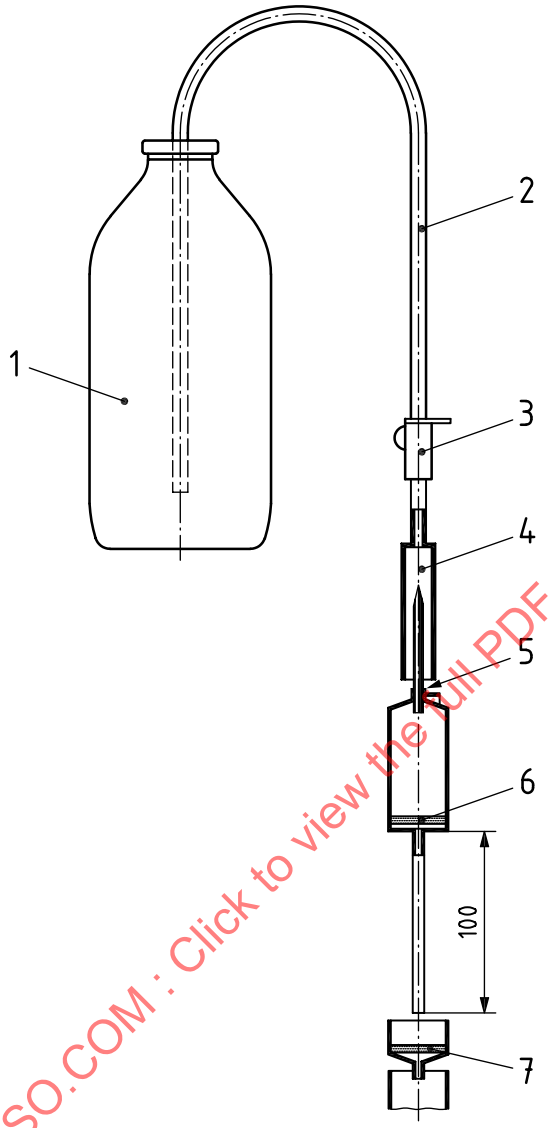
$$R = \left(1 - \frac{n_1}{n_0}\right) \times 100 \tag{A.5}$$

where

n_1 is the number of particles retained on the filter;

n_0 is the number of particles in the test fluid used.

Dimensions in millimetres



Key

1	storage bottle	5	piercing device
2	transfer tube	6	fluid filter
3	flow regulator	7	membrane filter
4	connecting piece		

Figure A.2 — Example apparatus setup for testing the efficiency of the fluid filter

A.7 Test of the injection site

Place the injection site in a horizontal, stress-free position. Fill the infusion set with water in such a manner that no air bubbles are trapped and apply a pressure of 20 kPa above the atmospheric air pressure. Perforate the injection site at the foreseen area using a hypodermic needle with an outside diameter of 0,8 mm and which conforms to ISO 7864. Keep the needle in position for 15 s. Remove the needle and immediately dry the perforated site. Over a period of 1 min, observe whether there is any leakage from the injection site. In the case of an alternative injection site design, the test should be performed by injection into the site in accordance with the instructions provided by the manufacturer.

Annex B
(normative)

Chemical tests

B.1 Preparation of extract solution S_1 and blank solution S_0

B.1.1 Extract solution S_1

Make a closed circulation system composed of three sterilized infusion sets and a 300 ml borosilicate glass boiling flask. Fit to the flask a thermostat device that maintains the temperature of the liquid in the flask at (37 ± 1) °C. Circulate 250 ml of water, conforming to ISO 3696 grade 1 or grade 2, through the system for 2 h at a rate of 1 l/h, e.g. using a peristaltic pump applied to a piece of suitable silicone tubing that is as short as possible. Collect all of the solution and allow to cool.

B.1.2 Blank solution S_0

Blank solution S_0 is prepared as described for extract solution S_1, but omitting the infusion sets from the circuit. The extract solution S_1 and the blank solution S_0 shall be used for the chemical tests.

B.2 Tests for reducing (oxidizable) matter

Add 10 ml of extract solution S_1 to 10 ml of potassium permanganate solution, $c(KMnO_4)$ = 0,002 mol/l and 1 ml of sulfuric acid solution, $c(H_2SO_4)$ = 1 mol/l, shake and allow to react for 15 min at (23 ± 2) °C.

After 0,1 g of potassium iodide has been added, titrate the solution against a sodium thiosulfate standard volumetric solution, $c(Na_2S_2O_3)$ = 0,005 mol/l, until it turns light brown in colour. Add 5 drops of starch solution and continue to titrate until the blue colour has disappeared.

Carry out a blank test simultaneously.

Calculate the difference, in millilitres, of the volume of 0,005 mol/l $Na_2S_2O_3$ solution for the extract solution S_1 and the volume of $Na_2S_2O_3$ solution for blank solution S_0.

B.3 Test for metal ions

Test 10 ml of extract solution S_1 for metal ions, using procedures endorsed by the national pharmacopoeia. Determine the degree of colouration.

B.4 Test for titration acidity or alkalinity

Add 0,1 ml Tashiro indicator solution to 20 ml of extract solution S_1 in a titration flask.

If the colour of the resulting solution is violet, titrate with sodium hydroxide standard volumetric solution, c(NaOH) = 0,01 mol/l, and if green, with hydrochloric acid standard volumetric solution, c(HCl) = 0,01 mol/l, until a greyish colour appears.

Express the volume of sodium hydroxide solution or hydrochloric acid solution used, in millilitres.