Aluminium (*2.4.17*): maximum 10 ppb, if intended for use in the manufacture of dialysis solutions.

Prescribed solution. To 400 ml of the water to be examined add 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *distilled water R*.

Reference solution. Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *distilled water R*.

Blank solution. Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *distilled water R*.

Bacterial endotoxins (*2.6.14*): less than 0.25 IU/ml.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of dialysis solutions.

01/2009:0008

WATER, PURIFIED

Aqua purificata

H_2O M_r 18.02

DEFINITION

Water for the preparation of medicines other than those that are required to be both sterile and apyrogenic, unless otherwise justified and authorised.

Purified water in bulk

PRODUCTION

Purified water in bulk is prepared by distillation, by ion exchange, by reverse osmosis or by any other suitable method from water that complies with the regulations on water intended for human consumption laid down by the competent authority.

Purified water in bulk is stored and distributed in conditions designed to prevent growth of micro-organisms and to avoid any other contamination.

Microbiological monitoring. During production and subsequent storage, appropriate measures are taken to ensure that the microbial count is adequately controlled and monitored. Appropriate alert and action levels are set so as to detect adverse trends. Under normal conditions, an appropriate action level is a microbial count of 100 CFU/ml, determined by filtration through a membrane with a nominal pore size not greater than 0.45 µm, using R2A agar and incubating at 30-35 °C for not less than 5 days. The size of the sample is to be chosen in relation to the expected result.

R2A agar

Yeast extract	0.5 g
Proteose peptone	0.5 g
Casein hydrolysate	0.5 g
Glucose	0.5 g
Starch	0.5 g
Dipotassium hydrogen phosphate	0.3 g
Magnesium sulphate, anhydrous	0.024 g
Sodium pyruvate	0.3 g
Agar	15.0 g
Purified water	to 1000 ml

Adjust the pH so that after sterilisation it is 7.2 ± 0.2. Sterilise by heating in an autoclave at 121 °C for 15 min.

Growth promotion of R2A agar

– *Preparation of test strains.* Use standardised stable suspensions of test strains or prepare them as stated in Table 0008.-1. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot. Grow each of the bacterial strains separately as described in Table 0008.-1. Use buffered sodium chloride-peptone solution pH 7.0 or phosphate buffer solution pH 7.2 to make test suspensions. Use the suspensions within 2 h, or within 24 h if stored at 2-8 °C. As an alternative to preparing and then diluting a fresh suspension of vegetative cells of *Bacillus subtilis*, a stable spore suspension is prepared and then an appropriate volume of the spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2-8 °C for a validated period of time.
– *Growth promotion.* Test each batch of ready-prepared medium and each batch of medium, prepared either from dehydrated medium or from the ingredients described. Inoculate plates of R2A agar separately with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 0008.-1. Incubate under the conditions described in the table. Growth obtained must not differ by a factor greater than 2 from the calculated value for a standardised inoculum. For a freshly prepared inoculum, growth of the micro-organisms must be comparable to that obtained with a previously tested and approved batch of medium.

Table 0008.-1. – *Growth promotion of R2A agar*

Micro-organism	Preparation of the test strain	Growth promotion
Pseudomonas aeruginosa such as: ATCC 9027 NCIMB 8626 CIP 82.118 NBRC 13275	Casein soyabean digest agar or casein soyabean digest broth 30-35 °C 18-24 h	R2A agar ≤ 100 CFU 30-35 °C ≤ 3 days
Bacillus subtilis such as: ATCC 6633 NCIMB 8054 CIP 52.62 NBRC 3134	Casein soyabean digest agar or casein soyabean digest broth 30-35 °C 18-24 h	R2A agar ≤ 100 CFU 30-35 °C ≤ 3 days

Total organic carbon or oxidisable substances. Carry out the test for total organic carbon (*2.2.44*) with a limit of 0.5 mg/l or alternatively the following test for oxidisable substances: to 100 ml add 10 ml of *dilute sulphuric acid R* and 0.1 ml of *0.02 M potassium permanganate* and boil for 5 min; the solution remains faintly pink.

Conductivity. Determine the conductivity off-line or in-line under the following conditions.

EQUIPMENT

Conductivity cell:

– electrodes of a suitable material such as stainless steel;
– cell constant: the cell constant is generally certified by the supplier and is subsequently verified at suitable intervals using a certified reference solution with a conductivity less than 1500 $\mu S \cdot cm^{-1}$ or by comparison with a cell having a certified cell constant; the cell constant is confirmed if the value found is within 2 per cent of the certified value, otherwise re-calibration must be performed.

Conductometer: accuracy of 0.1 µS·cm^{-1} or better at the lowest range.

System calibration (conductivity cell and conductometer):

– against one or more suitable certified reference solutions;
– accuracy: within 3 per cent of the measured conductivity plus 0.1 µS·cm^{-1}.

Conductometer calibration: calibration is carried out for each range of measurement to be used, after disconnection of the conductivity cell, using certified precision resistors or equivalent devices with an uncertainty not greater than 0.1 per cent of the certified value.

If in-line conductivity cells cannot be dismantled, system calibration may be performed against a calibrated conductivity-measuring instrument with a conductivity cell placed close to the cell to be calibrated in the water flow.

Temperature measurement: tolerance ± 2 °C.

PROCEDURE

Measure the conductivity without temperature compensation, recording simultaneously the temperature. Temperature-compensated measurement may be performed after suitable validation.

The water to be examined meets the requirements if the measured conductivity at the recorded temperature is not greater than the value in Table 0008.-2.

Table 0008.-2. – *Temperature and conductivity requirements*

Temperature (°C)	Conductivity (µS·cm^{-1})
0	2.4
10	3.6
20	4.3
25	5.1
30	5.4
40	6.5
50	7.1
60	8.1
70	9.1
75	9.7
80	9.7
90	9.7
100	10.2

For temperatures not listed in Table 0008.-2, calculate the maximal permitted conductivity by interpolation between the next lower and next higher data points in the table.

Heavy metals. If purified water in bulk complies with the requirement for conductivity prescribed for *Water for injections (0169)* in bulk , it is not necessary to carry out the test for heavy metals prescribed below.

CHARACTERS

Appearance: clear and colourless liquid.

TESTS

Nitrates: maximum 0.2 ppm.

Place 5 ml in a test-tube immersed in iced water, add 0.4 ml of a 100 g/l solution of *potassium chloride R*, 0.1 ml of *diphenylamine solution R* and, dropwise with shaking, 5 ml of *nitrogen-free sulphuric acid R*. Transfer the tube to a water-bath at 50 °C. After 15 min, any blue colour in the solution is not more intense than that in a reference solution prepared at the same time in the same manner using a mixture of 4.5 ml of *nitrate-free water R* and 0.5 ml of *nitrate standard solution (2 ppm NO_3) R*.

Aluminium (*2.4.17*): maximum 10 ppb, if intended for use in the manufacture of dialysis solutions.

Prescribed solution. To 400 ml of the water to be examined add 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *distilled water R*.

Reference solution. Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *distilled water R*.

Blank solution. Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *distilled water R*.

Heavy metals (*2.4.8*): maximum 0.1 ppm.

To 200 ml add 0.15 ml of *0.1 M nitric acid* and heat in a glass evaporating dish on a water-bath until the volume is reduced to 20 ml. 12 ml of the concentrated solution complies with test A. Prepare the reference solution using 10 ml of *lead standard solution (1 ppm Pb) R* and adding 0.075 ml of *0.1 M nitric acid*. Prepare the blank solution adding 0.075 ml of *0.1 M nitric acid*.

Bacterial endotoxins (*2.6.14*): less than 0.25 IU/ml, if intended for use in the manufacture of dialysis solutions without a further appropriate procedure for removal of bacterial endotoxins.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of dialysis solutions.

Purified water in containers

DEFINITION

Purified water in bulk that has been filled and stored in conditions designed to assure the required microbiological quality. It is free from any added substances.

CHARACTERS

Appearance: clear and colourless liquid.

TESTS

It complies with the tests prescribed in the section on Purified water in bulk and with the following additional tests.

Acidity or alkalinity. To 10 ml, freshly boiled and cooled in a borosilicate glass flask, add 0.05 ml of *methyl red solution R*. The solution is not coloured red.

To 10 ml add 0.1 ml of *bromothymol blue solution R1*. The solution is not coloured blue.

Oxidisable substances. To 100 ml add 10 ml of *dilute sulphuric acid R* and 0.1 ml of *0.02 M potassium permanganate* and boil for 5 min. The solution remains faintly pink.

Chlorides. To 10 ml add 1 ml of *dilute nitric acid R* and 0.2 ml of *silver nitrate solution R2*. The solution shows no change in appearance for at least 15 min.

Sulphates. To 10 ml add 0.1 ml of *dilute hydrochloric acid R* and 0.1 ml of *barium chloride solution R1*. The solution shows no change in appearance for at least 1 h.

Ammonium: maximum 0.2 ppm.

To 20 ml add 1 ml of *alkaline potassium tetraiodomercurate solution R*. After 5 min, examine the solution down the vertical axis of the tube. The solution is not more intensely coloured than a standard prepared at the same time by adding 1 ml of *alkaline potassium tetraiodomercurate solution R* to a mixture of 4 ml of *ammonium standard*

solution (1 ppm NH_4) R and 16 ml of *ammonium-free water R*.

Calcium and magnesium. To 100 ml add 2 ml of *ammonium chloride buffer solution pH 10.0 R*, 50 mg of *mordant black 11 triturate R* and 0.5 ml of *0.01 M sodium edetate*. A pure blue colour is produced.

Residue on evaporation: maximum 0.001 per cent.

Evaporate 100 ml to dryness on a water-bath and dry in an oven at 100-105 °C. The residue weighs a maximum of 1 mg.

Microbial contamination

TAMC: acceptance criterion 10^2 CFU/ml (*2.6.12*). Use casein soya bean digest agar.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of dialysis solutions.

01/2009:0359

WHEAT STARCH

Tritici amylum

DEFINITION

Wheat starch is obtained from the caryopsis of *Triticum aestivum* L. (*T. vulgare* Vill.).

CHARACTERS

Appearance: very fine, white or almost white powder that creaks when pressed between the fingers.

Solubility: practically insoluble in cold water and in ethanol (96 per cent).

Wheat starch does not contain starch grains of any other origin. It may contain a minute quantity, if any, of tissue fragments of the original plant.

IDENTIFICATION

A. Examined under a microscope using equal volumes of *glycerol R* and *water R*, it presents large and small granules, and, very rarely, intermediate sizes. The large granules, 10-60 µm in diameter, are discoid or, more rarely, reniform when seen face-on. The central hilum and striations are invisible or barely visible and the granules sometimes show cracks on the edges. Seen in profile, the granules are elliptical and fusiform and the hilum appears as a slit along the main axis. The small granules, rounded or polyhedral, are 2-10 µm in diameter. Between orthogonally orientated polarising plates or prisms, the granules show a distinct black cross intersecting at the hilum.

B. Suspend 1 g in 50 ml of *water R*, boil for 1 min and cool. A thin, cloudy mucilage is formed.

C. To 1 ml of the mucilage obtained in identification test B add 0.05 ml of *iodine solution R1*. A dark blue colour is produced, which disappears on heating.

TESTS

pH (*2.2.3*): 4.5 to 7.0.

Shake 5.0 g with 25.0 ml of *carbon dioxide-free water R* for 60 s. Allow to stand for 15 min.

Foreign matter. Examined under a microscope using a mixture of equal volumes of *glycerol R* and *water R*, not more than traces of matter other than starch granules are present. No starch grains of any other origin are present.

Total protein: maximum 0.3 per cent of total protein (corresponding to 0.048 per cent N_2, conversion factor: 6.25), determined on 6.0 g by sulphuric acid digestion (*2.5.9*) modified as follows: wash any adhering particles from the neck into the flask with 25 ml of *sulphuric acid R*; continue the heating until a clear solution is obtained; add 45 ml of *strong sodium hydroxide solution R*.

Oxidising substances (*2.5.30*): maximum 20 ppm, calculated as H_2O_2.

Sulphur dioxide (*2.5.29*): maximum 50 ppm.

Iron (*2.4.9*): maximum 10 ppm.

Shake 1.5 g with 15 ml of *dilute hydrochloric acid R*. Filter. The filtrate complies with the test.

Loss on drying (*2.2.32*): maximum 15.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 90 min.

Sulphated ash (*2.4.14*): maximum 0.6 per cent, determined on 1.0 g.

Microbial contamination

TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).