2.6. BIOLOGICAL TESTS

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2.6.1. STERILITY

The test is applied to substances, preparations or articles which, according to the Pharmacopoeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating micro-organism has been found in the sample examined in the conditions of the test. Guidance for using the test for sterility is given at the end of this text.

PRECAUTIONS AGAINST MICROBIAL CONTAMINATION

The test for sterility is carried out under aseptic conditions. In order to achieve such conditions, the test environment has to be adapted to the way in which the sterility test is performed. The precautions taken to avoid contamination are such that they do not affect any micro-organisms which are to be revealed in the test. The working conditions in which the tests are performed are monitored regularly by appropriate sampling of the working area and by carrying out appropriate controls (such as those indicated in the appropriate European Community Directives and associated guidance documents on GMP).

CULTURE MEDIA AND INCUBATION TEMPERATURES

Media for the test may be prepared as described below, or equivalent commercial media may be used provided that they comply with the growth promotion test.

The following culture media have been found to be suitable for the test for sterility. Fluid thioglycollate medium is primarily intended for the culture of anaerobic bacteria; however, it will also detect aerobic bacteria. Soya-bean casein digest medium is suitable for the culture of both fungi and aerobic bacteria.

Other media may be used provided that they pass the growth promotion and the validation tests.

Fluid thioglycollate medium

L-Cystine	0.5 g
Agar, granulated (moisture content not in excess of 15 per cent)	0.75 g
Sodium chloride	2.5 g
Glucose monohydrate/anhydrous	5.5 g/5.0 g
Yeast extract (water-soluble)	5.0 g
Pancreatic digest of casein	15.0 g
Sodium thioglycollate or	0.5 g
Thioglycollic acid	0.3 ml
Resazurin sodium solution (1 g/l of resazurin sodium), freshly prepared	1.0 ml
Water R	1000 ml

pH of the medium after sterilisation 7.1 ± 0.2

Mix the L-cystine, agar, sodium chloride, glucose, water-soluble yeast extract and pancreatic digest of casein with the *water R* and heat until solution is effected. Dissolve the sodium thioglycollate or thioglycollic acid in the solution and, if necessary, add *1 M sodium hydroxide* so that, after sterilisation, the solution will have a pH of 7.1 ± 0.2. If filtration is necessary, heat the solution again without boiling and filter while hot through moistened filter paper. Add the resazurin sodium solution, mix and place the medium in suitable vessels which provide a ratio of surface to depth of medium such that not more than the upper half of the medium has undergone a colour change indicative of oxygen uptake at the end of the incubation period. Sterilise using a validated process. If the medium is stored, store at 2-25 °C in a sterile, airtight container. If more than the upper third of the medium has acquired a pink colour, the medium may be restored once by heating the containers in a water-bath or in free-flowing steam until the pink colour disappears and cooling quickly, taking care to prevent the introduction of non-sterile air into the container. Do not use the medium for a longer storage period than has been validated.

Fluid thioglycollate medium is to be incubated at 30-35 °C.

Soya-bean casein digest medium

Pancreatic digest of casein	17.0 g
Papaic digest of soya-bean meal	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose monohydrate/anhydrous	2.5 g/2.3 g
Water R	1000 ml

pH of the medium after sterilisation 7.3 ± 0.2

Dissolve the solids in *water R*, warming slightly to effect solution. Cool the solution to room temperature. Add *1 M sodium hydroxide*, if necessary, so that after sterilisation the medium will have a pH of 7.3 ± 0.2. Filter, if necessary, to clarify, distribute into suitable vessels and sterilise using a validated process. Store at 2-25 °C in a sterile well-closed container, unless it is intended for immediate use. Do not use the medium for a longer storage period than has been validated.

Soya-bean casein digest medium is to be incubated at 20-25 °C.

The media used comply with the following tests, carried out before or in parallel with the test on the product to be examined.

Sterility. Incubate portions of the media for 14 days. No growth of micro-organisms occurs.

Growth promotion test of aerobes, anaerobes and fungi. Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from the ingredients. Suitable strains of micro-organisms are indicated in Table 2.6.1.-1.

Inoculate portions of fluid thioglycollate medium with a small number (not more than 100 CFU) of the following micro-organisms, using a separate portion of medium for each of the following species of micro-organism: *Clostridium sporogenes, Pseudomonas aeruginosa, Staphylococcus aureus.* Inoculate portions of soya-bean casein digest medium with a small number (not more than 100 CFU) of the following micro-organisms, using a separate portion of medium for each of the following species of micro-organism: *Aspergillus niger, Bacillus subtilis, Candida albicans.* Incubate for not more than 3 days in the case of bacteria and not more than 5 days in the case of fungi.

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.

The media are suitable if a clearly visible growth of the micro-organisms occurs.

VALIDATION TEST

Carry out a test as described below under Test for sterility of the product to be examined using exactly the same methods except for the following modifications.