Sterility of antibiotics and preparations containing antibiotics

SECTION 1. GENERAL

1.1 Use the applicable of the procedures given in Sections 1 to 4 (inclusive) of SABS Method 2.

SECTION 2. PROCEDURE

NOTE: Test in accordance with 2.1.2 and 2.1.3 all penicillin and penicillin derivatives that can be inactivated with penicillinase and that contain no other antibiotics.

Test in accordance with 2.3 all penicillin preparations that cannot be inactivated with penicillinase or that also contain other antibiotics.

2.1 Penicillin group products that can be inactivated by penicillinase and that contain no other antibiotics

2.1.1 Preparation of Penicillinase solution

a) Dissolve 10 g of casein hydrolysate, 2,72 g of monopotassium phosphate and 5,88 g of sodium citrate in 200 mℓ of water and mix well. Adjust the pH value to 7,2 at 25 °C with an aqueous solution of sodium hydroxide (200 g/ℓ), and dilute to 1 000 mℓ with water.
b) Dissolve 410 mg of magnesium sulphate in 5 mℓ of water and add 1 mℓ of an aqueous solution of ferrous ammonium sulphate (16 g/ℓ) and sufficient water to produce 10 mℓ .
c) Sterilize both solutions prepared in (a) and (b) above, by heating for 15 min in an autoclave at a temperature of 121 °C .
d) Cool and mix the solutions, then distribute the mixture in thin layers at the bottom of sterile conical flasks.
e) Inoculate with *Bacillus cereus* SATCC Bac 92 (similar to NCTC 9946). Keep the flasks at 18-37 °C until growth is apparent and then maintain the temperature at 35-37 °C for 16 h, agitating continuously to ensure maximum aeration.
f) Centrifuge, then decant and sterilize the supernatant liquid by filtration through a suitable membrane filter.

NOTE: In this method reference is made to the latest issues of SABS Methods 2 and 3.

g) Store the penicillinase solution at 0-2 °C and use within 2-3 d .

NOTE: 1,0 mℓ of the penicillinase solution should, at a pH value of 7 and at 30 °C, hydrolyse benzylpenicillin to benzylpenicilloic acid at a rate of at least 500 mg/h, provided that the concentration of benzylpenicillin does not fall below the level necessary for enzyme saturation. The Michaelis constant of the penicillinase for benzylpenicillin in the penicillinase solution, prepared as in (c) above, is approximately 12 g/mℓ . Freshly prepared penicillinase solution should be stored at 0-2 °C and used within 2-3 d . When freeze dried, the sealed ampoules may be stored for several months.

2.1.2 Test for bacterial contamination of the product

a) Withdrawal of sample, and culturing. To a sufficient number of tubes of fluid thioglycollate medium (see Subsection 3.2 of SABS Method 2) add enough sterile solution of penicillinase (see 2.1.1) to inactivate completely a quantity of penicillin equal to the quantity of penicillin to be tested in each tube, and incubate at 32 °C for at least 48 h . After incubation, examine the tubes and discard any tube which shows evidence of bacterial contamination. If the product under test is a liquid, proceed in accordance with Subsections 5.1-5.3.2 (inclusive) of SABS Method 2 and if the product is a solid, proceed in accordance with Subsection 2.1.2 of SABS Method 3, using those tubes containing penicillinase that do not show evidence of contamination. After adding the sample penicillin to the tubes, allow them to stand at room temperature for 2 h but shake frequently. To one of these tubes add 1,0 mℓ of a 1:1 000 dilution of an 18-24 h broth culture of a sensitive strain of *Staphylococcus aureus* (SATCC Sta 9) to serve as a control for completeness of inactivation.

b) Incubation. Incubate the tubes at 32 °C for at least 14 d .

c) Examination. Examine the tubes in accordance with Subsection 5.5 of SABS Method 2. If the control tube shows no growth, repeat the entire test, increasing the quantity of penicillinase used until the control tube shows growth.

2.1.3 Test for mould and yeast contamination of the product

a) Withdrawal of sample, and culturing. To sufficient tubes of soya-bean casein digest medium (see Subsection 3.3 of SABS Method 2) add enough sterile solution of penicillinase (see 2.1.1) to inactivate completely a quantity of penicillin equal to the quantity of penicillin to be tested in each tube, and incubate at 32 °C for at least 48 h . After incubation, examine the tubes and discard any tube which shows evidence of microbiological contamination. If the product under test is a liquid, proceed in accordance with Subsections 5.1-5.3.2 (inclusive) of SABS Method 2 and if the product is a solid, proceed in accordance with Subsection 2.1.2 of SABS Method 3, using the tubes containing penicillinase that do not show evidence of contamination. After adding the sample penicillin to the tubes, allow them to stand at room temperature for 2 h but shake frequently.

b) Incubation. Incubate the tubes at 25 °C for 14 d .

c) Examination. Examine the tubes daily for growth in accordance with Subsection 5.5 of SABS Method 2. Moulds or yeasts or some bacteria may grow.

2.2 Antibiotics other than those belonging to the penicillin group

2.2.1 Apparatus

NOTE: Use a filtration apparatus and a membrane filter so designed that the solution being examined can be introduced and filtered under aseptic conditions and that either the membrane filter can be removed for transfer to the appropriate culture medium, or the incubation can be carried out in the apparatus itself after addition of the appropriate culture medium.

a) Membrane filter. A membrane filter that has a diameter of approximately 50 mm (but at least 45 mm), a nominal pore size not exceeding 0,45 µm and whose effectiveness in retaining micro-organisms has been established. Filters composed of appropriate esters or mixtures of esters of cellulose are recommended for the filtration of aqueous, oily or alcoholic solutions.

NOTE: Individually packed sterile membrane filters that are provided by a reputable firm and that have been sterilized by any method that yields proper performance of the membrane filter may be used.

b) Membrane filter holder. Assemble a suitable membrane filter holder, plug the openings with non-absorbent cotton wool or other suitable closure, wrap the holder in brown paper, and sterilize by autoclaving at 121 °C for 20 min .

2.2.2 Procedure for membrane filtrations

NOTE: Use a separate sterile filter holder and membrane filter for each container to be tested. Ensure that the filters do not leak.

a) Sterile sodium chloride solutions. Prepare a solution containing 9 g of sodium chloride in 1 ℓ of water. Sterilize the solution by heating in an autoclave at 121 °C for 15 min .

b) Filtration of sample. Aseptically attach the sterile filter holder to a filter flask. Loosen the locking device of the funnel and, using sterile forceps, place a sterile membrane filter over the porous disc of the filter holder. Replace the funnel and tighten the locking device. Moisten the membrane filter with sterile distilled water or the sterile sodium chloride solution. In the case of a solution, immediately filter from each container the well-mixed volume (see Table 1 of SABS Method 2) at least twice through the moistened sterile membrane filter. In the case of a powder, aseptically add to each of the containers under test the volume stated on the label or, if this is not stated, a suitable volume of sterile distilled water or the sterile sodium chloride solution. As soon as the solution or the suspension is complete, filter the appropriate well-mixed quantity (see Table 1 of SABS Method 3) at least twice through the moistened sterile membrane filter. To remove residual antibiotics, filter through the membrane filter a sufficient number of 100 mℓ quantities of sterile distilled water, the sterile sodium chloride solution, or other suitable solvent which is not bactericidal or bacteriostatic.

c) Treatment of membrane filters. Using a sterile implement, aseptically transfer each of half of the number of membranes tested to separate tubes (preferably of nominal diameter 35 mm and of nominal length 200 mm) containing 45 mℓ of fluid thioglycollate medium (see Subsection 3.2 of SABS Method 2) and similarly transfer the remaining filter membranes to tubes containing 45 mℓ of soya-bean casein digest medium (see Subsection 3.3 of SABS Method 2).

Alternatively, halve each membrane filter, and aseptically transfer one half to a tube of the size given above, containing 45 mℓ of fluid thioglycollate medium (see Subsection 3.2 of SABS Method 2) and the other half to a tube (of similar dimensions) containing 45 mℓ of soya-bean casein digest medium (see Subsection 3.3 of SABS Method 2).

d) Incubation

1) Tubes containing fluid thioglycollate medium: Incubate at 32 °C for at least 14 d .

2) Tubes containing soya-bean casein digest medium: Incubate at 25 °C for at least 14 d .

e) Examination. Visually examine the tubes daily for growth of micro-organisms. Confirm any visible growth of micro-organisms by microscopical examination of stained smears. Fix smears from fluid thioglycollate medium with methanol. If, after visual examination, the presence or absence of growth of micro-organisms cannot be determined, prepare subcultures within 2 d and incubate at the same temperature as the original culture (see (d) above). Then proceed as in Subsection 5.5.2 of SABS Method 2.

2.3 Antibiotic mixtures containing penicillin and penicillin derivatives

2.3.1 Apparatus. As in 2.2.1.

2.3.2 Penicillinase solution. Prepare the penicillinase solution as in 2.1.1.

2.3.3 Procedure

a) Addition of penicillinase. To a sufficient number of tubes of fluid thioglycollate medium and soya-bean casein digest medium (see Subsections 3.2 and 3.3 of SABS Method 2) add enough sterile solution of penicillinase (see 2.3.2) to inactivate completely a quantity of penicillin equal to the quantity of penicillin to be tested in each tube.

Incubate at 32 °C for at least 48 h . After incubation, examine the tubes and discard any solution which shows evidence of bacterial contamination.

b) Filtration of sample. Proceed in accordance with 2.2.2, using the media tubes prepared as described in (a) above.

c) If the sample cannot be membrane filtered, follow the procedure described in Subsection 5.3.3(c) of SABS Method 2, using the media containing penicillinase described in (a) above.

2.4 Examination, interpretation of results, and retesting. Examine the tubes in accordance with Subsections 5.5, 6.1 and 6.2 of SABS Method 2.

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