Table 27-3 Formulations of Sterility-Test Media

Fluid thioglycollate medium	
L-Cystine	0.5 g
Sodium Chloride	2.5 g
Dextrose monohydrate/Anhydrous	5.5/5.0 g
Agar	0.75 g
Yeast extract (water soluble)	5.0 g
Pancreatic digest of casein	15.0 g
Sodium thioglycollate	0.5 g
Or Thioglycolic acid	0.3 mL
Resazurin sodium solution (1 in 1000)	1.0 mL
Freshly prepared	
Purified water	1000 mL
Soybean-casein digest medium (aka trypticase soy broth)	
Pancreatic digest of casein	17.0 g
Papaic digest of soybean meal	3.0 g
Sodium chloride	5.0 g
Dextrose monohydrate/Anhydrous	2.5/2.3 g
Dibasic potassium phosphate	2.5 g
Purified water	1000 mL

TSB container). TSB has a slightly higher pH (7.3 $\pm$ 0.2) than does FTM (7.1 $\pm$ 0.2), considered a better nutrient for fungal contaminants. TSB promotes growth of fungi and bacteria, and is also considered a better medium for slow-growing aerobic microorganisms than FTM.

Other media have been proposed to replace or be substituted for FTM and/or TSB and these can be found in the USP. For example, concentrated brain heart infusion broth has been suggested as an alternative to FTM and TSB when large-volume parenterals are directly inoculated with culture medium.

After preparation of culture media solutions, a validated steam sterilization process is applied. If media are to be stored, storage temperature should be between 2°C and 25°C in sterile, airtight containers. The length of storage time must be validated.

When membrane filtration is used for the sterility test, a diluting fluid must be used to rinse the filtration assembly in order to ensure that no microbial cells remain anywhere but on the filter surface. The diluting fluid may also be used to dissolve a sterile solid prior to filtration. Diluting fluid A, D, and K formulas are listed in the USP. Diluting fluids are intended to minimize the destruction of small populations of vegetative cells during the pooling, solubilizing, and filtering of sterile pharmaceutical products.

Both FTM and SCD media need to be modified for sterility testing by direct transfer of penicillin and cephalosporin antibiotics. To containers of each medium, transfer aseptically a quantity of β-lactamase sufficient to inactivate the amount of antibiotic in the specimen under test.

TIME AND TEMPERATURE OF INCUBATION

No ideal incubation time and temperature condition exists for the harvesting of all microorganisms. Most organisms grow more rapidly at 37°C than at lower temperatures. However, a temperature of about 23°C may reveal the presence of some organisms that might remain undetected if incubations were done at higher temperatures. The Division of Biologics Standards of the National Institutes of Health discovered that a pseudomonad contaminant in plasma grew in FTM at 25°C, but was killed at 35°C (3). As a result of this finding, the incubation temperature range of FTM was lowered from 32°C–35°C to 30°C–35°C as required by the USP.

The current time and temperature incubation requirements of the USP and EP sterility tests are found in Tables 27-4A and 27-4B. Incubation in TSB is accomplished at 20°C to –25°C because of favorable growth of fungal and slow-growing aerobic contaminants at this temperature range. The time of incubation for sterility testing by membrane filtration is 14 days