Table 27-4A Time and Temperature Incubation Requirements of the USP Sterility Test

Medium	Test procedure	Time (Days)[a]	Temperature (°C)	How product is sterilized
FTM	Direct transfer	14	30–35	Steam or aseptic process
	Membrane filtration	7	30–35	Terminal moist heat
	Membrane filtration	14	30–35	Aseptic process
TSB	Direct transfer	14	20–25	Steam or aseptic process
	Membrane filtration	7	20–25	Terminal moist heat
	Membrane filtration	14	20–25	Aseptic process

[a]Time is the minimum number of incubation days. Additional incubation time may be required if the nature of the product is conducive to produce a "slow-growing" contaminant.

Table 27-4B Time and Temperature Incubation Requirements of the EP Sterility Test

Medium	Test procedure	Time (Days)[a]	Temperature (°C)	How product is sterilized
FTM	Direct transfer	21 (14 + 7)	30–35	Steam or aseptic process
	Membrane filtration	7[b]	30–35	Terminal moist heat
	Membrane filtration	14	30–35	Aseptic process
TSB	Direct transfer	21 (14 + 7)	20–25	Steam or aseptic process
	Membrane filtration	7[b]	20–25	Terminal moist heat
	Membrane filtration	14	20–25	Aseptic process

[a]Time is the minimum number of incubation days. Additional incubation time may be required if the nature of the product is conducive to produce a "slow-growing" contaminant.
[b]A seven-day incubation period is only permissible where authorized or dictated in the European Medicines Evaluation Agency (EMEA) submission. In general, a 14-day incubation period is required for all products, which are required to meet the EP sterility test.

plus four more days to detect growth in media used as negative controls after adding a challenge organism.

Optimal detection conditions for 5 to 50 CFU of nine different microorganisms (aerobic and anaerobic bacteria and molds) were reported to be 22°C to 32°C over 14 days using soybean-casein digest and thioglycollate broths (4).

STERILITY-TEST METHODS

The USP and EP sterility tests specify two basic methods for performing sterility tests—— the direct transfer or direct inoculation method and the membrane filtration method, with a statement that the latter, where feasible, is the method of choice. In fact, in some cases, membrane filtration may be the only possible choice.

Direct Transfer Method

The direct transfer (DT) method is the more traditional sterility-test method. Basically, the DT method involves three steps:

1. Aseptically opening each sample container from a recently sterilized batch of product.
2. Using a sterile syringe and needle to withdraw the required volume of sample for both media from the container.
3. Injecting one-half of the required volume sample into a test tube containing the required volume of FTM and the other half volume of sample into a second test tube containing the required volume of TSB.

The DT method is simple in theory, but difficult in practice. The technician performing the DT test must have excellent physical dexterity and the proper mental attitude about the concern for maintaining asepsis. The demand for repetition in opening containers, sampling, transferring, and mixing can potentially cause fatigue and boredom with a subsequent deterioration in operator technique and concern. As this occurs, the incidence of accidental product sterility-test contamination will increase.