container or packaging system. If microbial growth occurs in a negative control and there is an absence of evidence of accidental contamination, there becomes a clear indication for retesting the product. USP has added verbiage that the sterility test must be carried out under aseptic conditions. Aseptic conditions are achieved without affecting any microorganisms that should be revealed during 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.

Bacteriostatic and Fungistatic Testing

If a sterility test is negative (no growth), there must be the assurance that growth was not inhibited by the antimicrobial properties of the product itself. The USP provides a procedure for determining the level of bacteriostatic and fungistatic activity of a product or material prior to its being tested for sterility by the direct transfer or membrane filtration test. Basically, the procedure calls for adding product to containers of culture media in volumes corresponding to those that would be used for testing the product containing 10 to 100 of the microorganisms listed in Table 27-6 and comparing with medium-inoculum controls without the product. If the material possesses bacteriostatic or fungistatic activity, then the product-media will show decreased or no microbial activity compared to control culture media. If this is the case, then procedures must take place for the proper inactivation of these bacteriostatic/fungistatic properties. Either a suitable sterile inactivating agent must be found or the material and medium must be adequately diluted to overcome the static effects. If at all possible, the membrane filtration test should be applied for those materials found to be bacteriostatic or fungistatic. Where membrane filtration is used, similar comparisons are made of incubated filters through which product and suitable diluting fluid have been passed, each containing the same added microorganisms.

Controls for Membrane Filtration Techniques

The MF test relies on the ability to produce sterile equipment and to have aseptic conditions under which to conduct the test. Three basic control procedures are recommended in separate experiments:

1. The membrane filters are challenged after their sterilization cycle for their ability to retain microorganisms.
2. The exposure times for agar settling plates used to monitor the environment are validated.
3. The cleaning procedures used to remove bacteriostatic and/or bactericidal residues from equipment following the MF test must be validated. This is especially important for the equipment involved in the sterility testing of antibiotics.

VALIDATION OF THE STERILITY TEST

For every product that is tested for sterility, the sterility-test method must be validated for that product. What this means, simply, is that prospective validation studies must be performed to collect data to prove that the sterility test can detect microbiological contamination in the product. Validation of the sterility test for a particular product involves adding small but known concentrations ($\leq$100 CFU) of various microorganisms to the final rinse and then demonstrating recovery of the organisms using the sterility-test methodology. Table 27-6 provides the test organisms required by USP and EP. The EP and USP chapters on sterility testing are now considered to be "harmonized", if the practitioner desires to test a product for sterility release, and that product is required to meet EP and USP requirements, there are several key points to consider.

Organisms from Table 27-6 will have to be used in bacteriostasis and fungistasis test, and *Bacillus subtilis* must be tested in both FTM and TSB. Upon incubation of the challenge containers, all bacteria must show visible growth within three days, and all fungi must show visible growth within five days of the test.

Even if the product is terminally sterilized, the final sterility test must incubate for 14 days (if the membrane filtration technique is used) to satisfy the EP requirement. ATCC 19404 must be used for the *C. sporogenes* challenge to satisfy the EP requirement, while ATCC 11437 must be used *for the C. sporogenes* to satisfy the USP requirement when performing the bacteriostatic and