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MICROBIAL TESTING IN SUPPORT OF ASEPTIC PROCESSING

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Abstract
Sterile products produced in staffed cleanrooms are subject to microbial contamination from the environment in which the process is carried out. The role of microbial testing to ensure the sterility of aseptically filled sterile products is explained, from the product development phase to in-process monitoring to finish product testing. Microbial testing is conducted in the sterile pharmaceutical industry in support of sterile product development; for in-process monitoring during aseptic processing and filling operations; and for testing finished products. In this study will be discussed the role that microbial testing plays in promoting sterility and assurance of aseptically filled sterile products.
Key words: microbial testing, sterile product.

Product development

The objective of the product development process is to take successfully progressing discovery leads from preclinical trials through development with the purpose of defining the formulation, delivery system, manufacturing process, and product specifications. The following microbial tests can be used during sterile product development and scale-up:

- microbial limits and bioburden testing
- bacterial endotoxin testing
- antimicrobial effectiveness testing
- container and closure integrity testing
- bacterial challenge testing for sterilizing filters
- aseptic processing validation using media fills

Pharmaceutical ingredient and packaging component evaluation.

Microbial considerations play a key role in the successful development of new sterile drug products. During formulation development, the potential microbial and endotoxin content of the active pharmaceutical ingredients and excipients should be considered. The testing used to evaluate the ingredients should comply with *USP* General Tests <61> "Microbial Limit Tests" and <82> "Bacterial Endotoxins Tests" [1,2]. Typically, USP- or NF-grade raw materials are selected for use in the formulation and the possible contibution each ingredient would make the product bioburden are evaluated. Recently, United States Pharmacopeia (USP) has begun adding bacterial endotoxin requirements on the basis of maximum human dosage for monograph ingredients that may be used in sterile products. In some cases, the blanket compendial Microbial Limits for the Total Aerobic Microbial Count not more than 1000 cfu/g or mL, and Total Combined Yeast and Mold Count not not more than 100 cfu/g or mL found in the draft *USP* General Chapter <1111> may be too loose for some sterile products [3]. The contribution that each individual ingredient may make to the presterile filtration bioburden, in terms of its concentration in the formulation, must be evaluated to minimize the bacterial challenge to the sterilizing filter and the endotoxin content of the product. Information about the properties and specifications of pharmaceutical ingredients is available in the *Handbook of Pharmaceutical Excipients* [4].

The bioburden of packaging components must be evaluated with respect to the sterilization process that will be used in the manufacture of sterile products. The bacterial endotoxin levels and the potential populations of gram-positive, spore-forming bacteria associated with stoppers and vials are a consideration. Vials must be inspected and packaged for shipment to the customer in a controlled environment. Individual vials must be separated with non-shredding dividers and shrink-wrapped to prevent glass-to-glass contact and particulate contamination. Vials are washed to remove particulates, depyrogenated to remove bacterial endotoxins, and sterilized before aseptic filling. The maximum temperature and belt speed are established for a depyrogenating tunnel that adequately depyrogenates and concurrently sterilizes the vials as they move through the tunnel into the aseptic filling area. Stopper preparation methods should physically remove bacterial endotoxins and nonviable particulates before siliconization. The cleaning and siliconization process should not contribute to the bioburden. The sterilization cycle ensures that the stoppers are sterile and dry so they can be stored before the aseptic filling operation. It should be noted that steam sterilization is not a depyrogenation step. Typically, stoppers are steam sterilized in heat-sealed, nonwoven, high-density polyethylene bags