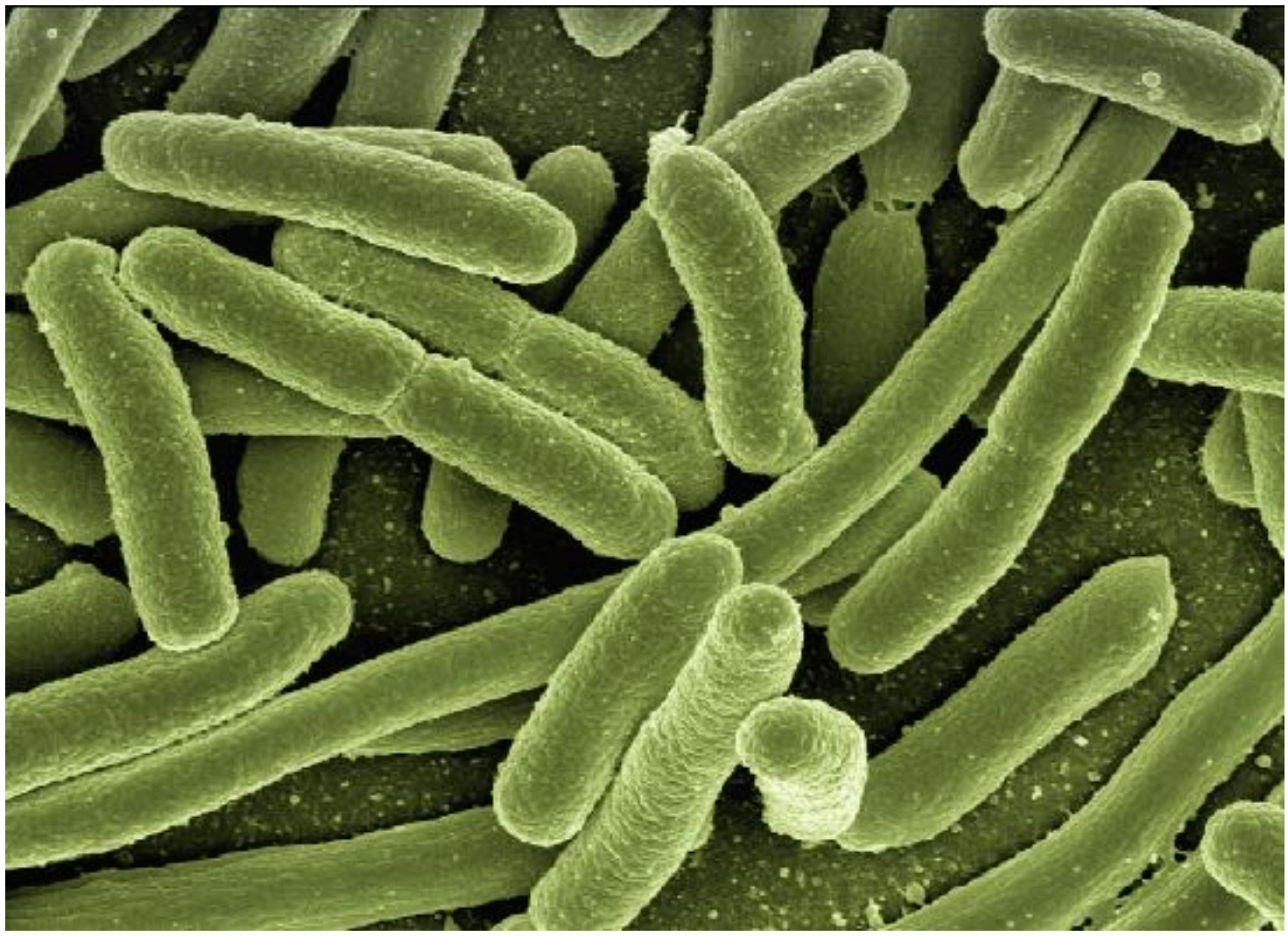
THINKING ABOUT USP CHAPTER <61> MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS: MICROBIAL ENUMERATION TESTS



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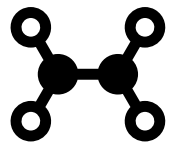

TEACHING METHOD

The general approach taken in this ebook is that training based first and foremost upon compliance, in this case with general chapter <61> of the USP, does not adequately prepare people to handle the unexpected because that mode of training does not emphasize what should always be of paramount importance to technical people: asking scientific questions. A thorough description of the educational philosophy followed may be found in the paper Training for Compliance Employing Scientific/ Skeptical Thinking at http://www.microbiologyforum.org/content/file/White Papers/PMF - Training for Compliance Versus Training for Thinking v6(1).pdf

<61> INTRODUCTION

The methods in this chapter are designed to provide a quantitative enumeration of mesophilic microorganisms that may grow aerobically. Both fungal and bacterial species are included. It is important to remember that these compendial tests are intended to determine whether a compendial article (substance or preparation) complies with an established specification for microbiological quality. Where are such specifications provided in the USP? Is it absolutely essential that the methods described in this chapter are used, or are alternative methods acceptable, and under what conditions could they be shown to be acceptable?

GENERAL PROCEDURES

It should come as no surprise that the materials to be tested must be handled in a manner that avoids extrinsic contamination, and they should be handled in such a way that does not interfere with any microorganisms that may be in the articles to be evaluated. What are some ways that extrinsic contamination may occur, and what are some ways of handling that could interfere with microorganisms that are present in the articles to be tested? What should be done if the articles to be tested contain antimicrobial agents?

ENUMERATION METHODS

The preferred methods of enumeration described in the chapter are the Membrane Filtration method or one of the various Plate-Count methods. They are preferred over the Most Probable Number method (MPN). Why is the MPN method not preferred, and in what possible circumstances is it nonetheless the best choice? The nature of the product and the required limit (where is the required limit specified?) to be met influences the choice of method. What qualifies as a sufficient sample size? Does the chosen method need to be **validated**, or does its **suitability** need to be determined?

GROWTH PROMOTION TEST, SUITABILITY OF THE COUNTING METHOD AND NEGATIVE CONTROLS

The compendial methods describe in the USP related to microbial detection require that any microorganisms present be capable of growth under the experimental conditions. Thus the ability of the media used to support growth must be established. While methods provided in the USP are considered validated (where can you find text in the USP confirming this?), articles under test must be shown to be suitable for use with the validated method. If the article under test cannot be shown to be suitable for use with the compendial method, does that mean the manufacturer no longer needs to be concerned with meeting the limits as stated in the related compendial monograph?

The chapter lists five species of microorganisms that should be used in demonstrating growth promotion and the suitability of the method. Three are bacterial species, and two are fungal species. They should be maintained with seed-lot techniques. Why is it important to include bacterial and fungal species in the list of five species to be evaluated?

The negative control should be using the diluent instead of the test article. What should be observed with the negative control?

Growth Promotion

The two types of media for bacteria should be inoculated with $\leq$ 100 cfu of bacteria, and the fungal medium with $\leq$ cfu of the designated fungal species. Adequate growth must be observed. Why is it important to use a low number of cfu in the inoculations?

Suitability of the Counting Method in the Presence of Product

The chapter describes a number of methods to be used in the preparation of the samples. To be acceptable, the method must not interfere with the growth of any microorganisms present in the sample, or with the growth of the test inocula (the five species). If none of the provided methods work, does this mean the manufacturer does not need to be concerned about microbial enumeration?

Prepared test samples should be inoculated with $\leq$ 100 cfu of the required microbiological species as used in the growth promotion portion, and the volume of the inocula should not exceed 1% of the sample. Why is that important?

With certain samples, additional protocols must be developed due to poor solubility, or appreciable antimicrobial activity. In some cases, the inocula could be added after neutralization, dilution, or filtration of the antimicrobial activity.

The chapter provides a table containing possible neutralizing agents.

In case no suitable means of eliminating the antimicrobial properties of the test article can be found for a given species, this indicates a low likelihood of contamination with that particular species. Does this mean the manufacturer does not need to be concerned with potential growth of other possible contaminating species?

Recovery of Microorganisms in the Presence of Product

The chapter describes various methods to be used in the enumeration of microorganisms. They include Membrane Filtration, two Pour Plate methods, and the Most Probable Number method (MPN was mentioned earlier). The counts obtained for each test species from the Membrane Filtration or either of the Plate Count methods should not differ be more than a factor of 2 from the cfu added by the inocula. The calculated MPN value must be within 95% confidence limits of the results obtained with the control. What should be done if the criteria cannot be met for one or more of the test species?

TESTING OF PRODUCTS

10g or 10 mL of sample to be tested should be used. Are there circumstances where this sample size would be unacceptable for a manufacturer, and if so, what can be done? Specifics for the various counting methods (Membrane Filtration, Pour Plate, Most Probable Number) are proved with a common caveat pertaining to the Growth Promotion Test and Suitability of the Counting method section. What is this caveat?

Interpretation of the Results

Acceptance criteria (described where?) are interpreted thusly:

10^1 cfu maximum acceptable count = 20;

10^2 cfu maximum acceptable count = 200;

10^3 cfu maximum acceptable count = 2000;

Why is the two-fold difference from what is provided in the monograph specification allowable?

FINDING ANSWERS

Many questions were asked in this e-book. They are intended to get your scientific thinking processes fully engaged. Answers are not always clear cut for some of these questions, and it is important to remember that much of science exists in grey areas, including portions of regulatory science, and answers specific to your needs may need to be developed with the best possible scientific thinking, but understanding that there may not be absolute answers to your questions. Remember that for many questions pertaining to the compendia, and specifically the USP in this case, the chapter itself has many answers, as does the USP section General Notices and Requirements.