[PPT PDF] Pharmaceutical Water System Design Validation - Microbial Testing of Water is discussed in detail in this article.

Pharmaceutical Water System: Classical Culture Approach for microbial testing of water

Classical culture approaches for microbial testing of water include but are not limited to pour plates, spread plates, membrane filtration, and most probable number (MPN) tests. These methods are generally easy to perform, are less expensive, and provide excellent sample processing throughput. Method sensitivity can be increased via the use of larger sample sizes. This strategy is used in the membrane filtration method. Culture approaches are further defined by the type of medium used in combination with the incubation temperature and duration. This combination should be selected according to the monitoring needs presented by a specific water system as well as its ability to recover the microorganisms of interest: those that could have a detrimental effect on the product or process uses as well as those that reflect the microbial control status of the system.

There are two basic forms of media available for traditional microbiological analysis: “high nutrient” and “low nutrient”. High-nutrient media such as plate count agar (TGYA) and m-HPC agar (formerly m-SPC agar), are intended as general media for the isolation and enumeration of heterotrophic or “copiotrophic” bacteria. Low-nutrient media such as R2A agar and NWRI agar (HPCA), may be beneficial for isolating slow growing “oligotrophic” bacteria and bacteria that require lower levels of nutrients to grow optimally. Often some facultative oligotrophic bacteria are able to grow on high nutrient media and some facultative copiotrophic bacteria are able to grow on low-nutrient media, but this overlap is not complete. Low-nutrient and high-nutrient cultural approaches may be concurrently used, especially during the validation of a water system, as well as periodically thereafter. This concurrent testing could determine if any additional numbers or types of bacteria can be preferentially recovered by one of the approaches. If so, the impact of these additional isolates on system control and the end uses of the water could be assessed. Also, the efficacy of system controls and sanitization on these additional isolates could be assessed.

Duration and temperature of incubation are also critical aspects of a microbiological test method. Classical methodologies using high nutrient

media are typically incubated at 30 to 35 for 48 to 72 hours. Because of the flora in certain water systems, incubation at lower temperatures (e.g., 20 to 25) for longer periods (e.g., 5 to 7 days) can recover higher microbial counts when compared to classical methods. Low-nutrient media are designed for these lower temperature and longer incubation conditions (sometimes as long as 14 days to maximize recovery of very slow growing oligotrophs or sanitant injured microorganisms), but even high-nutrient media can sometimes increase their recovery with these longer and cooler incubation conditions. Whether or not a particular system needs to be monitored using high- or low-nutrient media with higher or lower incubation temperatures or shorter or longer incubation times should be determined during or prior to system validation and periodically reassessed as the microbial flora of a new water system gradually establish a steady state relative to its routine maintenance and sanitization procedures. The establishment of a "steady state" can take months or even years and can be perturbed by a change in use patterns, a change in routine and preventative maintenance or sanitization procedures, and frequencies, or any type of system intrusion, such as for component replacement, removal, or addition. The decision to use longer incubation periods should be made after balancing the need for timely information and the type of corrective actions required when an alert or action level is exceeded with the ability to recover the microorganisms of interest.

The advantages gained by incubating for longer times, namely recovery of injured microorganisms, slow growers, or more fastidious microorganisms, should be balanced against the need to have a timely investigation and to take corrective action, as well as the ability of these microorganisms to detrimentally affect products or processes. In no case, however, should incubation at 30 to 35 be less than 48 hours or less than 96 hours at 20 to 25 .

Normally, the microorganisms that can thrive in extreme environments are best cultivated in the laboratory using conditions simulating the extreme environments from which they were taken. Therefore, thermophilic bacteria might be able to exist in the extreme environment of hot pharmaceutical water systems, and if so, could only be recovered and cultivated in the laboratory if similar thermal conditions were provided. Thermophilic aquatic microorganisms do exist in nature, but they typically derive their energy for growth from harnessing the energy from sunlight, from oxidation/reduction reactions of elements such as sulfur or iron, or

indirectly from other microorganisms that do derive their energy from these processes. Such chemical/nutritional conditions do not exist in high purity water systems, whether ambient or hot. Therefore, it is generally considered pointless to search for thermophiles from hot pharmaceutical water systems owing to their inability to grow there.

The microorganisms that inhabit hot systems tend to be found in much cooler locations within these systems, for example, within use-point heat exchangers or transfer hoses. If this occurs, the kinds of microorganisms recovered are usually of the same types that might be expected from ambient water systems. Therefore, the mesophilic microbial cultivation conditions described later in this chapter are usually adequate for their recovery.

"Instrumental" Approaches for microbial testing of water : Pharmaceutical Water System

Examples of instrumental approaches include microscopic visual counting techniques (e.g., epifluorescence and immunofluorescence) and similar automated laser scanning approaches and radiometric, impedometric, and biochemically based methodologies. These methods all possess a variety of advantages and disadvantages. Advantages could be their precision and accuracy or their speed of test result availability as compared to the classical cultural approach. In general, instrument approaches often have a shorter lead time for obtaining results, which could facilitate timely system control. This advantage, however, is often counterbalanced by limited sample processing throughput due to extended sample collection time, costly and/or labor-intensive sample processing, or other instrument and sensitivity limitations.

Furthermore, instrumental approaches are typically destructive, precluding subsequent isolate manipulation for characterization purposes. Generally, some form of microbial isolate characterization, if not full identification, may be a required element of water system monitoring. Consequently, culturing approaches have traditionally been preferred over instrumental approaches because they offer a balance of desirable test attributes and post-test capabilities.

Suggested Methodologies : Pharmaceutical Water System

The following general methods were originally derived from Standard Methods for the Examination of Water and Wastewater, 17th Edition,

American Public Health Association, Washington, DC 20005. Even though this publication has undergone several revisions since its first citation in this chapter, the methods are still considered appropriate for establishing trends in the number of colony-forming units observed in the routine microbiological monitoring of pharmaceutical waters. It is recognized, however, that other combinations of media and incubation time and temperature may occasionally or even consistently result in higher numbers of colony-forming units being observed and/or different species being recovered.

Drinking Water:	POUR PLATE METHOD OR MEMBRANE FILTRATION METHOD[1]
	Sample Volume—1.0 mL minimum[2] Growth Medium—Plate Count Agar[3] Incubation Time—48 to 72 hours minimum Incubation Temperature—30° to 35°
Purified Water:	POUR PLATE OR MEMBRANE FILTRATION METHOD[1]
	Sample Volume—1.0 mL minimum[2] Growth Medium—Plate Count Agar[3] Incubation Time—48 to 72 hours minimum Incubation Temperature—30° to 35°
Water for Injection:	MEMBRANE FILTRATION METHOD[1]
	Sample Volume—100 mL minimum[2] Growth Medium—Plate Count Agar[3] Incubation Time—48 to 72 hours minimum Incubation Temperature—30°C to 35°C

The extended incubation periods that are usually required by some of the alternative methods available offer disadvantages that may outweigh the advantages of the higher counts that may be obtained. The somewhat higher baseline counts that might be observed using alternate cultural conditions would not necessarily have greater utility in detecting an excursion or a trend. In addition, some alternate cultural conditions using low-nutrient media tend to lead to the development of microbial colonies that are much less differentiated in colonial appearance, an attribute that microbiologists rely on when selecting representative microbial types for further characterization. It is also ironical that the nature of some of the slow growers and the extended incubation times needed for their development into visible colonies may also lead to those colonies being largely nonviable, which limits their further characterization and precludes their subculture and identification.

Methodologies that can be suggested as generally satisfactory for monitoring pharmaceutical water systems are as follows. However, it must be noted that these are not referee methods nor are they necessarily optimal for recovering microorganisms from all water systems. The users should determine through experimentation with various approaches which methodologies are best for monitoring their water systems for in-process control and quality control purposes as well as for recovering any contraindicated species they may have specified.

Pharmaceutical Water System: Drinking Water:

POUR PLATE METHOD OR MEMBRANE FILTRATION METHOD1

Sample Volume—1.0 mL minimum2

Growth Medium—Plate Count Agar3

Incubation Time—48 to 72 hours minimum

Incubation Temperature—30 to 35

Purified Water:

POUR PLATE OR MEMBRANE FILTRATION METHOD1

Sample Volume—1.0 mL minimum2

Growth Medium—Plate Count Agar3

Incubation Time—48 to 72 hours minimum

Incubation Temperature—30 to 35

Water for Injection:

MEMBRANE FILTRATION METHOD

Sample Volume—100 mL minimum2

Growth Medium—Plate Count Agar3

Incubation Time—48 to 72 hours minimum

Incubation Temperature—30 C to 35 C

1 A membrane filter with a rating of 0.45 µm is generally considered preferable even though the cellular width of some of the bacteria in the sample may be narrower than this. The efficiency of the filtration process still allows the retention of a very high percentage of these smaller cells and is adequate for this application. Filters with smaller ratings may be used if desired, but for a variety of reasons the ability of the retained cells to develop into visible colonies may be compromised, so count accuracy must be verified by a reference approach.

2 When colony counts are low to undetectable using the indicated minimum sample volume, it is generally recognized that a larger sample volume should be tested in order to gain better assurance that the resulting colony count is more statistically representative. The sample volume to consider testing is dependent on the user's need to know (which is related to the established alert and action levels and the water system's microbial control capabilities) and the statistical reliability of the resulting colony count. In order to test a larger sample volume, it may be necessary to change testing techniques, e.g., changing from a pour plate to a membrane filtration approach. Nevertheless, in a very low to nil count scenario, a maximum sample volume of around 250 to 300 mL is usually considered a reasonable balance of sample collecting and processing ease and increased statistical reliability. However, when sample volumes larger than about 2 mL are needed, they can only be processed using the membrane filtration method.

3 Also known as Standard Methods Agar, Standard Methods Plate Count Agar, or TGYA, this medium contains tryptone (pancreatic digest of casein), glucose and yeast extract.