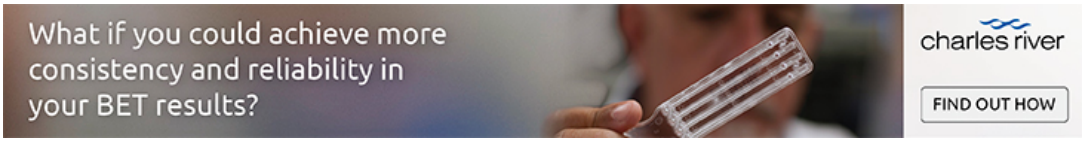

Justification of Incubation Conditions Used for Environmental Monitoring

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Introduction

Recently, a number of different documents have been making statements similar to "incubation conditions and media used must be justified". Many companies were able to cite USP <1116> as a rationale for using two different incubation conditions including 20°C-25°C and 30°C-35°C. However, the most recent revision to USP states: "Time and incubation temperatures are set once the appropriate media have been selected. Typically, for general microbiological growth media such as SCDM, incubation temperatures in the ranges of approximately 20°C–35°C have been used with an incubation time of not less than 72 hours." [1]. With a 15°C range in temperature, it is not likely that a single study will provide adequate data to justify the incubation conditions use. As such, companies need to generate data to support the incubation conditions utilized.

Background

For many years, pharmaceutical companies have used two different media for conducting monitoring of the environment. One of the media was designed for the recovery of bacteria and the other was chosen to isolate fungi (mold and yeast). Typically the bacterial media was incubated at 30°C-35°C for a set number of days and the fungal media was incubated at 20°C-25°C for a specified number of days.

Marshall, et al. published an article entitled "Comparative Mold and Yeast Recovery Analysis (The Effect of Differing Incubation Temperature Ranges and Growth Media)". This article described the studies performed to support the use of a single type of media to isolate both bacteria and fungi under a specified set of conditions that included two different temperature ranges. [2]

In the process of evaluating a novel sterility test method, Kielpinski, et al. studied the appropriate incubation temperature to use. Their data indicated that an incubation temperature of 32°C provided improved detection of the microorganisms in the sterility test method over the values obtained using the incubation conditions in the compendia. [3]

In 2012, the USP revised monograph <1116> from "Microbiological Evaluation of Clean Rooms and Other Environments" to a chapter entitled "Microbiological Control and Monitoring of Aseptic Processing Environments". As part of the change, this indicates that when using two-temperature incubation of environmental monitoring plates, if one chooses to incubate at the lower temperature first, the recovery of Gram-positive cocci may be compromised.

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In addition to the other variables associated with monitoring, the media used may contain neutralizing agents, like polysorbate 80 (Tween 80) and/or lecithin to improve the recovery of microorganisms from areas that may have been exposure to sanitizers or other antimicrobial substances.

Other types of concerns with environmental monitoring include: whether or not anaerobic monitoring should be conducted routinely, whether different culture media should be utilized, whether the incubation conditions selected can recover slow-growing microorganisms, and so forth.

This article describes a testing methodology that can be used to evaluate the environmental monitoring procedures utilized to justify the methods used. In most cases, a company would find the use of a single medium and a single set of incubation conditions the most cost effective approach to use if it were acceptable.

A two-phase approach to the protocol was developed to justify the environmental monitoring conditions. In this approach, Phase 1 involves testing a variety of samples in the laboratory to select an appropriate media and set of incubation conditions to evaluate *in situ*. Phase 2 is a comparative side-by-side study *in situ* looking at the existing environment monitoring conditions used and the proposed monitoring method selected in Phase 1.

Testing Scheme – Phase 1 Laboratory Testing to Select the Proposed Test Conditions

During this phase of testing, consideration should be taken to determine the various types of organisms and media conditions to be evaluated. Test organisms should include both laboratory stock cultures and environmental isolates from the facility. A rationale should be prepared to identify how organisms were selected, e.g., at least one each of a Gram-positive cocci and bacillus, a Gram negative cocci and bacillus, a mold and yeast. Additionally, prominent organisms previously isolated in environmental monitoring should be utilized. It is important to include a slow-growing microorganism like P. acnes in the test scheme. Some companies choose to include more organisms of a specific type that they routinely find in their environment, or have been associated with adverse trends or sterility failures.

If you have a program currently established, you will likely want to include those conditions routinely used in the test environment, especially if you intend to have acceptance criteria that compares to that same data.

Another consideration for the testing is the types of media to evaluate. It is common to look at a common nutrient media, like Trypticase Soy Agar. Fungal media such as Sabouraud Dextrose Agar or Potato Dextrose Agar can also be used. Some companies like to evaluate the performance of media with lower levels of nutrients, like R2A. There are companies that prefer Brain Heart Infusion broth/agar for recovery. Each of the media selected can be tested both with and without neutralizing agents, if desired. One might also try use of a designated anaerobic media (or regular media and an anaerobic system) to evaluate the difference in counts using anaerobic incubation.

Incubation conditions (temperature and time) should also be specified, e.g., 20°C-25°C for three days followed by 30°-35°C for an additional three days or continue to incubate for up to seven, 30°C-35°C for three days followed by 20°-25°C for an additional three days, 20°C-25°C for up to seven days (final incubation time to be determined at the end of phase 1 testing), 30°C- 35°C for up to seven days (final incubation time to be determined at the end of phase 1 testing), and so forth.

Following selection of the test organisms, test media, and incubation conditions, a series of studies are set up where each test organism is tested on each media for each of the indicated incubation conditions. For the extended incubation periods, such as up to seven days, it is worthwhile to record the counts daily to determine the optimal time for recovery of all specified organisms. While this is an extensive test, it provides a wealth of information for the facility.

The number of replicates to run for each test condition should also be specified. A higher number of replicates can be useful if using statistical analysis for the data.

Following collection of the data for all the test conditions, the results are analyzed to determine which test condition provides the optimal results. In most cases, it is costbeneficial to use a single media and a single incubation temperature. Some companies may choose to use different test conditions for different areas of the facility, e.g., use of media with neutralizers in areas where recovery may be stifled without the use of neutralizers.

Detailed instructions should be provided in the protocol to address how to select the desired test conditions, e.g.,

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slow-growing organisms be recovered in the test, do anaerobes need to be recovered, and so forth. Levels of recovery should also be addressed, e.g., if the counts must be the same or better than an existing method being used. There are different criteria that can be used for recovery levels, e.g., within 70% of a reference level (perhaps an existing methodology), within 0.5 log, or other method of choice. An important concern in this discussion is what to do if none of the media tested is successful in recovery for all of the identified acceptance criteria. It is important to seriously consider the criteria you will use for test condition acceptance, as you could design a study that precludes use of any of the test conditions identified. In some cases, you may also find that a variety of methods provide acceptable recovery and it becomes important to identify how to select the method to use.

Phase 2 – *in situ* testing: Comparative study

For a facility already conducting environmental monitoring, the new method selected (optimal method) will be compared to the existing methods (reference method) being used in the site, e.g., a biphasic, two media method. The purpose of this phase is to assess the appropriateness of the selected medium and incubation conditions *in situ*.

Environmental monitoring is conducted in accordance with the site monitoring procedures, however at every selected monitoring point, test samples are collected (using the optimum method selected in Phase 1) in addition to the required samples in the site standard operating procedure (SOP). The samples are incubated and evaluated per the appropriate methodology, i.e., the existing method plates are incubated as specified in the SOP while the test plates, using the conditions specified in Phase 1, are incubated as tested in Phase 1. A minimum of 20 separate monitoring locations should be identified for evaluation.

Figure 1. Determining Each Test Condition

For these types of comparisons, it is easier to include rooms where levels of recovery are expected, e.g., ISO 7 and 8 areas. This will aid in reducing variability if very low levels of microorganisms are isolated. The choice of sampling sites may be based upon previous recovery data. If no data exists, sampling sites may include lower level classified areas, hallways, or unclassified areas. Following incubation, the average recovered counts, in colony forming units (cfu) are determined. A comparison of the counts using the test method and the reference method will be conducted. Acceptance criteria for the comparison of data should be established. In most cases, the test method should give results that are equivalent and/or better than the existing method used. It is useful to maintain all the samples (refrigerated) until the end of the analysis, so that plates can be recounted or analyzed if necessary.

If a reference method is not available for comparison, the test method should be used *in situ* for a specified number of days much like one might do with an initial performance qualification of an environmental monitoring program.

Conclusion

This testing scheme involves a good deal of work but provides conclusive data on the appropriateness of the environmental monitoring conditions utilized. When audited, it provides documented evidence to support the methods selected and addresses the concerns of some regulators on which incubation condition should be initiated first. Lastly, in many cases companies can end up with a set of conditions that are more cost effective than the current methods.

Acknowledgement

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References

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Author Biography

Jeanne Moldenhauer is the Vice President of Excellent Pharma Consulting. She has over 25 years of experience in the pharmaceutical, biotechnological and device industries. She chairs the Microbiology/ Environmental Monitoring Interest Group of PDA and is on the Scientific Advisory Board. She has numerous publications and books.

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Duggirala Ratnakumari • a year ago
Hi mam I am Ratnakumari working as a Microbiologist in pharma company. Some of the Sirs asking me why you r keeping scda plates for fungal growth only 72 hrs. Actually as per gpt it's inbation period is 5-7 days then how you are saying 72 hrs enough for yeasts and molds count.
Please tell me the answer.
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THYAGARAJAN Parthasarathy • 2 years ago
Sir,
Actually my doubt is anyhow SCDA is a general media which can able to enhance the growth of both bacteria and fungi. So on that basis , the hypothetical test shall be performed by performing GPT on SCDA by inoculating different types of organisms including fungi, mold & isolates and incubated in 30 -35 degree centigrade for maximum 5 days.So every day while doing monitoring the growth of each organisms in SCDA plate, if it supports the growth more than 70% then we can use the environmental monitoring SCDA plates in single media with single incubation period.Is it acceptable?.
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VAIBHAV DESHPANDE • 2 years ago
Hi, I have one query, can we go for incubation of the Environmental monitoring plates at 30-35 degree as single media with single incubation condition for 5 days. Is it acceptable to regulators?
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Amit angaria ➚ VAIBHAV DESHPANDE • 2 years ago

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Goutam Saha • 2 years ago

A very useful and practical discussion. It has given clear picture on the scientific considerations for a holistic environmental monitoring approach to the pharma community.
Please provide some clarity on the following points for the selection of EM methodology for anaerobic organisms;
1. How the selection of the media and organism will be made? Is it from the product nature that it demands anaerobic condition in manufacturing and processing?
2. Will the assessment of EM statistical data and microbial analysis of input material give some clue (growth) to plan for anaerobic EM?
3. Whether process simulation study will be impacted and needed to perform for anaerobic media under anaerobic condition?
4. Can the recovery study of the identified isolates be performed under simulated condition in the microbiology laboratory?
5. The process of testing of the targeted in-house isolates and maintaining the condition suitable to grow them, makes the ground level working complex and time taking. How to make it practicable and friendly?

Kindly comment.

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