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Issues that Can Affect the Accuracy of Environmental Monitoring Data

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Introduction

In the last decades, the importance of implementing and maintaining an Environmental Monitoring (EM) Program that is compliant with current Good Manufacturing Practice (cGMP) has increased significantly, especially in manufacturing plants dedicated to the production of injectable drugs. Numerous guidelines have been issued on this topic and the interest regulatory authorities have shown concerning EM data, and how these data are generated, has been growing continually.

EM is generally recognized as a fundamental part of the sterility assurance system related to the manufacture of sterile drugs, especially by aseptic processing. Nevertheless, it is generally assumed that monitoring environmental conditions from a microbiological point of view can never lead to complete and objective knowledge of the quantity and quality of microbial flora in the manufacturing area. The sensitivity of traditional monitoring techniques, such as air sampling, swabs, or Rodac®, is well known to be very low, and, from a time and space perspective, there is no practical possibility of covering all the manufacturing processes with a monitoring program.

Although monitoring is similar to photographing the manufacturing environment using an old camera (with a delay of five to seven days in obtaining the image due to incubation time), the resulting “pictures” have great importance in maintaining the process under control and should reflect reality as closely as possible. Regarding EM data, it is essential to compare data obtained in a medium-long timeframe against expected performance to detect any drift from the normal status of the system (as it was assessed during the initial validation phase),and simply to provide evidence that things are still going as expected.

The ability of generating accurate, reliable, and consistent EM data is, therefore, a key aspect of laboratory compliance and a fundamental tool in sterility assurance, especially for aseptically filled products. Moreover, a lack of reliability in EM data could lead to serious business issues, since contamination caused by improper sampling techniques or non sterile media in a critical location within grade “A” areas may have a significant impact, at least in delaying batch release and in allocating time and resources for the necessary investigation. On the other hand, any question regarding the ability of the EM program to effectively detect environmental contamination, especially in critical areas, could raise serious doubts concerning the credibility of the aseptic process, of all sterility assurance levels, and could potentially lead to dramatic consequences such as the recall of batches already released.

Recent inspection focus has been moving from a simple revision of EM data to an accurate assessment concerning data generation in order to evaluate whether the data are really meaningful and representative of the actual situation.

Issues Related to EM Sampling Technique

The accuracy of EM data can be affected by many factors, such as the training of Quality Control (QC) operators or, generally speaking, the training of anyone responsible for the execution of monitoring activities; the calibration of monitoring equipment, including those dedicated to incubation and processing of samples; the choice and validation of culture media; the quality control of media; and the choice and validation of incubation conditions and documentation.

Regarding operator training, people conducting EM should understand the scientific basis of their activity including the factors that can affect microorganism proliferation. The educational background and experience of all operators should be adequate to support the understanding required by their duties.

More specifically, people conducting EM should be trained properly on the subjects of gowning procedures and aseptic techniques, in the use of sampling devices and equipment, and in all relevant cGMP documentation skills. Management of sampling activities is another critical issue that requires specific skills and training, since routine EM monitoring activities in an average-size clean room typically imply a huge number of samplings, with many different locations and frequencies.

While operating in grades A and B, people conducting EM may experience problems in communicating with their supervisors or colleagues who work outside the clean room. Therefore, it is important for EM technicians to be able to manage monitoring activities properly, according to defined Standard Operating Procedures (SOPs) and sampling plans. They must also react in appropriate ways to unexpected events in the clean room, performing extra monitoring, as necessary. An EM technician's witnessing of manufacturing activities and aseptic behaviors can often be useful in the development of investigations due to excursions from alert or action levels. Since monitoring activities are usually complicated, good skills in documentation are also required to be able to track all the performed activities correctly and in a timely manner.

Working with culture media in the clean room is critical, since these materials have been designed to promote the proliferation of exactly what we do not want in the clean room itself! Ideally, monitoring activities in critical areas should not be conducted by humans, who are by far the biggest source of contamination, but, since the outcome of automation in aseptic operations, including environmental monitoring, is still far away, we must rely on effective personnel training, the development of adequate skills, and the personal, professional commitment of the technicians.

EM operators must be aware of the significant potential impact of their activities on product sterility assurance. They should consistently apply proper clean room procedures. The personnel monitoring data of EM operators should be carefully reviewed and, at a minimum, their annual participation in successful media fills, with actual involvement in monitoring activities, is required. (The debate concerning the delegation of all or some monitoring to production staff, instead of dedicated people from the Quality Unit, is still open.)

In case you have production staff performing some or all EM activities, training and qualification issues become even more important. At minimum, the Quality Unit should have a system in place to monitor the performance of the production staff and their adherence to cGMP and internal SOPs.

Issues Related to EM Sampling Instrumentation and Incubation Equipment

Calibration of sampling instrumentation involved in EM is another key requirement in achieving a compliant EM program. Dynamic air samplers should be consistently able to sample the required volume of air (usually 1 m3). Air quality is ensured by a semiannual calibration, usually performed by the supplier following a validated procedure. The most relevant sampling parameter to be verified is the volumetric flow rate. Verification is usually performed by putting the sampler in series with a referenced flow meter.

It is well known that each kind of commercially available air sampler (slit to Agar samplers, surface air samplers, centrifugal air samplers, sterilizable microbiological atrium, and gelatin filter samplers) has its own characteristics and specific performance in terms of sampling and microbial recovery.1 It is, therefore, difficult to say which is the best one in absolute terms.

Again, the most meaningful exercise in terms of air sampling is the continuous comparison of data to expected results over a medium-long timeframe, in order to detect any deviation from the standard levels of environmental contamination. Comparison of data from different time periods is obviously possible only when the sampling parameters are consistently the same over a long timeframe. Therefore, it is essential (1) not to change the particular sampler you have selected and used to qualify your EM program and (2) to verify the sampling parameters with an adequate frequency in order to be assured that your equipment is still working as it did in the initial qualification phase.

Incubators used to process EM plates are obviously a key part of your data accuracy assurance. It is a sound practice to have dedicated incubators for EM samples that have been qualified and temperature mapped at the desired temperature ranges. Temperature mapping should be performed with the chamber empty and fully loaded. The selection of temperature probe locations should be scientifically defensible. Additional challenges to verifying the effect of some routine activities in the use of the equipment could be useful, such as the determination of the time required to return to the desired temperature range after

the opening of the incubator door and the subsequent temperature variation. As usual, data gathered during the validation activities should be used in writing the related SOP in a way that will guarantee reliable and consistent performance.

Temperature should be continuously monitored and recorded by a qualified system; any alarm (significant excursion from temperature range longer than a pre-defined time period) should be tracked and managed according to a specific SOP. Impact on the related samples should be formally assessed. The same consideration should be applied to other laboratory equipment, such as refrigerators used to store EM plates or other reagents, whose criticality is comparable to incubators.

Issues Related to Culture Media

Culture media is obviously the most critical material in EM activities. A robust quality system should be in place to guarantee that culture media are effectively able to support the growth of the microorganisms that are expected to be recovered in the clean room. Media are to perform as expected in actual operational conditions, from storage through final incubation.

It is quite uncommon, nowadays, to prepare culture media for clean rooms, internally, since the need for materials sterilized by ionizing radiation and wrapped in special clean room packaging has forced companies to widely utilize ready-to-use media for these applications. For ready-to-use media, choice and qualification of the supplier are obviously critical; many parameters should be taken into account in selecting an EM media supplier.

Suppliers should have a cGMP compliant quality system in place in order to guarantee consistency in product performance and quality. Particular care should be applied to guarantee the sterility of the media to avoid the accidental introduction of microbial contamination into the clean room and to minimize the differences in composition and quality between different batches (media are made from natural source raw material, which have great inherent variability). To achieve these goals, an adequate classified production environment should be ensured and many checks at different production phases, starting from raw materials, should be performed.

Another particularly critical step in the supply chain is the transportation of media from production site to client site. It is essential, but also difficult in practice, to maintain validated storage conditions for every plate throughout the journey to the final destination. Therefore, the supplier must consider particular worst-case situations that can actually occur in everyday activities, such as occasional heat-shock, during the shipping validation.

The biggest criticality inherent in the shipping of media, is usually one of the reasons that forces users to verify growth promotion properties, and all other QC tests, on each batch and each shipment of media. Typical tests confirmed internally in the user’s lab include: visual inspection, pH, pre-incubation (with the same incubation conditions as in routine monitoring), and Growth Promotion Testing (GPT). It is also a sound practice to include representative environmental isolates together with the standard American Type Culture Collection (ATCC) strains, among the ones used to challenge media in GPT. The scientific rationale in isolate selection should be clear, periodically reviewed, and properly documented.

Another good practice is to take into account the particular conditions of storage, exposure, and incubation present on site, since these parameters could have a dramatic impact on the actual performance of media. It is, therefore, good practice to validate microbial recovery on particularly “stressed” plates and to periodically perform GPT on plates that have been exposed and incubated in the clean room.

For air monitoring, since drying is a key factor for recovery, plate exposure is the most critical condition (i.e., aspiration of 1 m3 or more under Laminar Flow (LAF)), while surface monitoring inactivation of disinfectant residues should be assessed by GPT data (data collected from literature may also be supportive). The effect of decontamination procedures, such as introducing UltraViolet (UV) light or Hydrogen Peroxide into clean room pass-boxes, could also have an impact on media performances and should be included in these validation QC activities.

Minimum recovery expected in GPT is a debated issue. Usually a 50% recovery in respect to control plates can be acceptable, because it is the one guaranteed by most media suppliers, and it is in accordance with the variability inherent in all traditional microbiological testing. On the other hand, a higher recovery (70%) could be requested, based on the requirement of the microbial recovery test set in the United States Pharmacopoeia (USP) Chapter 1227.2

Incubation conditions have a great influence on the growth of the microorganism that can be recovered from a controlled environment. Although some standard temperature ranges do exist, incubation conditions (i.e., time and temperatures) may vary from site to site. Incubation conditions should be able to promote growth of the normal flora (the kind of bacteria and fungi

expected to be found in the environment). Growth promotion data and scientific literature should support the established conditions.

Usually, if you use a generic media such as Tryptic Soy Agar (TSA) for both bacteria and fungi, you are expected to incubate both at 30-35°C (ideal for mesophilic bacteria growth) and at 20-25°C (to support the growth of most yeast and molds species), but again, this is only one of the possible combinations of time and temperature.

Although a single media is accepted for routine monitoring, the performance, at least periodically, of some monitoring with a specific media for yeast and molds (i.e., Sabouraud Dextrose Agar typically incubated not less than 5 days at 20-25°C) is recommended, if data or environmental conditions e.g., high humidity) indicate the potential presence of molds. It is also recommended to use this specific media during the EM validation activity, together with TSA, to get more data on clean room normal flora levels and then to establish the most suitable conditions for routine monitoring.

Monitoring for obligatory anaerobes may be performed occasionally, again, depending upon historical data and on the process to be monitored. This activity is required especially when your process is at risk from these organisms (e.g., the product is oxygen sensitive and therefore filled under nitrogen) or when you have recovered them in sterility test media for anaerobes (Fluid Thioglicollate Media).

In the case of the recovery of molds or anaerobes, monitoring should be enforced in terms of frequency and number of locations in order to check whether the episode is an isolated incident or it is part of an adverse trend. If a substantial change in your normal flora occurs, relevant changes in the EM program may be necessary.

Issues Related to Isolation and Recovery of Microorganisms

Generally speaking, information about the quality of microorganisms recovered in the facility is essential, but as a matter of fact, you cannot identify each isolate, taking into account that in a standard size clean room hundreds of colonies are recovered every week (most of them from non-critical and ancillary areas).

Therefore, an identification (ID) program should be defined in your SOPs and should be rationally defensible. Practically, you must state what, how, and when you want to identify and up to which level (gram staining, genus, species).

What we should never forget in dimensioning an ID program is that the aim is to identify the normal flora related to the manufacturing environment and to detect any deviation from it. This because significant drifts from the normal flora could be interpreted as adverse trends, as well as a relevant increase in the number of organisms recovered.

Moreover, assessing the normal flora can help in identifying the most significant and representative strains to be used for: growth promotion testing of media for EM and media fills, validation of disinfectants, and the validation of sterility tests and other lab methods. In other words, an efficient and reliable ID program can help you in improving the quality of your data and, therefore, the control you have on the manufacturing area.

Issues Related to Documentation Practices

Documentation practices are another crucial issue in a compliant EM program. Since documentation provides the only track of the whole work, the more you document, the more you will be able to conduct an effective investigation in case of failure. Moreover, the more you document, the better you can assess the accuracy and meaning of your data.

Appropriate sampling data sheets can be a great help to operators in properly documenting their activity. Minimum data should include: the identification of sampling points, the number of samples to be taken for each location, and the sampling frequencies. Indicate the sampling method, sampling times or intervals, the method to be used in completing the forms, and any other relevant comments that will standardize the procedure and clarify what is expected of the operator(s).

Sampling locations should be clearly indicated in approved drawings, which are usually attached to the sampling SOP. A controlled copy of these drawings should be present in the clean room to help EM technicians in their activity.,

Incubation results should be recorded in laboratory notebooks according to procedure. These records should be easily correlated with sampling sheets to catch potential omissions and to standardize documentation performed by different operators. An exhaustive list of all information that should be documented in sampling sheets and in result notebooks can be

found in the Parenteral Drug Association (PDA) Technical Report about EM.3 Raw data must be signed by technicians and reviewed by a supervisor as a basic GMP practice.

Conclusion

As briefly reviewed in this article, accuracy of EM data can be influenced by many different factors each of which should be continually checked and taken into account in the management of an EM program. Because media is the primary material utilized in generating EM data, their selection, management, and control, is fundamental.

On the other hand, since EM - as with most clean room activities - is still a manual activity, we must rely almost exclusively on human behavior and commitment. Even the best-designed and validated EM program cannot be useful and effective if operators and supervisors do not apply it accurately and consistently day after day. Adequate time and resources should be spent in the training, qualification, control, and supervision of people's work, whose inherent and "natural" variability should never be underestimated.

References

1. USP 27 "Microbiological Evaluation of Clean Rooms and Other Controlled Environments"
2. USP 27 "Validation of Microbial Recovery from Pharmacopoeial Articles"
3. PDA Technical Report No. 13 "Fundamentals of an Environmental Monitoring Program," September 2001.

Also See:

Special Edition: Environmental Monitoring Handbook
