BIOMÉRIEUX

Automated Methods for Environmental Control (EM)

Application of Automated Microbiology for Environmental Monitoring of Clean Rooms

Your Ally in Advancing Quality

PIONEERING DIAGNOSTICS

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INTRODUCTION

Heavy investments across the entire bioproduction value chain – from R&D to industrialization and production relocation are behind the growing boom in biotechnology in Europe, to gain competitiveness and catch up with other regions such as the United States.

In this context, optimizing the control of bioproduction processes and the environmental monitoring of the biopharmaceutical industry is key to supporting the competitiveness of companies and no longer making quality control a bottleneck in the value chain of bioproduction but rather a real added value.

Microbiological controls such as environmental monitoring, bioburden tests, mycoplasma detection, in-process sterility controls, sterility tests on finished products, bacterial endotoxin tests, aseptic process simulations, as well as identifications of contamination etc. are a non-exhaustive part of the Smart Quality Monitoring (SQM) which can be set up in the pharmaceutical industry.

In routine SQM microbiological controls in bioproduction or ATMP manufacturing, there are 3 major applications for Rapid and/or Automated Microbiology technologies:

- Environmental Monitoring (EM)
- In Process Controls (IPC)
- Finished product controls

ENVIRONMENTAL MONITORING – EM

The regulatory requirements for the classification and monitoring of clean rooms are detailed in various reference documents such as GMP Good Manufacturing Practices (EU GMP Annex 1), the FDA guideline “cGMP Guidance for industry”, pharmacopoeia chapters such as USP <1116> *Microbiological Control and Monitoring of Aseptic Processing Environments*, ISO 14644 & 14698 standards and Technical Report PDA - TR13 “Fundamentals of an Environmental Monitoring Program”. These reference documents describe EM as a key element in ensuring that aseptic production environments are kept free from potential microbiological contamination, while ensuring the complete integrity of sample-related data from collection until contaminants are identified.

The reference method for monitoring this contamination of cleanrooms is to use irradiated culture media plates with different sizes (90mm and contact/55mm), making it possible to recover the environmental flora according to the different media formulations. Once the sample has been taken in a clean room and the culture media plate has been transported to the Quality Control laboratory, the inspection of the plate begins after incubation at appropriate temperatures and durations, in counting the Colony Forming Units (CFU) on the surface of the media by a qualified operator. To be visible and “detectable”, microorganisms must grow into distinct macroscopic colonies visible to the naked eye. EM control and numeration is therefore a visual and completely manual process, carried out by people with, by nature, variable – although qualified – counting performances.

The Annex 1 of the GMPs advocates the use of automation methods and alternative methods for the microbiological control of bioproduction environments.

NUMERATION ERRORS

Two papers (Sutton, Leblanc) published evidence about the natural variability amongst of the qualified operators who perform visual inspection of EM plates. In addition, between 2011 and 2018, among all the FDA Warning Letters about bad practices around the use of culture media for EM, 50% concerned counting errors!

The manual nature and the subjectivity of the human analysis for the detection of small microbiological "events" on culture media plates may create problems of FP or FN on the counting results of colonies on Petri dishes. The impact can be both industrial and economic in the case of FP, but extremely serious in terms of Public Health with FN results if contaminations are not detected in the production environments of injectable products in the controls of Class A environment or in an isolator.

DATA TRACEABILITY AND INTEGRITY ISSUES

Moreover, this manual reference method is also subject to many data traceability and integrity errors, due, amongst other things, to the handwriting of the operators and the manual writing on culture media plates or even on paper. The integrity of the data cannot therefore be completely preserved. An analysis of FDA Warning Letters was conducted between 2013 and 2018 and the proportion concerning "Data Integrity" issues increased from nearly 25% to 45%, which over the observed period represented more than a third of the deficiencies noted!

Throughout the whole EM process, many errors can be identified, for example:

- Errors in the preparation of labels
- Operators manual writing error
- Manual writing on EM plates and/or sampling sheet
- Use of an unsuitable culture medium
- Sampling time not respected or not traced
- Sampling point not respected
- Use of expired culture medium
- Culture media plate lost during transport
- Multiplication of the risks of incorrect information transcription (on the sampling sheets or in any Excel file or LIMS)
- Incorrect incubation cycle
- Variable reading performance depending on the operator

INDUSTRIAL INEFFICIENCY ISSUES

Beyond possible counting and traceability errors, manual methods also present many operational constraints that make them inefficient from an industrial point of view. Thus, throughout the whole EM process and mirroring the previous risks of error, many causes of operational and industrial optimization can be identified, mainly on the time allocated to low added-value tasks:

- Prepare traceability labels and sampling sheets
- Check the paper "to-do list"
- Manually write on Petri dishes and on paper documents
- Manually reconcile the quantity of sampled culture media plates in comparison to the sampling plan
- Load/unload Petri dishes from incubators
- Tedious manual counting of colonies
- Double verification with the "4 eyes" count
- Writing of manual reports
- Verification of the information transfer from paper to digital format

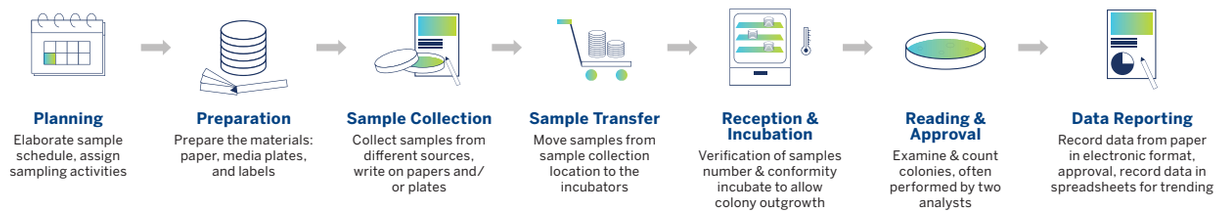

Figure 1. Schematic representation of the complete EM process workflow.

All of these limitations throughout the EM process can only be overcome with the implementation of digital and automated solutions, such as environmental control software, automated incubation and colony counting systems on EM culture media plates.

AUTOMATED MICROBIOLOGICAL METHODS FOR EM

The automation of EM involves mastering the entire value chain of environmental control: from the supply of Petri dishes to the approval of results of colony counting after incubation, all data must be traced and controlled to ensure data integrity while limiting and standardizing the number of human operations. EM automation, to solve those issues is now possible with completely integrated solutions.

bioMérieux has developed and validated a complete EM management solution covering the entire EM workflow: the 3P® CONNECT software manages the planning and traceability of data, and the 3P® STATION system (Figure 2) allows the incubation at different temperatures and automated counting of colonies on Petri dishes with early alerts for better reactivity.

Figure 2. 3P® STATION automated incubation and colony counting system.

This whole solution was optimized with a panel of pharmaceutical manufacturers [3,4] – who were able to develop it with us towards an operational and industrial design and above all to reach and demonstrate a high level of validated performance at least equivalent to human analysis.

TRACEABILITY ISSUE SOLVING

At the different stages of EM control (Figure 1) – Planning, Preparation, Sampling, Transport, Reception & Incubation, Reading & Approval, Data Reporting – previously manual actions can now be automated and digitalized through the use of EM management software such as 3P® CONNECT and through the automation and standardization of the incubation and colony counting with 3P® STATION also allowing to access to all data and pictures for secured data integrity.

Thus, over the entire EM process, almost 40% of the manual and low value-added steps can be eliminated, reducing the risk of information loss and data traceability/integrity issues. This resulted into securing 100% of the critical data integrity gaps identified. Indeed, the absence of paper documentation, the data integrity, the traceability of all the samples, the possibility of an audit trail (21 CFR Part 11) as well as the tools for trend analysis and issuance of reports are all factors reinforcing the control of EM and its security.

Finally, the possible integration of this solution with an existing and routinely used LIMS makes it even easier to integrate into a global management model for data traceability and supervision of the EM program in real time.

INDUSTRIAL INEFFICIENCY ISSUES RESOLUTION

Again, taking into account all of the EM steps (Figure 1), one study showed that the time saved thanks to digitalization could amount to a total of almost 3 minutes for each culture media plate analyzed. This study was carried out on a manufacturing line and Quality Control laboratory of a production site, and almost 99% of the complete EM workflow was evaluated with the use of the 3P® CONNECT software.

For EM programs of a few hundred, to several tens of thousands of culture media plates depending on the manufacturing site, saving time with an automated process can significantly improve global efficiency of the site.

Finally, industrial studies are underway on the use of a single incubation temperature and reduced incubation time for EM culture media plates in order to continue optimizing the EM process. The aim is to increase flexibility and efficiency from an industrial point of view. [6]

So, the automation of incubation at a single temperature and the automated colony counting on culture media plates is an opportunity to potentially reduce the incubation time by 1 to 2 days, on a cycle of 7 days of incubation for example, nearly 30% reduction in the incubation cycle, which represents a significant gain in terms of industrial efficiency.

SUMMARY ON EM

With regard to EM, depending on the production sites, hundreds of thousands of controls can be carried out each year on culture media plates to ensure the contamination control status of all production areas (Classes A, B , C, D). Traditionally, these manual EM controls require waiting for the microorganisms to grow, managing incubations at different temperatures, and counting the CFU on each plate manually. Upstream of these samples and the analysis of the results after incubation, the planning and manual management of the entire EM workflow are also key points which generate constraints of data integrity and issues of traceability of all the data.

This cumbersome process and these methods are subject to many potential errors due to their manual characteristics, such as critical errors of FP or FN during the CFU counting step by Quality Control technicians, but also traceability problems and data integrity management issues which are, in particular, increasingly reported by the FDA.

This is why EM management solutions such as 3P® CONNECT, ranging from sampling planning to automated counting with the 3P® STATION system, are necessary to overcome the problems identified throughout the workflow.

These automated and validated microbiology solutions now make it possible to obtain results that are standardized and faster than manual processes. It will be possible to manage different incubation temperatures and different incubation cycles to reproduce the same schemes as the current procedures in place: it is therefore not an alternative method but simply the automation of the traditional method in use by various biopharmaceutical manufacturers. It allows for faster, more reliable results and better traceability of the information, in particular to launch investigations or preventive/corrective actions if necessary.

The biopharmaceutical industry should consider the large-scale implementation of these automated solutions, replacing traditional manual and risky methods.

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GLOSSARY

ATMP: Advanced Therapeutic Medicinal Products
CFU: Colony Forming Unit
CGT: Cell and Gene Therapies
DI: Data Integrity
EM: Environmental Monitoring
FN: False Negative
FP: False Positive
IPC: In Process Controls
LOD: Limit Of Detection
SQM: Smart Quality Monitoring

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