Single temperature analysis for environmental monitoring samples

Determination of the feasibility and the impact of the single temperature for environmental monitoring microorganisms

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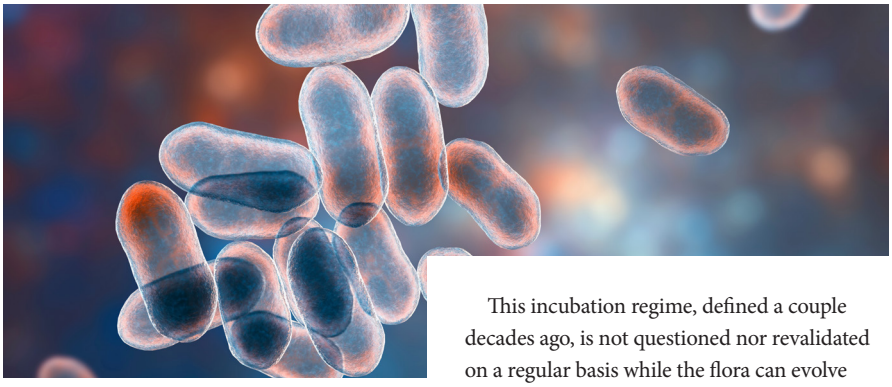


ENVIRONMENTAL MONITORING (EM) is one of the main microbiological controls that pharmaceutical industries perform to ensure the safety and efficacy of pharmaceutical products. To efficiently control the quality of these products, the presence of potential microbial contaminants must be monitored. Although the traditional method is extremely manual, variable and error-prone, it remains the standard procedure used in industry for hundreds of millions of samples per year.

While the level of requirements in data integrity and data traceability is increasing from regulations and inspections, pharma manufacturers are simultaneously willing to improve process efficiency and productivity thanks to time saving and deviation reduction. This enables traditional methods and practices to be questioned, such as the incubation period for EM samples.

Dual or single temperature?

Regulations for environmental monitoring provide wide guidance on temperatures and regime to follow[1-4] and to avoid any risk of missing a microorganism, it has been commonly adopted by pharmaceutical industries for several years that two sequential temperatures (usually 20-25°C, followed by 30-35°C) would match the main flora encountered. And indeed, a survey[5] from the Parenteral Drug Association (PDA) in 2017 revealed that 73 percent of polled pharmaceutical companies chose dual temperature incubation as their most common practice.

This incubation regime, defined a couple decades ago, is not questioned nor revalidated on a regular basis while the flora can evolve with a risk that some new microorganisms can be not detected during the EM campaign. Besides, today, more and more industries are considering adopting single temperature for environmental monitoring samples, as 70.8 percent of respondents currently using dual temperature are open to adopting single temperature[6] as it brings valuable benefits.

Processing single temperature decreases manual handlings of the samples during the transfers of incubators and manual transcription; therefore decreasing mistakes and related investigations.

Recent studies from several companies[7] have indeed determined that single temperature is at least equivalent or better to detect the flora they have. They also underlined that regardless of the final choice, the key aim is to ensure that it fits with the flora in place. Indeed, each microorganism has its own growth criteria in terms of culture media components, incubation temperature and time. Such optimal growth conditions linked to each microorganism's specificity are difficult to be conducted during EM and consensus may be made to find the good balance for the existing flora.

Overview of studies on single temperature incubation

In 2018, the 'One Media / One Temperature' PDA task force was launched with eight pharmaceutical manufacturers.[8] The '*in vitro* Study 2019-2020'[9] has showed that one temperature period at 27.5°C for seven days performed as well as the two dual temperature periods (22.5°C for four days and 32.5°C for three days / 32.5°C for three days and 22.5°C 4 for days) for 13 ATCC strains with eight bacteria, two yeasts and three molds. These PDA and bioMérieux/Sanofi studies have concluded that 27.5°C is the optimal growth temperature from both mesophilic aerobic bacteria and fungi tested.

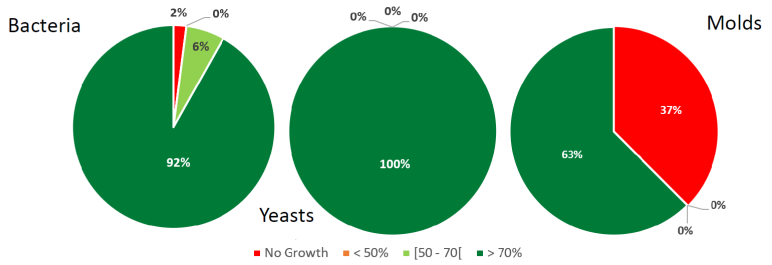
Complementary analysis[10] were performed to evaluate the suitability of a single temperature for EM on a higher panel of strains including wild/local microorganisms isolates. Over the tested fungi, *Cladosporium herbarum* was confirmed to be sensitive to high temperatures and has a better growth at 22.5°C and 25°C rather than 27.5°C. Besides, over the bacteria panel tested, *Corynebacterium striatum* has showed a recovery rate slightly below specifications at 22.5°C compared to 25°C after the seven days of incubation. These studies have demonstrated that the suitable temperature for EM incubation is between 25°C and 30°C.

Following this collaborative study, another comprehensive analysis[11] from bioMérieux in 2023 has compared different incubation conditions for 83 strains including 49 bacteria, 24 molds and 10 yeasts that are frequently found in EM program. The study was performed using the 3P® STATION as an automated petri dish incubator and kinetic reading every hour, comparing different single temperatures and allowing to visual each results thanks to the images taken every hour. The growth controls were performed on traditional incubators.

This study has confirmed that the incubation temperature has different effects on the strain recovery. **Figure 1**.

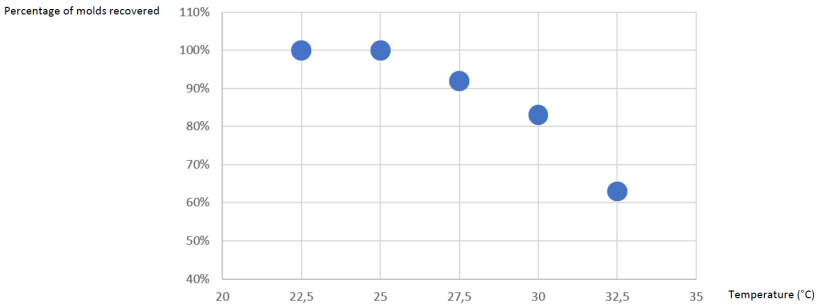
First, in the range of 22.5°C to 32.5°C, yeasts were found not sensitive at all. For bacteria, the lower incubation temperature of 22.5°C affected the recovery of four strains. A slight increase of temperature to reach 25°C was enough to recover all bacteria with good recoveries. Finally, the growth of the molds was more affected by high temperature as 32.5°C with 37 percent of the strains tested that did not recover, while 25°C was sufficient to have them all grow. **Figure 2**.

Figure 1:



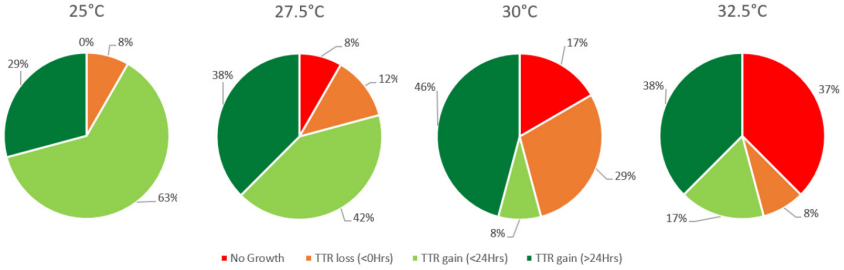
Impact of the temperature on the growth and recovery rates of the tested strains. Recovery rates are calculated for bacteria with enumerations observed at 22.5°C vs 32.5°C. Recovery rates are calculated for yeast and molds with enumerations observed at 32.5°C vs 22.5°C.

Figure 2:



Percentage of the molds tested recovered at the different incubation temperatures.

Figure 3:



Time to Result impacts of the studied temperatures (32.5, 25, 27.5 and 30°C) and the TTR at 22.5°C on molds strains

Times to results and to detect

While streamlining the incubation regime with a single temperature can provide significant benefits for pharma manufacturers, it can eventually be endorsed further with improvements over the Time to Results (TTR) and Time to Detect (TTD). The chosen temperature has indeed an impact on the growth evolution of the strains as demonstrated by this study[11]. For the bacteria, a decrease of growth detection has been observed at 22.5°C with 24 hours delay for 54 percent of them. The temperature of 25°C allowed to limit this effect. For the range of 27.5°C to 30°C, the detection time has not been different from 32.5°C. 38 percent of the 20 molds tested grew faster at 32.5°C while 37 percent were not growing at all. At 25°C, they all recovered and 92 percent had an improvement of the TTR. **Figure 3**.

Conclusion

Various studies have demonstrated that a single temperature between 25°C and 30°C could be suitable for an EM program: this temperature range seems the most adapted to a large panel of microorganisms encountered in clean areas. Coupled with TTR/TTD improvements for some strains, the use of current dual temperature practices can be questioned.

Single temperature analysis and validation can be eased with automated incubation and counting solutions as 3P® ENTERPRISE. It also provides a standardised reading of plates and alerts raised should a sample exceed its specification, allowing rapid corrective actions. From planning to data, EM is supervised at all stages of the process for earlier product release as well as faster turnaround of production lines after cleaning validation.

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Marion Louis is Global Market Manager in the Pharma Quality Control business of bioMérieux. She holds an engineer degree in Biotechnology from Polytech' Clermont Ferrand and a Master's degree in Management, France. For the last 15 years, she worked in microbiology for pharmaceutical applications and she is now supporting the key pharmaceutical industries worldwide in implementing digital and automated solutions dedicated for Environmental Monitoring.