Using a Single Temperature For Incubation: A Real Possibility to Foster Environmental Monitoring Outcomes

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INTRODUCTION

The environmental monitoring (EM) program is an important GMP control in pharmaceutical manufacturing. It must rapidly detect deviations from established alert/action limits that may compromise the state of control of the facility. The ability to recover from environmental stress depends on two main factors: the type and quality of the culture media and the adequacy of the incubation temperature (mainly two consecutive temperatures). Implementing a single incubation temperature in routine for environmental monitoring is a challenging task and is still discussed in the industry.

Recent initiatives such as the PDA "One Media, One Temperature" propose a simplification of the incubation regime using TSA incubated at a single temperature in the range of 25-30°C. In the bioMérieux "in vitro study"[1], which examined the growth of a wide range of microorganisms at different temperatures, the universal temperature at which all germs could be detected was 25°C.

bioMérieux has conducted a new study using real EM samples to compare the performance of single temperature incubation versus dual temperature incubation. The poster presents the results obtained and emphasizes the suitability of a single temperature incubation for routine use, but also shows that the selection of the unique temperature can promote the time to detection and improve the time to result of the EM tests.

MATERIAL AND METHOD

Sample Preparation

Microorganisms were collected in a clean but unclassified area to generate a relevant number of contaminated samples. Three main sampling methods were used: settle plates on 90mm diameter dishes (bioMérieux TSA 3PN ref. 43819), active air sampling with the airIDEAL® instrument (10min - 100L/min) and surface sampling with 55mm diameter contact plates (bioMérieux Count-Tact® LockSure 3P ref. 43699). All samples were run four (4) times (4 surfaces and 4 air samples) for each condition.

Incubation conditions

The evaluated temperatures were:

- Double incubation temperatures: 22.5°C followed by 32.5°C (3+2 d)
- Single incubation temperature: 25°C
- Single incubation temperature: 27.5°C

The samples were incubated for five (5) days in the 3P® STATION instrument.

Picture 1: bioMérieux 3P® STATION

The 3P® STATION is an automated Petri dish incubator/counter that can incubate plates in a temperature range between 20 and 35°C with an accuracy of +/- 1°C. The system can detect and track the growth of microorganisms present on the surface of the plates. A growth curve is also available, providing information on the growth kinetics throughout the incubation cycle of each growing microorganism.

MATERIAL AND METHOD

Samples were realized in seven (7) different runs for a total of 171 plates.

At the end of the incubations, enumerations were performed for each condition tested. The Time to Detection (TTD) of the different microorganisms recovered was extracted from the data generated by the 3P STATION. The macroscopic morphologies of the strains were also observed and compared.

Identifications were performed using the Vitek® MS instrument.

RESULTS AND DISCUSSION

Analysis of the microorganisms recovered during the study clearly shows that the diversity of microorganisms is equivalent between the different incubation temperatures. The proportion of molds and bacteria, and even the different types of bacteria, is approximately identical for the three (3) incubation regimes evaluated (Figure 1). Incubation temperature does not appear to have a dramatic effect on the diversity of microorganisms recovered from the environment.

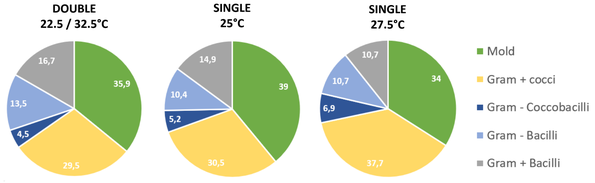

Figure 1: Repartition of the different microorganisms recovered with the different sampling techniques and at different incubation regimes on TSA culture media.

A closer look at the raw data reveals some dissimilarities between the different cycles. The total number of microorganisms recovered at double temperature and single temperature 25°C appears to be significantly higher than at 27.5°C (Table 1). This difference is entirely due to a significant reduction in the detection of molds when comparable numbers of bacteria are observed through the three incubation regimes.

CYCLE	N	SUM COUNT	SUM BACTERIA	SUM MOLD	PERCENTAGE BACTERIA	PERCENTAGE MOLDS
22.5 / 32.5 °C	56	1814	1252	562	69.02	30.98
25°C	56	1897	1150	747	60.62	39.38
27.5°C	59	1602	1146	456	71.54	28.46

CYCLE	N	MEAN COUNT	MEAN BACTERIA	MEAN MOLDS
22.5 / 32.5 °C	56	32.39	22.36	10.04
25°C	56	33.88	20.54	13.34
27.5°C	59	28.61	20.46	8.14

Table 1: Enumeration observed on plates per type of microorganisms and at different incubation temperatures.

This finding correlates with the conclusion of the first bioMérieux "in vitro" study, which demonstrated difficulties in recovering certain types of mold at 27.5°C. Furthermore, 25°C seems to be the best temperature to recover molds without reducing the detection of bacteria.

Air sampling (active and settle plates)

Figure 2 shows the number and reparation per microorganism type obtained with different incubations. Again, similarities of patterns are visible between double temperature and single temperature 25°C. Nevertheless, 27.5°C shows a lower number of colonies recovered and, surprisingly, a number of molds significantly higher than the other regimes. In addition, this discrepancy is only seen on settle plates. This finding raises the question of the influence of the sampling method in relation to the incubation cycle.

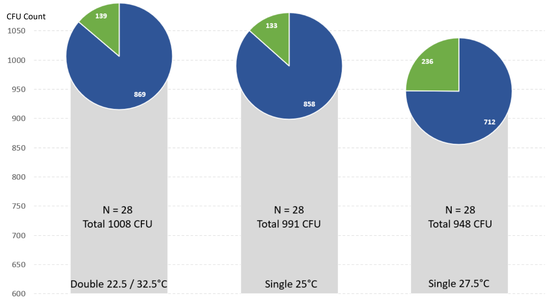

Figure 2: Repartition of the different microorganisms recovered from air sampling techniques and at different incubation regimes on TSA culture media. Bacteria are shown in blue and molds in green.

Surface sampling

The results vary from one incubation regime to another. Single temperature 25°C allows to recover more microorganisms and especially molds (Figure 3). Then, single temperature 27.5°C is the condition that permits to recover the highest number of bacteria but also leads to a dramatic decrease in the recovery of molds and at that point is the temperature showing significantly less colonies recovered. Double incubation is more balanced and recovers in the same proportion bacteria and molds but slightly less than single temperature 25°C.

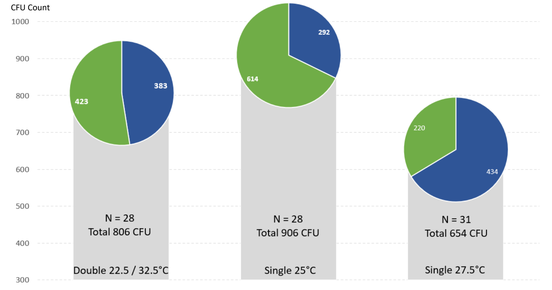

Figure 3: Repartition of the different microorganisms recovered from surface sampling and at different incubation regimes on TSA culture media. Bacteria are shown in blue and molds in green.

Time to Detection (TTD)

The results presented in Figure 4. a) show a visible effect of the incubation regime and the time of growth of the microorganisms. The longest time to detection is observed for the double incubation regime, where 80 hours are necessary to recover 90% of the flora. The single incubation regimes show an improvement in TTD, 27.5°C being the condition where the shortest TTD is measured with less than 50 hours. Although fewer microorganisms are recovered, they grow faster than the other conditions evaluated. The single incubation at 25°C also shows a good TTD improvement compared to the double incubation temperatures; a reduction of almost 1 day of incubation is then measured.

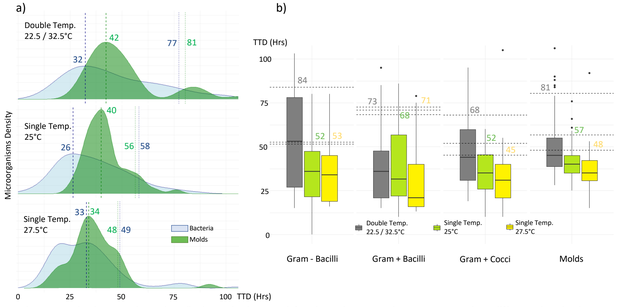

Figure 4: a) Time to detection (in hours) observed for the microorganisms recovered at different incubation temperatures (double, single 25°C and 27.5°C) at the maximum detection observed and at 90% of total growth - b) Box plots of TTD (in hours) per incubation temperature and microorganisms nature.

Figure 4. b) shows the differences observed between the different types of microorganisms. Surprisingly, a clear negative effect on TTD is observed for Gram bacilli when incubated at double temperatures. The other box plots confirm the superiority of single incubation over double incubation on TTD.

CONCLUSION

This study, conducted in an unclassified environment with microbial variability higher than that expected in a pharmaceutical cleanroom, presented a new method for pharmaceutical environmental monitoring based on a single temperature incubation cycle using the 3P® STATION instrument.

The results demonstrate that the use of a single temperature is suitable for an EM program. In fact, the temperature of 25°C was shown to recover environmental stressed microorganisms without major effects. Moreover, a significant reduction of TTD can be considered with a target of less than 3 days of total incubation. This represents a real improvement in EM processes compared to the current practice of double temperature (22.5°C followed by 32.5°C) and highlight the great added value of automated reading methods to improve data integrity, result reliability and process efficiency.

REFERENCE

1. Poster presented at the PDA Micro 2023 - Fact Checking On The Behavior Of Environmental Microbial Organisms In A Single Temperature Incubation Regime