“To Stress, or Not to Stress”

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Disclaimer: Some of the data shared in this presentation was generated by the Growth Direct® System, as such, the data is specific to the Growth Direct® System technology.

Introduction

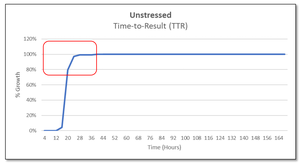
To stress or not to stress, a common debate about the relevance of using stressed microorganism when conducting environmental monitoring microbial test method validations across the pharmaceutical microbiology industry.

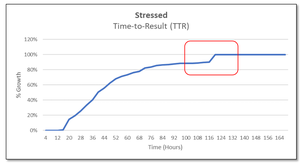
What is the right answer?

The goal of this study address this questions by evaluating Time-To-Result (TTR) for a variety of microorganisms under stressed and unstressed conditions. In this study TTR was defined as the following:

- **Time-To-Result (TTR):** The time required to detect 100% of colony forming units (CFU) based on a known standard.
- **Time-To-Result 85% (TTR85%):** The time required to detect 85% of colony forming units (CFU) based on a known standard.

This study was based on TTR85%.





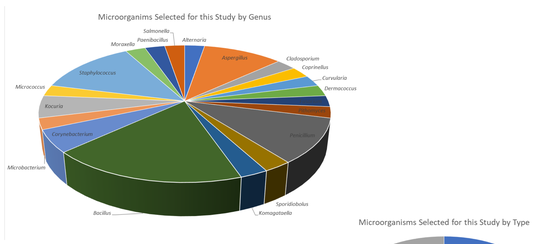
This data is an example of unstressed and stressed utilizing Time-To-Result (TTR). Stressed data shows a lag in Time-To-Results (TTR) compared to unstressed data.

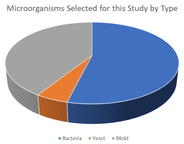
Materials and Methods

The microorganisms selected were found to be the most common recovered from clean room environments within the pharmaceutical industry. The list of microorganism were generated through a literature review. Following the review, the isolates were ranked and selected based on the frequency at which they showed up in the literature search and those found to be the most dominate Genus species recovered in clean rooms. This review resulted in a total of 39 microorganisms including bacteria, yeast, and molds.

All these microorganisms were exposed to both stressed and unstressed conditions and incubated on appropriate media at four different incubation conditions.

Panel of Microorganisms

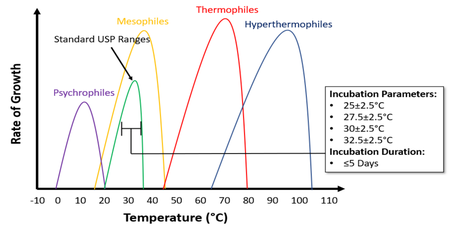




This data is a grouping of the microorganisms used in this study. Categorized for this poster presentation into Genus and other morphological characteristics.

Incubation Parameters

The common incubation schemes used in the pharmaceutical industry are single temperature and dual temperature. A single temperature incubation scheme was selected for this study. A total of four temperatures were selected between the standard USP incubation temperature ranges.



Growth Media

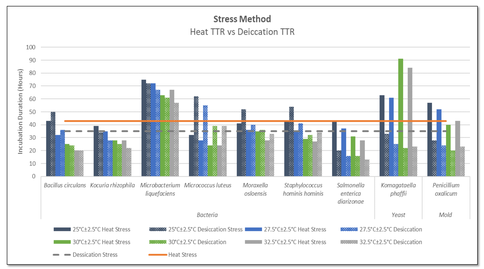
The standard USP base medium used in environmental monitoring to cultivate bacteria, yeast, and mold is Trypticase Soy Agar (TSA). The addition of neutralizers is commonly used to inhibit and promote growth in the presence of cleaning agents used in cleanroom environments. Two media types were selected for this study containing neutralizers.

- TSA with Lecithin and Polysorbate 80 (TSALP80)
- TSA with Lecithin, Polysorbate 80, Histidine, and Sodium Thiosulfate (TSALP80HT)

Stress Method

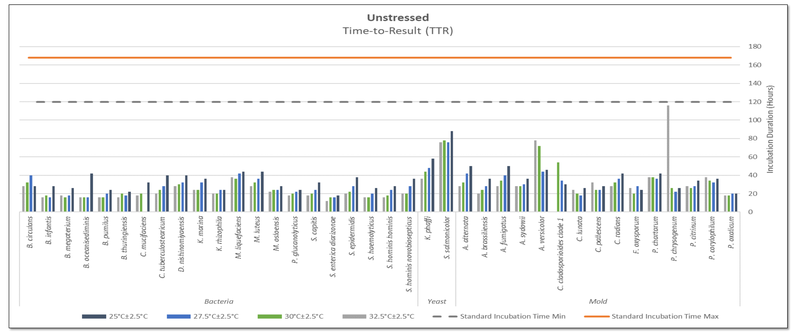
Two different types of physical stress methods were evaluated using a subset of microorganisms. Based on the evaluation one stress method was selected for this study.

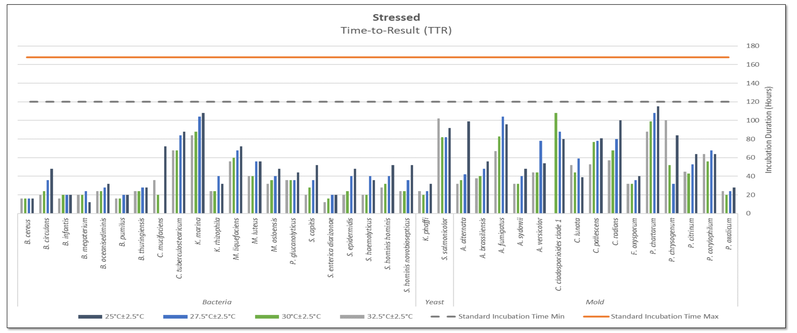
- **Desiccation Stress:** The physical removal of moisture from to stress microbial cells.
- **Heat Stress:** The use of temperature to stress microbial cells.

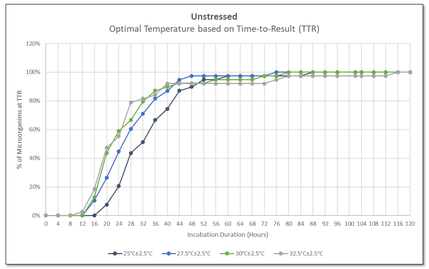


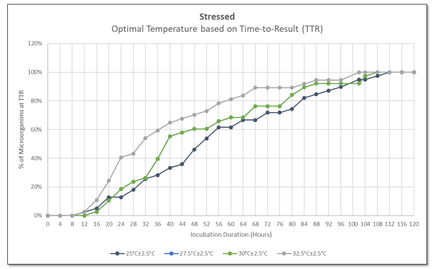
Heat stress mechanism demonstrated a more robust performance by producing higher reproducibility across all groups of microorganism. Additionally, it was more effective at stressing yeast and molds.

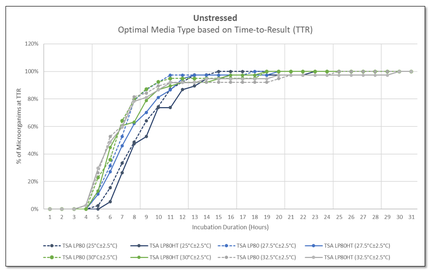
Results

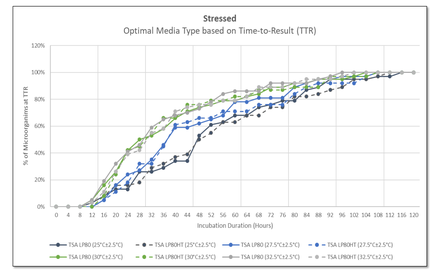












Discussion

Stressed vs Unstressed TTR

The collected data demonstrates that stress conditions impact the TTR and that the impact various mostly based on the microorganism and incubation scheme. For example, Spore-forming bacteria, such as, *Bacillus cereus*, remain consistent across different incubation schemes under stressed and unstressed conditions. Similar trends were observed among the molds *P. oxallicum, A. sydowii*, and *F. oxysporum.* However, this trend is not universal with all molds. For example, *A. fumigatus* and *C. cladosporioides clade 1* exhibited an increase in TTR when stressed. Conversely, in the case of the gram-positive non-spore-forming cocci *K. marina*, stress led to a significant increase in TTR compared to unstressed cells across all incubation schemes. A surprising observation was made with the yeast *K. phaffi*, which exhibited shorter TTR under when stressed compared to unstressed conditions.

Optimal Temperature Based on TTR

The incubation temperature has an observable impact on TTR for stressed populations. An increase in incubation temperature inversely correlates with a decrease in TTR. This inverse relationship indicates that TTR can improve the recovery of stress cells by adjusting the incubation temperature. This impact became less pronounced as the incubation duration increased. Table below summarizes the TTR based on temperature.

Optimal Media Type Based on TTR

The media type used does not demonstrate significant impact on TTR. However, there is a slight preference for TSALP80HT at lower temperatures and transitions to TSALP80 at higher temperatures. Table below summarizes the TTR based on media type.

Temperature	Optimal Temperature Range per Microorganism Panel (ATCC)	Unstressed Optimal Incubation Duration per TTR Study	Stressed Optimal Incubation Duration per TTR Study	Optimal Media Type per TTR Study
25°C ± 2.5°C	22.5°C – 37.0°C	88	116	TSA LP80HT
27.5°C ± 2.5°C		80	108	TSA LP80/TSA LP80HT
30°C ± 2.5°C		72	108	TSA LP80
32.5°C ± 2.5°C		80	102	TSA LP80

Conclusion

Based on the data collected from this study the use of stress microorganisms in microbial method validations seems to more critical when shorter incubation durations are validated. Those microorganism that are stressed have an increased TTR compared to unstressed microorganisms. However, in all cases the stressed microorganisms TTR is less than the standard USP incubation duration. Alternatively, when you are validating a shorter incubation duration it is important to understand the TTR of stressed microorganism to decrease the risk of missed recoveries in your routine environmental monitoring program. If the incubation duration is reduced, temperature has the potential increase TTR. Conversely it has the potential to decrease TTR. Therefore, it is important to select and validate an optimal temperature for the microorganism found in your cleanroom environment. Additionally, the use of neutralizers seems to have a slight impact on TTR. Although this is not significant t it is important to select a media and neutralizers that is applicable for the cleaning agents used in your facility.

So, what is the right answer?

It depends on the method. If you plan to use the standard USP incubation temperature, the use of stressed microorganism may not be relevant. However, if you are looking to reduce the incubation duration by using an alternative method, the use of stressed microorganism may be relevant to your application.

References

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Acknowledgements

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