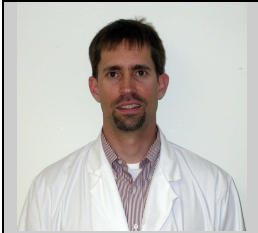


Spore News

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Reduced Incubation Time, What's the point?

We are often asked by our customers if they need to confirm the reduced incubation time (RIT) for biological indicators. The US FDA RIT protocol originated almost 30 years ago and, although the details have been debated, is still the only recognized protocol to reduce the incubation time for culturing biological indicators. The intent of the protocol is for the manufacturer to determine the appropriate amount of time necessary to recover low numbers of injured spores. The manufacturer's compliance with the protocol provides the end-user with confidence they can release product without fear of more biological indicators turning positive...the "what-if-we-missed-one" scenario. The RIT protocol is required for manufacturers of biological indicators who want to reduce the standard label claim to less than seven days. The seven day timeframe has little scientific basis, whereas the US FDA RIT protocol is scientifically and statistically-based.

Why do we as manufacturers want to reduce the incubation time? The answer is why not? Scientific advancements in sedimentation solutions have allowed manufacturers to produce spore crops with less vegetative debris, termed "clean spores". Furthermore, advances in sporulation techniques have yielded more consistent resistance and germination. Advances in cultivation media have also assisted with faster germination. Therefore, due to the scientific advances in cultivation and sporulation media as well as post-processing of the spores, the obvious next step is to take advantage of these advances to achieve an RIT of less than seven days. The RIT allows end users to release sterilized product based on biological data in less than seven days. This results in shorter process times which reduce inventory costs, allows faster turn-around time on critically-needed medical supplies. In short, it allows the end user to get the critical biological information faster so that decisions to reprocess or release can be made faster. From a manufacturing standpoint, it allows the manufacturer to have shorter process times during manufacturing, faster release testing turn-around time, which means they can get customer orders completed more quickly.

To perform an RIT, spores are injured during exposure in BIER vessels to sub-lethal times. One hundred biological indicators must be exposed per sub-lethal time. Thirty to

eighty units must survive when cultured. The most probable number of spores per BI for this test is calculated using the Halvorson Ziegler equation of $\ln n/r$. n is the number of units tested (i.e. 100) and r is the number of units sterile. When 30 units are positive, 70 units would be sterile. This would mean the most probable number of spores/BI = 0.36 spores. In the case of 80 units positive, 20 units would be sterile, therefore, the most probable number of spores/BI = 1.61. The RIT protocol is really analyzing the last surviving spore in the BI, a very conservative test. The RIT is the time when 97% of the data available in 7 days of incubation is available to the analyst. Time must be reproduced with three different lots of biological indicators. To get this type of control and reproducibility, every variable must be addressed. Consequently, performing an RIT is not trivial. It requires extensive control of critical experimental variables: the spores, the recovery medium, and the incubation conditions. The exposure conditions necessary to stress the BI to the last surviving spore must be precisely controlled.

What are the issues? Customers misinterpret the protocol and assume that they must repeat the RIT in their process vessels. There are three elements that can significantly influence the reduced incubation time results: (1) the spores – cultivation, cleaning, and preparation, which are all controlled by the manufacturer; (2) the media – highly specialized by the manufacturer to recover spores; (3) the incubator – only factor controlled by both the manufacturer and the user. Lack of control and consistency in any of these elements can lead to significant discrepancies in RIT.

The physical dynamics of a process vessel are chaotic when compared to the BIER vessels used by manufacturers. The chamber size, the load, the precise delivery of the sterilant, and the precise expulsion of the sterilant are just a few examples of process variables that make it nearly impossible to use a process vessel for RIT. This array of process variables is impossible to control to the extent that reproducible sub-lethal exposures can be performed.

The RIT protocol is not universally accepted. The international community proposes testing 200 biological indicators rather than 100. Furthermore, there are some who insist the RIT is the time when 100% of the biological indicators are positive, others proposing 97% and some 95%. Politics contribute to the discrepancies. TUV, an ISO-certifying body in Germany, will not allow an RIT of less than 7 days. ISO cannot get a consensus of opinion, FDA does not see any reason to change, and the debate continues.

The intent of the RIT is to supply the end-user with the assurance that if the spores were not killed during the sterilization process, those spores will germinate and grow to yield a positive BI within the specified RIT. The RIT is designed to evaluate the ability to recover the last surviving spore. When cycles fail and yield a positive BI, it is a catastrophic failure; thousands of spores survive not just one or two spores. The reason this can be said is that all sterilization cycles have a test point when all BIs are killed. As shown in Figure 1, additional exposure time is added for sterility assurance.

In order for a BI to survive, the cycle must lose all the lethality intended to be delivered in the sterility assurance portion. Typically, a BI failure occurs when recorded physical parameters are acceptable. Remember, it is the first positive biological indicator that fails the sterilization process, not the last. Customers will never see a sterilization failure with only one or two spores. It will be a catastrophic failure (Figure 1).

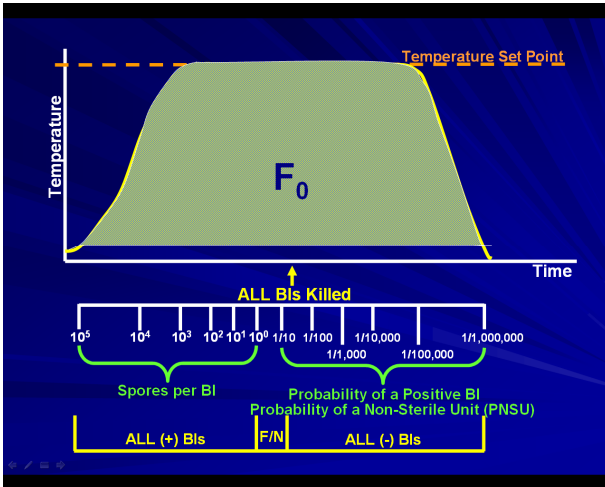


Figure 1. Graphical representation of accumulated process lethality versus biological lethality.

What would cause such a catastrophic failure? Validated cycles can fail for a multitude of reasons. These can include probe failure due to a calibration issue, a load configuration change causing air pockets, mechanical failure (i.e. steam traps, check valves), leaks, boiler issues, non-condensable gasses, poor vacuum, low ethylene oxide concentration, inadequate relative humidity, poor circulation, etc (Figure 2).

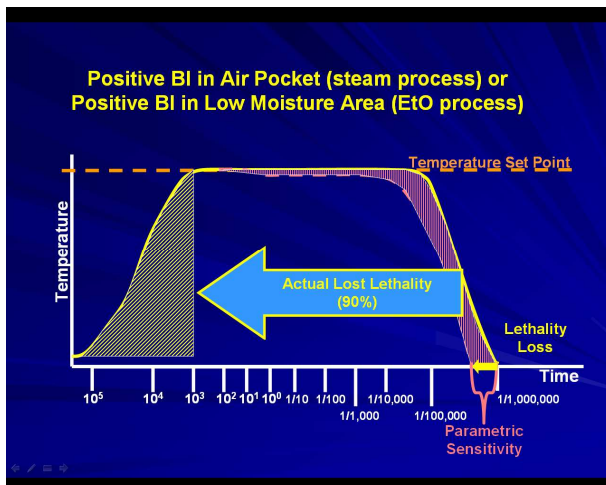


Figure 2. Catastrophic failure due to ineffective sterilant.

The impact of air pockets on steam efficacy in an autoclave can be significant. Dry heat (hot air) is 10 to 100 times less lethal than saturated steam. Air pockets can result from

packaging barriers, i.e. tortuous paths as found in catheters, lumens, etc., and tightly packed loads.

Finally, although RIT is important to establish, it is the manufacturer's responsibility, not the customer's. Customers (end users) will become very frustrated trying to verify the RIT in a process vessel. It is a protocol for manufacturers. We at SGM go to great lengths to control our spores, our culture media, our incubators, and our BIER vessels to achieve the reproducible data necessary to establish the RIT. We welcome the opportunity to provide any pertinent information that regulatory departments may require.

We recommend that all users have temperature distribution studies of their incubators to ensure that their incubation conditions meet manufacturer specifications. The spores are killed by exposure to lethal conditions. The lethal mechanism is not sterilizer dependent. The rate of lethality can vary in sterilizers but this test requires a lethal insult to reduce the spore challenge to approximately one spore...end of story.



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