Sterilization Monitoring and Validations of Liquid Loads in Steam Autoclave Cycles and the Importance of Appropriate BI Selection and Resistance.

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Various types of BI's (Biological Indicators) have been used in the Validation and **Monitoring of Liquid Loads for Steam Sterilization Cycles**. The types of BI's used over the past several years by **Sterile Services/ Central Supply** largely involve the use of Spore Strips in the chamber or the use of Self-Contained BI's. Each BI type is intended for a particular use and has characteristics that make it appropriate for that intended use. Most commercially prepared BI's are supplied with a Certificate of Analysis. This Certificate should provide the end user with information regarding the strain of bacterial spores on or in the BI, the population of the spores on or in the BI, its expiration date and other traceable information to link the BI with a Manufacturer and a specific Lot number assigned to that group of BI's. Should a problem occur with the BI or its use, this traceability is very important to the end user for problem resolutions. **Of equal importance is the information on the Certificate of Analysis that states the resistance of the BI and calculated Survival/Kill Time.** This information is extremely important to the end user and is unfortunately often overlooked. This is especially true with the Monitoring of Liquid Loads in Cycles involving a short exposure time. Without an appropriate resistance for the BI used, frequent and troublesome **False Positives** can occur. This is true for most Sterilization Cycles but it especially applies to **Liquid Loads**. To understand the importance of selecting an appropriate BI for a Sterilization Cycle, a brief review of the process of determining BI resistance may be helpful.

Typical Spore Strips

BI's are tested in a BIER Unit (Biological Indicator Evaluator Resistometer). A BIER Unit is a piece of equipment similar to a small chamber autoclave that is capable of very precise creations of sterilization parameters or conditions. An example would be a Steam BIER Unit that creates 'temperature and time' exposures within + or – 0.5 degrees of the desired exposure temperature. The Unit will also hit that temperature in less than ten seconds of starting to charge the chamber with steam. A 15-minute exposure at 121C is then very close to a 'real-time' 15 minutes of actual exposure at 121C. The Unit will not have the usual three or more minutes of come-up time, as is common with many steam sterilizers in Liquid Load Cycles. **THUS, a BIER Unit can determine very exact resistance (or the survival ability) of the BI in a specific sterilization cycle.** The length of time necessary to kill the BI under specific cycle parameters can be determined accurately through a series of gradually longer and longer cycle times until an 'all kill' point is reached. From this information, resistance or a **D-Value** can be calculated and assigned to the BI. The D-Value is expressed in minutes. It is the '**exposure time** necessary to achieve a 90% (1 Logarithmic) reduction in population of a BI's population under a specific set of Sterilization conditions. These conditions may be ' at 121C in saturated steam'.
A key point here is the **' at 121C'.** A D-Value of 2.0 minutes means that one would achieve a 1-Logarithmic kill of the BI population in 2.0 minutes **at 121C in Saturated Steam**. Thus a BI with a 2.0 times a Log 6 population (2.0 X 10^{6}**)** of spores would take 2.0 minutes kill time (D-Value) for each Log of population or a total of 12.6 minutes theoretically to produce an all kill at 121C exposure to saturated steam. Again, the key is the 'at 121C exposure'.

To demonstrate the importance of the BI resistance or D-Value when applied to a typical Liquid Load Sterilization Cycle, the following scenario will be used.

Sterilization Temperature: 121C
Load Configuration: 3 X 1L Flasks of Media/Product
Monitoring BI's Resistance: D-Value at 121C of 2.5 minutes with a population of 2.0 X 10^5

The above load is in place and the Autoclave Cycle 'Start' button is pushed. The chamber reaches 121C in 3 minutes and at that point the '15 minute exposure at 121C' is initiated. **The chamber was at 121C for 15 minutes** but the liquid to be sterilized would not have heated up that fast. When the chamber hit 121C, the liquid in the flasks may have only been at 90+C and by the end of exposure time, the liquid may have only been at 121C for a total of 4 to 8 minutes or less.

The BI used to Monitor the Sterility of the Cycle had a D-Value of 2.5 minutes and population of 2.0 Log 5. This information would be used to calculate a minimum of 13.2 minutes (Population Log X D-Value) at 121C to reach a total kill of the BI. Again, the key is '**13.2 minutes at 121C**'. The liquid was not at 121C for 13.2 minutes and a BI intended for use in Liquid Loads that was actually placed in the liquid may likely survive and grow up with positive results upon incubation. Spore Strips placed in the chamber that had the same resistance characteristics would have been killed in the 15 minute cycle. However, **one is not Monitoring the chamber, one is monitoring for Sterility Assurance Level of the Liquid Load.** Chamber conditions and those within the liquid load are very different. The Liquid Load's actual come-up time to reach 121C may be very long depending upon the individual volumes of liquid being sterilized. A 17 Liter container of liquid may take several hours for the liquid to reach 121C in a 121C Liquid Load Cycle.

Load Cycle Validations may utilize thermocouples and BI's to determine the exposure time necessary to increase the load temperature for adequate lethality of biologicals. In a Cycle with a long come-up time and a long come-down (exhaust/cooling) time, some lethality is being produced even though the temperature is not at 121C. The thermocouples and BI's can confirm total cycle time needed to produce lethality of a Log 6 BI. This will include the exposure to 100+C conditions during come-up and come-down time along with actual exposure time at 121C. Once Validated, future Monitoring of the Liquid Load Cycle may be done possibly with a Log 5 BI depending upon the Cycle Exposure Time established through review of Validation data and the method chosen to follow for Sterility Assurance.

Not all BI types are intended to be used in all types of Sterilization Cycles. A BI's 'intended use' is usually stated by the BI manufacturer somewhere on the package label or accompanying printed information that comes with the BI package. The carrier material and method used to determine the resistance of the BI should also be included on the Certificate of Analysis.

Many times a Spore Strip would be used to Monitor Sterilization Chamber conditions. D-Values listed for Spore Strips are usually determined on a 'dry spore strip' in its primary packaging of a glassine envelope. It would be subjected to various exposure times at a specific temperature in a Steam BIER Unit under saturated steam conditions and a D-Value at 121C calculated and the D-Value Certified for that Lot of Spore Strips. In a Liquid Load Cycle, the Spore Strips can Monitor the chamber conditions, but again they cannot monitor what conditions exist 'in the liquid load'. Spore Strips are not intended to be placed into a container of liquid for Monitoring a Liquid Load. The D-Value Certified for the Spore Strip will not be the same as when it is placed into a liquid container. This would not be its 'intended use'. The affect of the liquid, its volume and its content will drastically change the spore strips resistance characteristics. One would be working with a BI of 'unknown resistance' under the conditions of usage and Monitoring Record Data would be of little value to verify Sterility Assurance.

A type of sealed self-contained BI should be used in Liquid cycles. One that is sealed would not be affected by the liquid or its contents. A sealed glass ampoule type BI is ideal for use in liquids and is calibrated or has the resistance determined for the unit as a finished product. If it has a Certified Resistance of 2.0 minutes at 121C, that resistance will not change when placed in a flask of liquid as would a spore strip or unsealed self-contained. The come-up time to reach 121C in the Liquid Cycle will not change. It may take the first 10 minutes of the cycle to reach 121C but when the exposure duration is sufficient to kill the BI unit, one has demonstrated lethality of the given BI with a resistance of 2.0 minutes. **This can be documented as Sterility Assurance Monitoring with a BI of known resistance.**


Glass Ampoule BIs

For the above situation, if 'come-up time took 10 minutes and we wanted 15 minutes of exposure at 121C, one could run a 25 minute cycle and achieve the calculated kill time at 121C.' One may say ' I can't run a 25 minute 121C Cycle because the manufacturer's directions say to **Sterilize at 121C for 15 minutes**'.
Running the 25 minute cycle is accomplishing a 'Sterilization at 121C for 15 minutes' since the first part of the cycle was not at 121C.

Once a cycle time has been Validated, **the end user must now be careful to not accept for use a BI with a much higher D-Value than was used for the initial Validation**. If Validation and Monitoring was done with an Ampoule BI with a D-Value of 1.8 minutes, problems may likely occur if now BI's with higher D-Values of 2.5 are accepted for use. BI's should be selected to meet your Sterilization Cycle requirements rather than design your cycle to meet a BI's requirements (Kill Time). If minimal cycle times are used and a Cycle was Validated around a Log 6 BI with a D-Value of 1.6 minutes, the actual **exposure time needed at 121C** would be approximately 10 to 12 minutes. Total Cycle time would be longer, but the exposure time of the liquid at 121C would need to be 10 to 12 minutes. If at a later date BI's are accepted that have a D-Value of the 2.5 minutes, the needed exposure at 121C is now up to 16 to 18+ minutes. A Validated 15 to 18 minute 'Total Time Cycle' will very likely not Kill the more resistant BI now used. This higher resistant BI may cause problems with the Validated Cycle due to positives occurring even though no positives have occurred with this Autoclave for many years. There is nothing wrong with the Validated cycle, an inappropriate BI Resistance was accepted and used. This is why **it is important to select and purchase your BI's based upon 'intended use and Certified Resistance'.** Each time a new Lot of BI's come in for use, the end user must carefully review the Certificate of Analysis to insure that both population and stated resistance are appropriate for the Cycle being run. Far too often BI Resistance is overlooked by the end user. Cycles fail, the Autoclave Manufacturer is called to check the Autoclave for calibration and needless headaches begin.

If a self-contained, sealed glass ampoule type BI is used to Monitor Liquid Loads, **it is important to select a BI that monitors the most difficult area of the load to sterilize.** This does not just mean the front area of the Autoclave above the drain but **the most difficult area of each liquid container or flask**.

Using a Kaye Validator, thermocouples were placed into several 2 Liter flasks of TSB to be sterilized. The flask volume was divided into 4 quadrants. Upper most quadrant was labeled #1, slightly lower quadrant was #2 with the lowest bottom area being #4.

A fifth thermocouple was also used to monitor the chamber temperature. A 25-minute Liquid Loads Cycle was run at 121C and the temperature of the chamber and various depths of the liquid in the flasks were recorded. **Figure #1 Graph** shows the five-thermocouple readings. The top line (#5) is the 'Chamber Temperature'. The next four lines represent the temperature readings of the thermocouples in the 2 Liter flask of liquid. The upper line (#1) is the thermocouple located near the surface of the liquid with the other lines representing thermocouples at lower depths in the liquid. Thermocouple #4 is located near the bottom. One can see that as the depth location of the thermocouple increases, it takes longer for that liquid area of the flask to increase in temperature. The 121C Cycle was started at 14:01:40 pm. The upper line shows that the Chamber reached 121C at 14:04:20 and at that point the Exposure Cycle started. The upper surface area of the liquid hit 121C at 14:14:40, not until approximately ten minutes later and 10 min. into the Cycle. The lower area of the liquid did not hit 121C until 14:27:00. This point was 24 minutes into the Cycle with 1 minute left in the Cycle. This was 13 minutes after the surface area hit 121C. If the Cycle Exposure had set for 15 minutes, the majority of the liquid would never have hit 121C. In this 25-minute cycle, there was a 13-minute difference between when the top area hit 121C and when the bottom area of the liquid hit 121C. Data also shows that almost 10 minutes into the cycle the lower area was barely at 100C. Spore lethality is starting to be achieved at this point. However, the 13-minute difference between the time required for the upper portion of the flask to hit 121C and the bottom portion is significant.

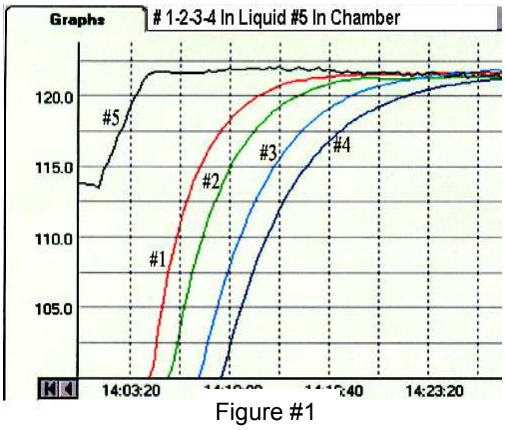

Figure #1

This 'temperature difference' in thermocouple location can now be applied to **proper BI placement**. If an ampoule BI type were used to monitor 'sterilization conditions' **in the liquid**, an ampoule that floated on the surface of the liquid would likely be killed while the lower area of the liquid being sterilized did not meet full sterilization parameters. This could create a situation where the full liquid content was not sterile yet the BI used was negative for growth. A sealed ampoule that could be maintained in the lower area of the flask for the cycle duration would more accurately measure that 'sterilization conditions were met' and the BI verification of this during Monitoring would be accurate.

In **Figure #2** is a photo showing proper BI ampoule placement for the most accurate Monitoring of Sterilization conditions. Most, if not all Standards regarding the use of Biological Indicators clearly state to '…. place the BI in the most difficult area of the load to Sterilize'. This example shows that the ampoule is held in place by a string. The ampoule is lowered into the center and lower portion of the liquid and then the string is held in place with a piece of autoclave tape. This is the most difficult area of the load to be sterilized. When the cycle is complete, the ampoule is removed and incubated for Growth/ No Growth testing.


Figure #2

Proper Sterilization Monitoring can be achieved and many headaches eliminated through the use of an appropriate BI if attention is paid to BI Type Selection and Resistance acceptance.

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