Steam Sterilization and BI Monitoring
Liquid Loads/ Liquid Media

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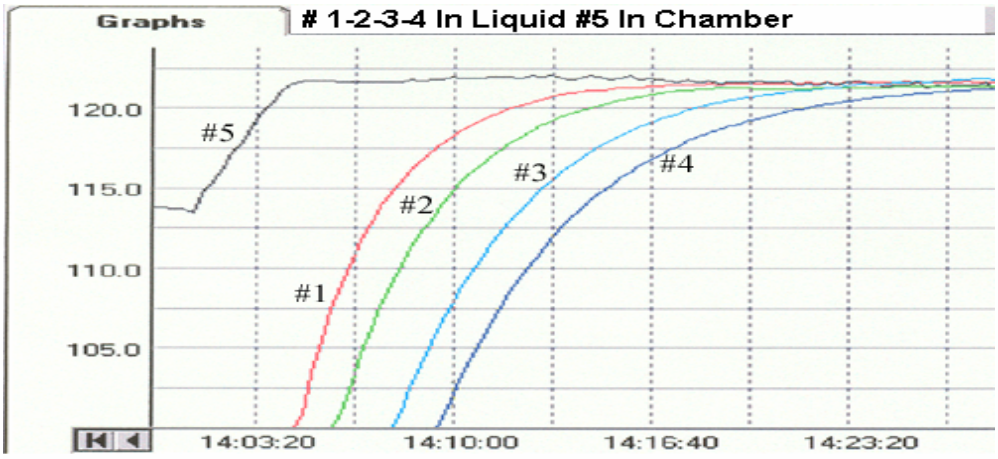
When attempting for the first time to implement a sterilization monitoring program or to validate a liquid load cycle using Biological Indicators, numerous problems can and usually do occur. Foremost is the inability of the sterilization cycle parameters to actually kill the BI being used. 'Something's wrong, I know my media is sterile but the BI survives the cycle.' More than likely, that statement is true. However, in validation with BI use, the BI must be killed. This must be done in order to meet several regulatory or certification requirements. This can be accomplished and a major problem solved!

When running liquid loads of media and other liquid volumes, several points need to be clear. Unlike hard goods, the liquid load is not mimicking the chamber conditions immediately. There can be a tremendous time lag for the liquid to reach sterilization temperature as compared to the chamber reaching sterilization temperature. As an example, the graph below shows the chamber temperature (TC #5) as it increases following the start of a sterilization cycle. Inside the chamber is a 2 Liter flask of tryptic soy broth (TSB). Inside the flask are located 4 thermocouples. Thermocouple (TC) #1 is located near the top of the liquid in the flask. TC #4 is located near the bottom of the flask. TC #3 slightly higher or mid-area of the liquid and TC #2 located about 4" below the surface of the liquid.



One can see that the autoclave chamber reaches 121°C approximately 3 minutes after the cycle has started. However, the lower portions of the liquid do not reach 121°C until the cycle has run for almost 20 minutes. It is important to keep in mind that the liquid was not at 121°C for a majority of the cycle duration.

This brings up several important points.

a. The liquid was not at 121°C for at least the first 15 minutes of the cycle.
b. Chamber temperature and liquid load temperature, for at least the first 15 minutes, are not similar.
c. The upper portion of the liquid in the flask heats up faster than the liquid in the lower portion of the flask.

We can now apply the above information to an actual sterilization cycle and see why the BI was not killed. In this example we will use a 2 liter flask of media in a 15 minute cycle and we are monitoring the flask of media with a BI suitable for monitoring liquid loads. Such a BI should be placed in the actual liquid to be sterilized and sealed so that the liquid will not come into contact with the spores and affect their performance. The BI should be placed or located near the lower portion of the flask since this is the area that heats up the slowest. The ampoule should not just float on the liquid surface where it would heat up the fastest and not be representative of the entire liquid volume temperature. Such a BI as pictured below can be used.

A thin wire can be tied around the neck of the ampoule and then lowered into the liquid and held in the desired location. As long as the

ampoule sinks in the liquid, it could just be dropped into the liquid and at least it would be monitoring the lower liquid portion for temperature.

In our example, the cycle time is 15 minutes. During this entire 15 minute cycle, the majority of the liquid contents did not reach 121°C. If the BI being used had a kill time of 15 minutes, it would survive this cycle. The BI certificate of performance said *killed in 15 minutes* but the BI was not dead and upon incubation it grew. The key here is the '15 minutes at 121°C'. The BI isn't killed in 15 minutes as a magic time; the time of 15 minutes must be at 121°C. This cycle parameter was not achieved and the BI was not dead. Some lethality is accumulated during the come-up time to reach 121°C but not enough to produce total lethality for the BI.

To achieve a 15 minute kill at 121°C, the overall cycle should be around 25 minutes. This would allow for a 10 to 15 minute come-up to 121°C and then an additional 10 minutes at 121°C to kill the BI.

Most media manufacturers state in the media preparation to sterilize at 121°C for 15 minutes. Again, this is not a 15 minute cycle. To sterilize at 121°C for 15 minutes, one can see that the cycle overall length needs to be close to 25 minutes. This would allow for come-up time plus 15 minutes at 121°C.

So if your BI is not dying, it is telling you that the cycle parameters necessary for BI spore lethality necessary for that particular BI were simply not met or achieved. If you had a BI with a Log 5 population and a 1.6 minute D-Value, the minimum time necessary to kill it would be around 9 to 10 minutes at 121°C. Including come-up time, a minimum overall cycle time of 20 to 25 minutes would be necessary to kill this BI.

The D-Value of the BI (how tough or resistant it is to moist heat), its population and the temperature of the liquid being sterilized prior to entering the chamber will all have an affect upon the cycle time needed. Be consistent with your BI use and liquid loading patterns. By this I mean consistently use a BI with a D-Value limit. Only purchase an acceptable BI with a known resistance that your cycle can kill. Let's say we set a D-Value limit of 1.8 minutes. Don't accept anything higher from your vendor. For population, choose a Log 4 or 5 and stick with it. Don't routinely monitor with a Log 4 ampoule and then throw in some Log 6 ampoules. You will likely run into trouble with BI and cycle failures. If you have consistently demonstrated that your cycle can kill a Log 5 BI with a D-Value of 1.8 minutes, don't order or accept a new lot of BI's that has either higher population or D-Value.

A good understanding of liquid cycles and BI consistency will help to eliminate present and future problems.