The 'Unique' Autoclave Load: Sterilizing Liquid Media

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When it comes to the steam sterilization of liquid media, there are a number of user concerns that are handled in various ways. Monitoring these cycles with biological indicators (BIs) and the various restrictions and cycle modifications that are done can lead to a false positive or failed cycle. Here are only a few of the cycle modifications done or restrictions applied by users for the steam sterilization of liquid media:

- *I want to keep the media flask in a container while being sterilized so the boil-over does not get all over my autoclave.*
- *According to the manufacturer's instructions, I'm to sterilize the media at 121°C for 15 minutes. I can't find a BI that will die in this short cycle.*
- *I can't run a longer cycle time since the media is heat sensitive and may not promote growth if a longer cycle time is used.*
- *Our protocol states to run only in a 15 minute, 121°C cycle.*
- *We have determined a cycle exposure time for a 1L flask of TSB. Now, what time should I use for a 2L flask, double the time?*

Many unique questions arise when working with liquid loads that would never come up if the cycle was for hard goods or wrapped goods. Most of the items would not be temperature sensitive, so there would be no worry about boil-over or questioning of BI placement. Yet with liquid loads there are no concerns about pre-vac air-removal or steam penetration as into a porous load. So, why do so many problems occur with liquid media sterilization and BI lethality?

Some of the methods used to address the concerns expressed above actually can contribute to a failed cycle or a cycle where the BI is positive. This largely happens due to the fact that most of the liquid load/media cycle configurations used in many clinics, universities or hospitals were not validated. Validating a load configuration cycle provides "documented procedure for obtaining, recording and interpreting the results required to establish that a process will consistently yield product complying with predetermined specifications"[1]. In sterilizing media, the desired outcome is to produce media with certain qualities such as pH range, growth promotion ability and sterility.

When performing a validation of a selected exposure time/temperature liquid media cycle to be used, it needs to be determined how long it takes from the start of the cycle to get the media up to 121°C. This would be recorded as the *come-up time* required for that particular cycle. If this was done with 1L flasks of media, a separate validation would be needed for cycles including larger volumes (2L flasks for example). To determine come-up time for a load of two 1L flasks of TSB, a temperature recorder could be put into each flask of media to record the media's temperature during the entire cycle duration. This activity would be repeated for at least two more cycles with each containing newly prepared flasks of media. The come-up times of all three cycles should be very similar. If, for example, the come-up times for the three cycles (to hit a temperature of 121°C) were 16 minutes, 12 minutes and 14 minutes respectively, this data could be compared, and it could then be determined that a worst-case come-up time would be 16 minutes.

[1] ISO/TS 11139:2006, Definitions 2, 55

There is now data that supports that, with a certain number of 1L flasks of a particular media, the temperature of the media will reach 121°C within 16 minutes of the start of the cycle. The number of flasks and the volume in each along with their position placement within the autoclave chamber should be documented and must remain the same for additional cycles used in the future unless a continued validation is performed using other placement areas or fewer flasks. It is necessary to validate a worst-case load to be able to predetermine that all other loads will also hit 121°C temperature within the specified 16 minute come-up time.

A very easy to use thermistor or data logger (Figure 1) for recording the media's temperature during a sterilization cycle can easily be placed inside the actual flask of media. By doing so, the actual temperature of what is being sterilized can be determined. Placing the data logger on a shelf inside the autoclave chamber would not show what is actually going on inside the media flask. The logger must actually be placed inside the media flask. To demonstrate the very large temperature difference between chamber and media come-up time, a data logger was placed inside the autoclave chamber while another was placed inside the media flask. The cycle was set for 20 minutes and both temperatures were recorded simultaneously during the exposure.

Figure 1: Various Data Loggers

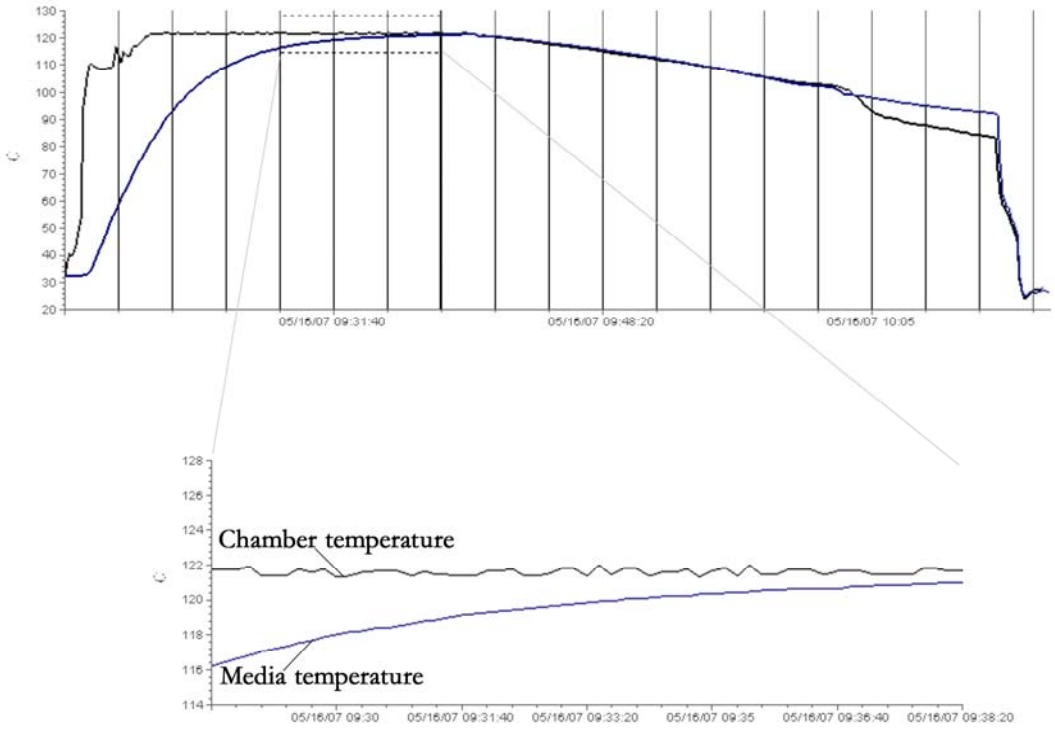

Figure 2: Data logger readout

Figure 2 shows that the chamber reached the temperature of 121°C within 4 minutes while the temperature of the media flask did not reach a temperature of 121°C until 19 minutes into the cycle. If this 20 minute cycle were to be used for the media sterilization, the media would only have been at the sterilization temperature of 121°C for approximately 1 minute. If a BI ampoule would have been placed inside the media flask during the cycle (Figure 3), it

would not have been killed. Thus if a 15 minute cycle had been used, the media would have only reached 117°C by the cycle end. Obviously this is not a suitable cycle for this load.

USP clearly states that with media sterilization of 121°C for 15 minutes, the 15 minute exposure time is for the media temperature once it hits 121°C.[2] Therefore, if it takes 20 minutes for the flask of media to reach 121°C, it must now remain there for a full 15 minutes. This makes a total cycle time of 20 minutes for come-up and 15 minutes for exposure or a full 35 minutes for that cycle. Anything less and the manufacturer's instructions for media sterilization are not being followed.

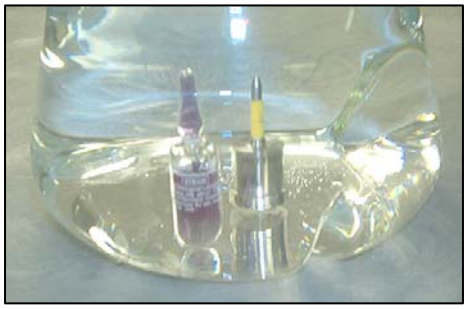

Figure 3: BI Ampoule and Logger in a flask

The first concern mentioned the placement of the media flask into a pan or container to catch any boil over media may be a cause for a failed cycle. This should not be done. During a liquid loads cycle, upon steam charge, the steam supply enters the autoclave chamber usually from an inlet located in the upper rear of the chamber. The steam is to push forward and down to gradually push all the air out of the chamber and down the drain. The replacement of air with steam is necessary so that a high steam quality environment exists and the transfer of heat will be much more efficient with steam being the conveyor of energy transfer. Air is an extremely poor conductor for heat transfer, especially when compared to steam transfer. In this situation, if air is still present in the chamber, it will act as an insulator and actually reduce and slow the transfer of heat energy. If the media flask is placed into a container to catch boil-off from the flask, air can be trapped in the container and insulate the lower portion of the flask from efficient heat energy transfer. Steam is trying to replace the air but can not push the air out of the container and may actually push the air down and compact it so it remains in the container protecting the flask from getting efficient heat transfer. Thus the flask will take much longer to heat up due to being placed into a container and may result in a failed cycle.

If boil-over is a real concern, place a low height pan (like a cookie sheet) under the lower rack of the autoclave chamber. The pan will still catch boil-off and will also be under the media flask without protecting it. Better yet, if it is possible to reset some of the cycle set points, slow down the post exposure exhaust ramp so that pressure is released at a slower rate and boil-over will be eliminated.

Running media in a longer cycle will likely not adversely affect the media and growth promotion abilities, after all, running a 35 minute cycle where it takes a 20 minute come-up time would actually be a *15 minute cycle at 121°C*. If there are concerns about cycle length, run the cycle and then test the media and verify that it still performs as expected and that growth promotion has not been compromised.

Running different volumes per flask or different numbers of flasks in a cycle are situations where additional validation work would need to be done. Running 1L flasks may need a 35 minute cycle where several 2L flasks may need additional time for come-up and perhaps a 45 minute cycle or more. By using a data logger, the various exposure times needed to get differing volumes of media up to 121°C can easily be determined.

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[2] USP 30, NF25 Volume 1, Chapter 1117, "Media Preparation and Quality Control", page 596