Overview Of Sterilization Methods V1

Choosing the proper method of media sterilization is very important because failing to do so establishes significant limitations to developing and producing media to effectively support biotech development. To do this, it is necessary to understand the characteristics of the most popular methods at a high level as well as how this relates to R&D. The table below shows high-level compatibility of the most common methods of sterilization for both liquid and solid-setting media (agar-based and gelled).

Interestingly, the most common comparison is between filtration and the other methods. Overall, filtration is truly the most gentle, having virtually no impact of the media quality. Its most significant challenge is that real-time validation is not possible. There is no way to know that it is working properly while being used. Failures are not detected for a considerable period after use. The filter must be removed, tested to confirm function, and replaced often. This is a fundamentally difficult characteristic that often limits the use of filtration.

Method	Bioscience Compatibility	Validation	Scale-Up	In-Package Treatment Style	Influence of Package/Batch Size
Heat-Batch	Liquid media that are not prone to thermal degradation.	Only retroactively with Biological Indicators (BI)	Complicated by batch size. Often not feasible due to media degradation.	Liquid In vessel	Yes Significant
CTS Continuous - Flow Sterilization	All Liquid media. Agar based media. Other gel-based media. (Sans egg).	Real-time validation & records.	Excellent	Liquid and fill with aseptic techniques	None
Filtration	Liquid media that are prone to thermal damage.	Cannot be validated in real time.	Excellent	Liquid and fill with aseptic techniques	None
Irradiation	Devices	Only retroactively with Biological Indicators (BI)	Complicated By batch Size. Often not feasible due to quality loss. Principally used for devices.	Not common	Yes

Thermal processing does not suffer from this issue. In particular, continuous flow thermal processing (also called Continuous Thermal Sterilization or CTS processing), is *monitored, recorded and validated in real time*. The actual media exposure to heat is tightly controlled and very reproducible. This well-established method is especially

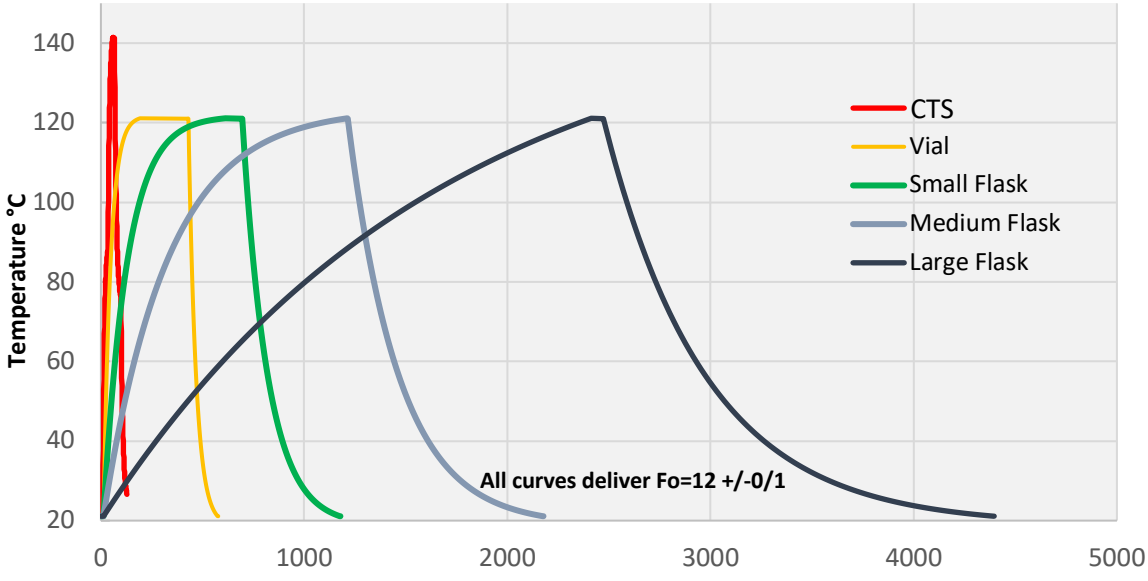
useful because it avoids all of the variability and most of the thermal damage caused by batch methods. Batch sterilization methods have been around for many years and are used extensively. As we move into more refined media, their quality tends to suffer as they also tend become more sensitive to heat processing. Many media sterilized via batch methods lose quality and often brown severely when sterilized in larger batches because the size of the vessel or batch determines the length of the treatment. Larger vessels or batches simply require longer heating times that lead to media failure because of excessive damage in sterilization. The result is that many media work well using the small batches methods in R&D and fail in scale-up. This severely restricts successful development of highly promising refined media.

Challenges to R&D

What is done in manufacturing originates in R&D and the sterilization technologies must be compatible for laboratory discoveries to be reasonably reproduced in manufacturing. This is very often overlooked, usually because of a degree of naivete about the critical details of sterilization and scale-up, *with the result of its critically hampering R&D*. A responsible discussion of sterilization must include how it is to be properly executed in R&D and successfully scaled-up for manufacturing for each type of media.

This is often complicated by differences between different types of media. Some are durable and tolerate to extreme exposure to heat. Results with these media usually translate well from R&D to manufacturing using batch and CTS sterilization. Still others are more sensitive. Their results change considerably as batch sizes are increased, and they often fail when scaled-up for manufacturing. *This is due to the significantly larger heating and cooling times required for large manufacturing batches*. Examples of this shown in Figure 1.0.

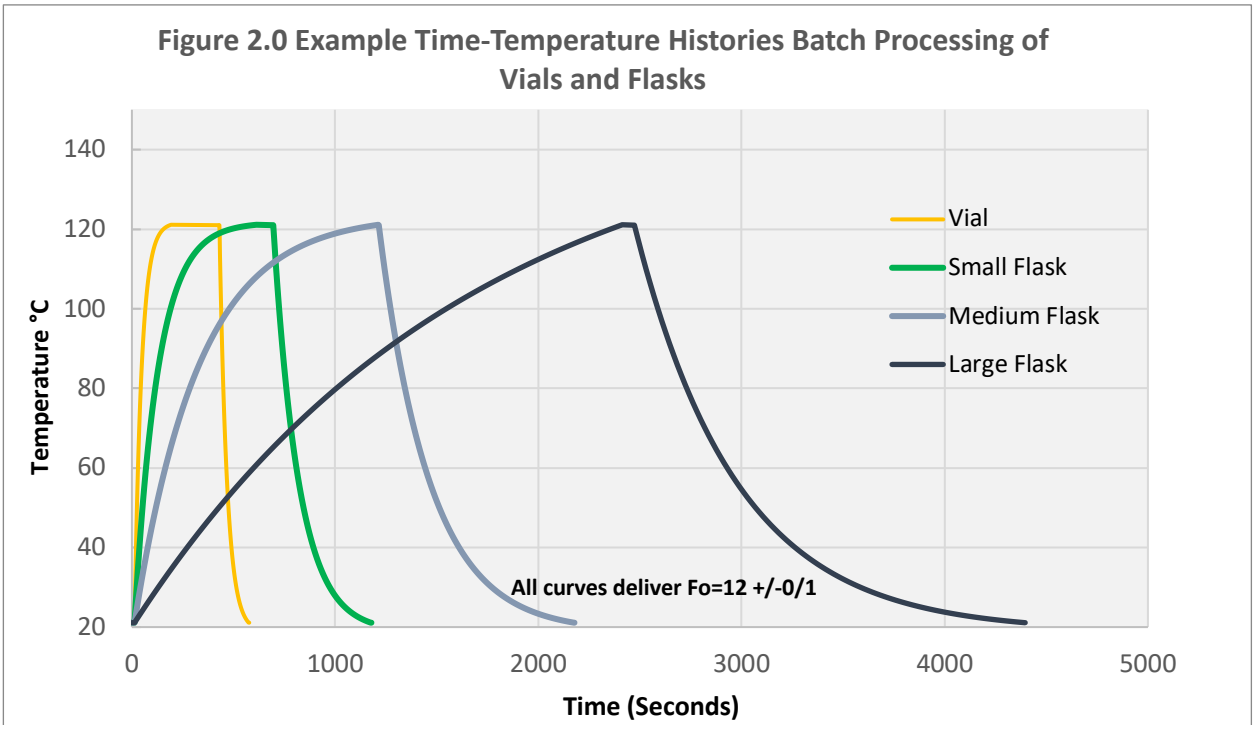
Figure 1.0 Example Time-Temperature Histories For Continuous-Flow CTS Process and Batch Processing of Vials and Flasks



Many manufacturing sites adopt CTS continuous-flow sterilization to address this. CTS technology maintains media functionality where large batch methods often do not. This greatly improves the success of durable and sensitive media at the manufacturing level and eliminates considerable variability in their performance. It also creates significant work in scale-up. *If R&D is conducted using autoclave sterilization. The media will often need to be greatly reformulated and revalidated when being taken to manufacturing, adding greatly to schedules and expenses. Simply put, results do not translate between the two methods*.

Fundamental differences between batch and CTS continuous-flow sterilization are the cause. Batch sterilization simply takes a long time to heat and cool. Even small vessels require at least 15-20 minutes when CTS sterilization usually takes less than 3-4. Batch sterilization in large vessels takes even more time and has even more impact on media quality. Thus, batch methods introduce variability due to batch size (and often failure of otherwise potentially useful media) because, media in each different size vessel undergoes a different thermal exposure.

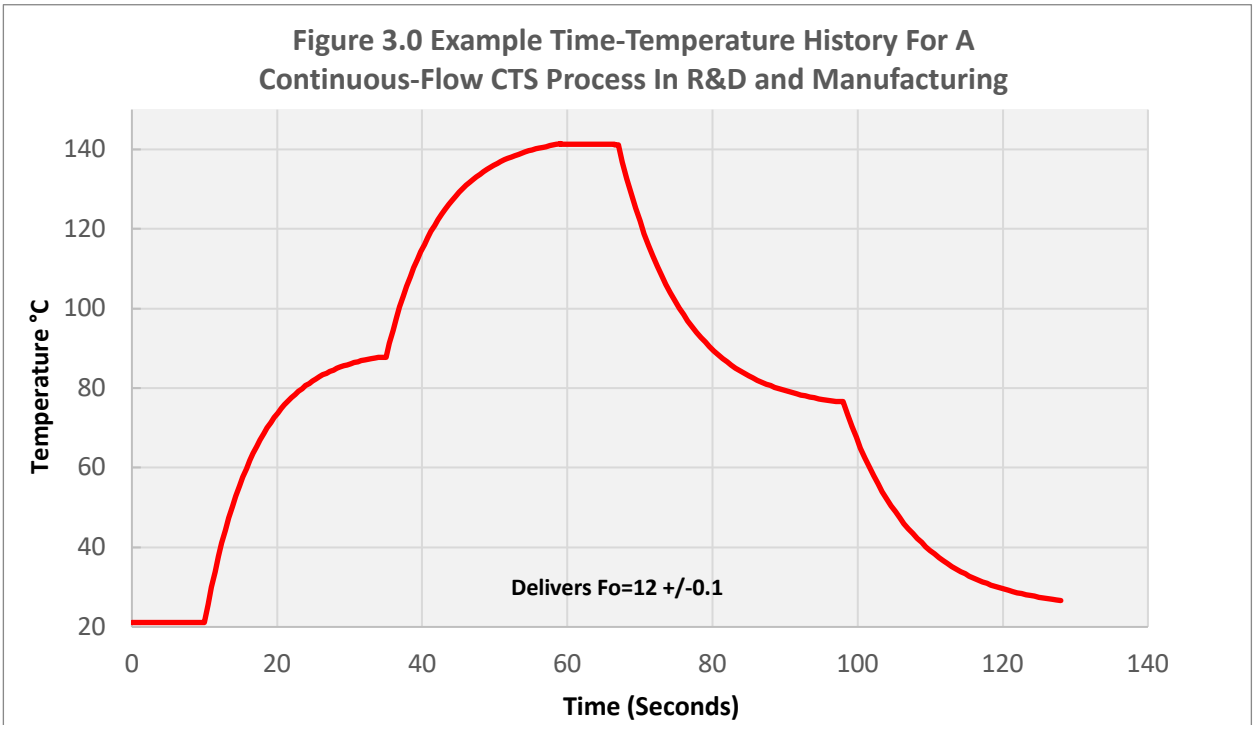
Figure 2.0 below shows this in terms of comparative Time-Temperature Histories for media sterilization in a series of vessels ranging from vials to large flasks. Each one of these delivers the proper heat exposure for a 12-log reduction in the target organism used for sterilization. Consequently, each is considered to be sterile. It doesn't take advanced mathematics to understand that each of these exposures will produce different quality media, especially in the longer exposure times. Many of these will not perform as required. Simply put, the media is sterile, and overcooked.



It should also be noted that variation in media quality/performance is even caused within a single type of vessel by variation in fill levels as well as autoclave/sterilizer loading and cycle design. There are many sources if variation in batch processes that influence media quality and often lead to poor performance.

CTS Continuous Sterilization

CTS continuous sterilization applies just one thermal exposure and eliminates the variability of batch sterilization. See the "CTS" Curve in Figures 1.0 and 3.0 for comparison to the batch temperature histories. As a continuous flow process, the media are processed at *steady state* and every drop receives the same thermal treatment regardless of the container/batch size. This also greatly simplifies scale-up of the sterilization process from R&D to manufacturing because there is just one thermal exposure. *This, of course, requires that the CTS process is used in R&D.* MTI BioScience is the global leader in providing laboratory-scale CTS processing systems and scale-up to manufacturing. *This is the key element in the path from R&D to manufacturing.*



The Hidden Benefit of CTS Sterilization

CTS sterilization has additional benefits that make it even more important for R&D and small-scale production. The CTS sterilization process, *although higher in temperature than batch methods, is so brief that it maintains more media quality and better performance*. Although this is counter-intuitive, it is true even at very severe sterilization levels. This means critically useful media, that fail in batch methods, are often successful using CTS sterilization.

Thus, placing the CTS process into the lab enables broadened and accelerated discovery, and development. This is, in fact, why MTI Bioscience was established!