STERIS
Life Sciences

Topic: Maximizing Sterility Assurance – Sterile Hold Time Testing for Autoclaved Items within Parenteral Drug Manufacturing

United States Code of Federal Regulations and European Union EudraLex "Annex 1" are in alignment that hold times must be established and validated for sterilized equipment. However, details on the acceptable storage duration and validation test criteria are lacking. STERIS benchmarked manufacturers within the pharma and biopharma industry to learn if common practices exist for establishing hold times for autoclaved items used within aseptic fill / finish manufacturing.

Sterilization wrapping is used widely throughout the industry. For items required to be sterile, the wrapping allows for steam penetration during autoclaving and maintains a microbial barrier post sterilization. The wrapping allows for materials to be moved throughout the facility and within the clean rooms without compromising the critical product contacting surfaces. Double wrapping or using a material that is compatible with surface disinfectants can be methods of transfer into a higher classified space. For these reasons, defining the sterilization wrapping system (material of construction and closure method) is essential.

Likewise, the sterile hold time depends on the sterilization wrapping system. After sterilization occurs, materials are to be used within a timeframe that is tested and validated. This allowable hold time is established for each sterilized item and associated sterilization wrapping system and is finally confirmed by an aseptic process simulation.

To test and confirm sterilization wrapping system performance, STERIS designed experiments using various materials and closure methods over a defined hold time. Biological indicators were used to confirm sterilization at time zero and sterility testing was performed throughout the hold time duration. Stainless steel coupons as well as common items used in fill / finish manufacturing were used as the test subjects. This unique experimental data is the basis for best practice recommendations for sterilization wrapping systems to assist in validation work for site specific aseptic fill / finish items.

Maximizing Sterility Assurance- Sterile Hold Time Testing for Autoclaved Items within Parenteral Drug Manufacturing

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Introduction

For items required to be sterile, wrapping allows for steam penetration during autoclaving and maintains a microbial barrier post sterilization. The wrapping allows for materials to be moved throughout the facility and within the clean rooms without compromising the critical product contacting surfaces. Double wrapping or using a material that is compatible with surface disinfectants can be methods of transfer into a higher classified space. For these reasons, defining the sterilization wrapping system (material of construction and closure method) is essential.

Likewise, the sterile hold time depends on the sterilization wrapping system, operators and sterilization cycle performance. After sterilization occurs, materials are to be used within a timeframe that is tested and validated. This allowable hold time is established for each sterilized item and associated sterilization wrapping system and is generally confirmed by an aseptic process simulation/validation. STERIS benchmarked manufacturers within the pharma and biopharma industry to learn if common practices exist for establishing hold times for autoclaved items used within aseptic fill / finish manufacturing.

To test and confirm sterilization wrapping system performance, STERIS has additional studies using various materials and closure methods over a defined hold time. Biological indicators were used to confirm sterilization at time zero and sterility testing was performed throughout the hold time duration.

What is a Sterile Hold Time?

The time between sterilization and use of an item in aseptic manufacturing. It is the expiration duration that is supported by data.

Regulations

Annex 1: Draft 13	
8.48	• Where materials, equipment, components and ancillary items are sterilised in sealed packaging or containers, the packaging should be qualified for minimizing the risk of particulate, microbial, endotoxin/pyrogen or chemical contamination, and for compatibility with the selected sterilisation method. The packaging sealing process should be validated. The validation should consider the integrity of the sterile protective barrier system and the maximum hold time before sterilisation and maximum shelf life assigned to the sterilised items. The integrity of the sterile protective barrier system for each of the sterilised items should be checked prior to use.
8.46	• Where possible, materials, equipment and components should be sterilised by validated methods appropriate to the specific material. Suitable protection after sterilisation should be provided to prevent recontamination. If sterilised items are not used immediately after sterilisation, these should be stored using appropriately sealed packaging. A maximum hold time should also be established.

USP 1211	
Equipment Section	• The procedures used for the cleaning and sterilization of direct contact surfaces, including dirty, clean, and sterile hold times, must be validated to ensure they do not adversely impact essential product quality attributes as well as to verify the effectiveness of the cleaning procedure and that no microbial recontamination/proliferation occurs during equipment storage.

Operational Advantage

Hold time testing is ensuring package integrity to protect the sterile part or material. Packages being held in a cleanroom post sterilization can be ready to use when needed without extra preparation time. Confirming a hold time allows for less reprocessing of autoclaved parts/ materials if not used within production on the same day as sterilization.

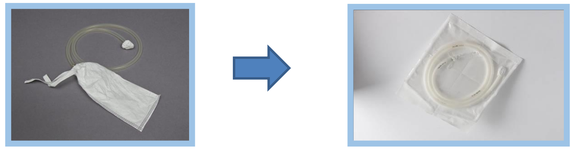




Designing Experiments

Sterility assurance is maximized by confirming hold times of parts or materials used for aseptic manufacturing. Each end user is required to create a validation based on routine wrapping configurations using representative parts and equipment for a time range that fits the individual process. The sterile hold time will be confirmed during aseptic process simulations/validations.

Materials chosen during a study will also determine the wrapping configurations to be tested. Wrapping material should be low particulate generating to limit the risk to the product.



Filling Needle and Tubing Assembly with elasticized covers in a self seal autoclave pouch



Stopper bowl with elasticized cover and autoclave bag with drawstrings using a gooseneck closure



Pipe openings with elasticized covers in a heat sealed autoclave pouch

Closure methods are to be tested within a sterile hold study. Best practice closures do not use any cellulose-based materials, such as autoclave tape, to limit particulate generation within the aseptic area. The best practice closure methods allow for removal of parts from the packaging with limited contact with critical areas and less risk to product contamination.

Types of Wrapping and Closure Methods Used				
Material Type	Heat Seal	Self Seal	Gooseneck	Elasticized Cover
Tyvek/ PET film pouch	X	X	N/A	X
Tyvek/ HDPE film pouch	X	X	N/A	X
All-Tyvek Bag	N/A	N/A	X	X

Elasticized covers are used as initial barriers but are not considered a full microbial barrier. They allow for extended protection when installing materials that are product contacting to limit contamination by personnel handling.

The time frame of the study is dependent on processing restrictions and parts. Replications at the same timepoint are tested for repeatability. Multiple time points can be tested to increase confidence in longer sterile hold times.

Data Overview

Results	
BI	• 8 tested in load, all negative for growth
Day 10	• 8 samples negative, 1 positive
Day 20	• 23 samples negative, 1 positive
Day 30	• 29 samples negative, 2 positive

Positive results for all days are including positive controls that were in place to confirm growth outside of wrapping materials where the storage occurred. The extra positive sample was expected due to a closure method that does not create a complete seal or tortuous path.

Summary

Completing a hold time study maximizes the sterility assurance of your parts and equipment as well as your product. It helps to reduce the reprocessing of sterile parts and materials. This testing allows end users to store sterilized materials within the clean area to limit the amount of sterilization required on the day of processing. STERIS data can be used to aid in the design of the study protocol as well as confidence in the package integrity time frame. Parts tested and wrapping configurations should mimic routine wrapping styles. Justification can be used to limit the number of parts that are required to be tested using a risk-based approach. Testing has been completed to support a 30-day sterile hold time using Tyvek bags, pouches and covers.

References:

1. EudraLex: The Rules Governing Medicinal Products in the European Union. Volume 4, EU Guidelines to Good Manufacturing Practice, Medicinal Products for Human and Veterinary Use. Annex 1- Draft 15 (2022)
2. United States Pharmacopeia 1211: Sterilization and Sterility Assurance of Compendial Articles (2013)
3. STERIS AST Laboratory Reports, Brooklyn Park, MN (2022)
4. STERIS Protocol and Final Report (2022)