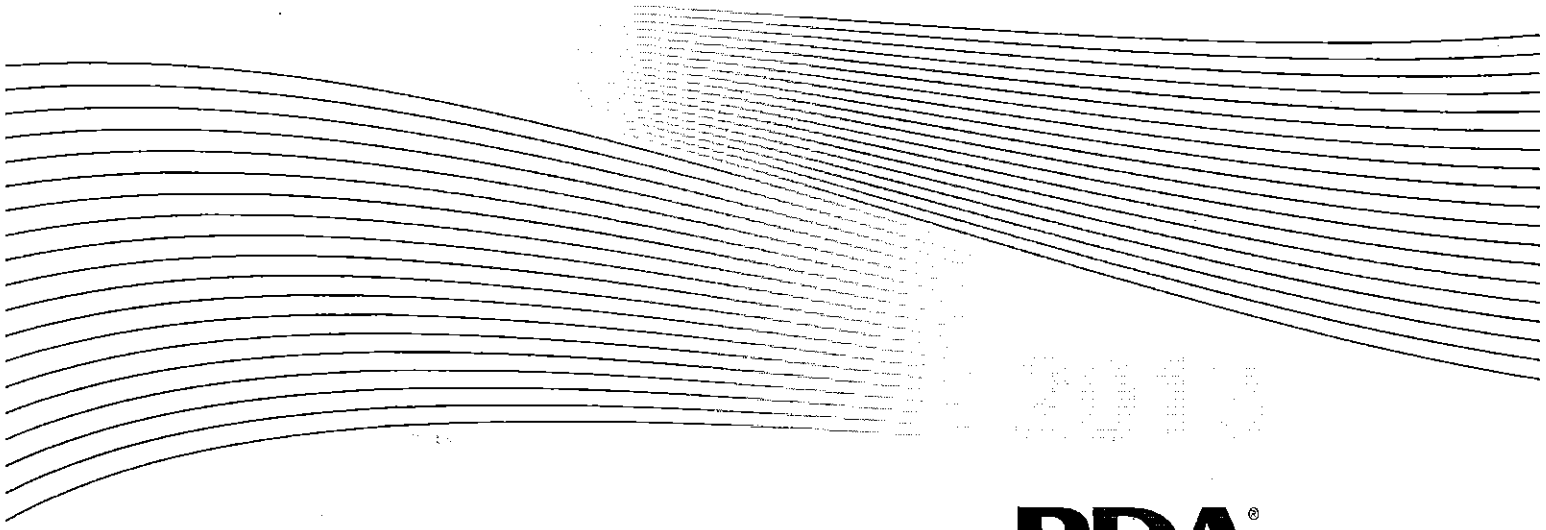
Technical Report No. 61

Steam In Place



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PDA Technical Report No. 61: Steam in Place Task Force Members

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The Steam in Place Task Force would like to dedicate this technical report in memory of Lance Morien.

The content and views expressed in this Technical Report are the result of a consensus achieved by the authorizing Task Force and are not necessarily views of the organizations they represent.

Steam In Place

Technical Report No. 61

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Table of Contents

FIGURES AND TABLES INDEX

1.0 Introduction

PDA Technical Report No. 1, *Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control*, updated in 2007, focuses on the microbiology and engineering concepts of moist heat sterilization and the general approach to sterilization science in batch sterilizers (autoclaves) *(1)*. This technical report is intended to complement PDA Technical Report No. 1 and will focus on steam in place (SIP) processes.

The primary objective of the task force responsible for this technical report was to develop a scientific technical report on SIP processes that provides recommendations for use by industry and regulators. References to appropriate and up-to-date scientific publications, international regulatory documents, journal articles, technical papers, and books are used to provide more detail and supportive data can be found.

Steam in Place was chosen as the title because this document focuses on the various applications of steam for in situ sterilization for "sterile" applications and for in situ sanitization and other bioburden control applications widely used for systems that do not claim to be "sterilized" via steam. We also differentiate "steam in place" from the more generic term "sterilize in place" used to describe in situ sterilization using various types of gaseous or liquid sterilizing agents including steam *(1)*.

The task force was composed of European, North American, and South American industry professionals to ensure the methods, terminology, and practices of SIP reflect sound science and can be applied globally. This technical report was disseminated for public review and comment prior to publication, to provide the widest possible review and ensure its suitability as a guide to industry.

SIP is often a pivotal step of aseptic processing for sterile product manufacture, and as such, may benefit from the application of risk management methodologies. The characterization, evaluation, and assessment of risk are useful to direct overall efforts for cycle development and subsequent validation. After development of a risk assessment, more resources can be focused on mitigating risk for systems, equipment, or processes that have the highest potential for product contamination. The management of risk may be employed throughout the lifecycle of SIP equipment and processes to efficiently focus and allocate resources commensurate with the probability of impacting final product purity and safety. Descriptions of the specific steps and tools for risk management are available from a variety of sources *(2,3)*.

1.1 Scope

The scope of this technical report is limited to discussion of SIP processes that provide moist heat sterilization and/or sanitization of equipment and systems supporting the manufacture of medicinal products. The principles discussed in this report may also be applied to those systems where portable equipment is steamed at a fixed station (steam out of place).

Application of the concepts presented in this technical report to laboratories or other non-CGMP applications, including hospitals, is not intended.

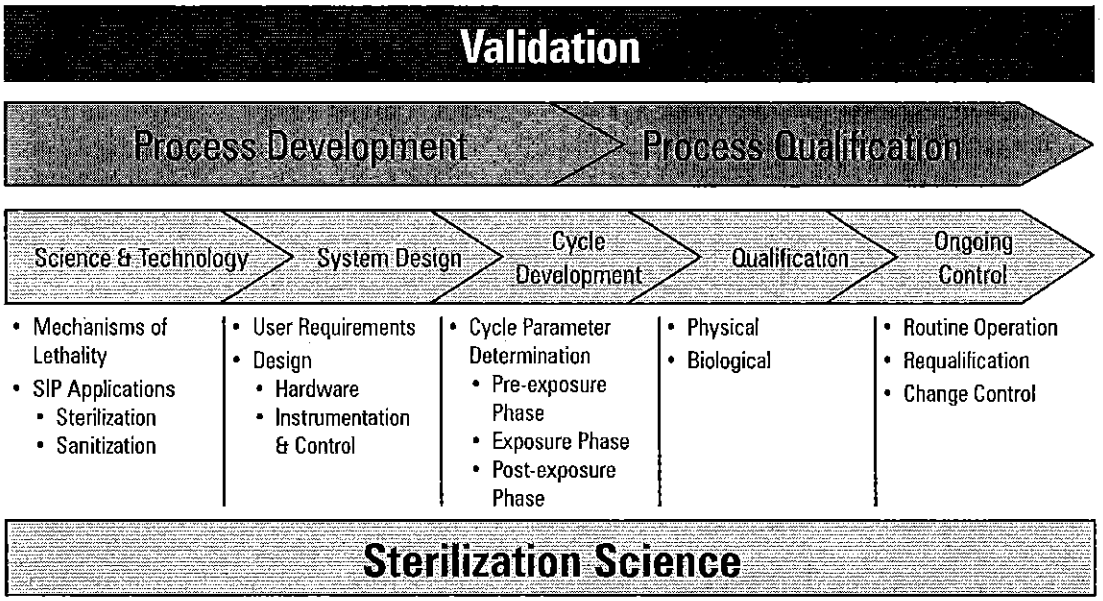
The following concepts are out of scope:

- Clean-in-Place (except where related to SIP)
- In situ media sterilization
- Product Sterilization
- Design and qualification of utilities

This technical report is organized in a logical progression from the essential elements of SIP system design through SIP cycle development, qualification, and ongoing operation.

In the interest of clarity, the report provides a glossary of technical terms, and begins with a discussion of the SIP Life Cycle as depicted in **Figure 1.1-1.**

Figure 1.1-1 Steam in Place Life Cycle



Sterilization science for SIP systems will be discussed to expand on the concepts developed in PDA Technical Report No. 1. The System Design section will cover the design considerations for an SIP process including hardware (e.g., pipes, tanks, filters, valves) and controls (e.g., monitoring and control instruments). Example process parameter tables for SIP cycles are provided to support assessment of risk associated with different cycle phases. The Cycle Development section applies theoretical concepts that are developed into the practical application of a comprehensive SIP process.

The Performance Qualification section focuses on the application of physical and biological approaches used to demonstrate the efficacy of particular SIP processes as they relate to intended use.

Finally, the Ongoing Process Control section discusses ways to establish and maintain a continuous state of control after the SIP process is implemented. This section includes recommendations for procedural controls, records management, change control, requalification, and maintenance practices.

2.0 Glossary of Terms

Term usage may differ from company to company, and some terms may be subject to change in the future. However, the terms used in a sterilization program must be clearly defined and well understood within the company. Regulatory guidelines may offer other definitions that should be considered. This technical report uses the following terms, listed here with their definitions and synonyms where applicable.

Bioburden
Viable microorganisms on or in a pharmaceutical product or in the manufacturing environment.

Biological Indicator (BI) Challenge System
A test system containing viable microorganisms of a pure specified strain providing a defined resistance to a specified sterilization process *(4)*. **[Synonyms:** BI challenge system, microbial challenge, microbiological challenge system.]

Biological Qualification
A component of performance qualification that demonstrates, by use of biological indicators, that the required lethality (F_{BIO}) or spore log reduction (SLR) is achieved consistently throughout the sterilized or sanitized portion of the SIP system.

Bracketing Approach
A scientific approach for defining characteristics (e.g., Tank sizes, system configurations filter sizes and types) that are tested (in a qualification study or validation study) at upper and/or lower limits.

Calibration
The demonstration that an instrument or device produces results within specified limits when compared to those produced by a reference standard or a standard that is traceable to national or international standards, over an appropriate range of measurements.

Cold Spot
The location within an SIP system that achieves the lowest process lethality (F_0) during a SIP process. **Note:** When lethality values are not available or not applicable (e.g., a sanitization process operating at less than 100 °C) the cold spot is the location with the lowest temperature profile during the SIP cycle.

Cool-down Phase
The phase of an SIP cycle that occurs after completion of the exposure phase. Parameters (e.g., time, temperature, pressure) of a cool-down phase are typically defined in order to meet applicable user requirements for system cooling and drying.

Critical Control Point
A step at which control can be applied and is essential to prevent or eliminate a pharmaceutical quality hazard or reduce it to an acceptable level *(3)*.

Cycle Development
A series of activities performed for the purpose of defining or confirming the cycle parameters (e.g., time, temperature, pressure) necessary to ensure sanitization or sterilization.

Deadlegs
An area of entrapment in the vessel or piping run that could lead to contamination of the product due to insufficient exposure to moist heat *(5)*.

D_T Value
The time in minutes required for a one-logarithm, or 90%, reduction of the population of microorganisms used as a biological indicator under specified lethal conditions. For steam sterilization, the D-value should always be specified with a reference temperature, D_T. For example, a BI system with a $D_{121°C}$ of 1.4 minutes requires 1.4 minutes at 121 °C to reduce the population by one logarithm. **[Synonym:** D-value]

Exposure Phase
The phase of the SIP cycle in which the appropriate parameters (e.g., time, temperature, pressure) are maintained within defined ranges for the time (exposure time or dwell period) determined to be necessary to achieve the desired lethality.

F-Value (Lethality Factor)
A measurement of sterilization effectiveness, the F-value is the calculated equivalent lethality (using a specified z-value), in terms of minutes at a reference temperature (T_{ref}), delivered by a sterilization cycle.

F_0

A term used when the *specific* reference conditions of $T_{ref} = 121.1°C$ and $z = 10°C$ are used to calculate the equivalent lethality. For example, when the z-value of the BI is 10°C, a cycle with an $F_{(T=121.1°C, z=10°C)}$, or F_0, equal to 8 minutes is equivalent (in terms of delivered lethality) to a square wave cycle of 8 minutes at 121.1°C. A square wave cycle that provided an exposure of 25.9 minutes at 116°C would also yield an F_0 of 8 minutes.

Note: The reference temperature used in calculating F_0 is 121.1°C, which is the approximate mathematical equivalent of 250°F. The reference temperature of 121°C for F_0 will be used throughout this report for brevity.

$F_{Biological}$ (F_{BIO})

A term used to describe the empirically derived lethality of microorganisms on or in a BI challenge system. The F_{BIO}-value is calculated as D_T x LR, where D_T is the D-value of the BI system at the reference temperature (T) and LR is the actual logarithmic reduction ($\log N_0 - \log N_F$) of the BI population achieved during the cycle. Where N_0 is the initial population of the BI and N_F is the remaining population after the sanitization/sterilization cycle.

$F_{Physical}$ (F_{PHYS})

A term used to describe the delivered equivalent lethality that is calculated based on the physical parameters of the cycle. The F_{PHYS} value is calculated for a reference temperature (T_{ref}) and z-value using the equation:

$$F_{PHYS} = i\sum_{t=0}^{n} 10^{\frac{T_i - T_{ref}}{z}}$$

Where,

i = time interval between readings,

T_i = temperature reading for that interval,

T_{ref} = reference temperature

z = temperature change required to change the D-value by a factor of 10

Flash Steam

A mixture of steam and water that occurs when hot water under pressure moves to a region of lower pressure.

Gravity Displacement Process

A sterilization process based on the principle that air within the system is more dense than steam entering the system. As steam enters the system, air is pushed out the bottom drain/vent/trap and exits with the condensate.

Hazard

The potential source of harm *(3)*.

Heat-up Phase

The phase of an SIP cycle that occurs prior to the exposure phase. Process parameters (e.g., air removal, preheating, uniform temperature distribution)are developed for this phase in order to meet applicable user requirements for system conditioning. **[Synonym:** come-up time, heat-up time.]

Installation Qualification (IQ)

Documented verification that the equipment or systems, as installed or modified, comply with the approved design, the manufacturer's recommendations, and/or user requirements *(6)*.

Leak Test

See System Integrity Test

Minimum Acceptable Cycle (MAC)

The minimum cycle conditions (in terms of delivered minimum lethality or minimum time and temperature) that would be considered acceptable.

Operating Parameters

Values (e.g., time, temperature, pressure) that are controlled and/or measured that collectively define each phase of an SIP cycle (e.g., heat-up, exposure, and cool-down).

Critical Parameters

Values (e.g., time, temperature, and pressure) that are controlled and/or measured to ensure the efficacy of a steam in place cycle. Failure to meet a critical parameter should result in rejection of the cycle.

Key Parameters

Values that are controlled and/or measured and are used to assure the ongoing "state of control" of steam in place cycles. Failure to meet a key process parameter should result in an investigation.

Operational Qualification (OQ)
Documented verification that the equipment or systems, as installed or modified, perform as intended throughout the anticipated operating ranges *(7)*.

Overkill Design Approach
A sterilization method where the steam in place cycle is capable of meeting or exceeding both an F_{BIO} and F_{PHYS} of 12 minutes and worst case bioburden assumptions are made to demonstrate an SAL of 10^{-6}. (Note: For typical SIP systems, the F_{PHYS} will need to be greater than the F_{BIO}.)

Partial-Cycle Qualification
A qualification method that uses less than the full exposure time to demonstrate sterilization or sanitization cycle efficacy. **[Synonym:** fractional cycle.**]**

Performance Qualification
Documented verification that the equipment and ancillary systems, as connected together, can perform effectively and reproducibly based on the approved process method and specifications *(7)*.

Pre-Vacuum Process
A process in which air is removed by applying a vacuum (i.e., negative pressure) or pulses of vacuum to precondition the system prior to the exposure phase.

Pure Steam
Steam in which the condensate complies with the Compendial monograph, Water for Injection (WFI) *(8,9)*. **[Synonyms:** clean steam, high quality steam]

Routine Operational Cycle
Parameters that are specified for ongoing SIP operations used in production. The operational cycle is typically controlled to produce additional lethality over the qualified minimum acceptable cycle in order to provide increased sterility assurance.

Sanitization
A process that reduces the number of viable microorganisms to a defined level.

Saturated Steam
Steam that is at a temperature and pressure that corresponds to the vaporization curve of water. It is in a state of equilibrium between being a liquid and a gas with no entrained liquid water.

Spore Log Reduction (SLR)
The number of log reductions (10-fold changes) of spores from the initial population. For the overkill sterilization method, one targets a spore log reduction of 12 to achieve 1×10^{-6} probability of a survivor when using a biological indicator having a population of 1×10^{6}.

Steam in Place Cycle
A sequence of defined steps and operating parameters (e.g., time, temperature, and pressure) performed in situ on equipment and/or systems to provide a given sterility assurance level (SAL) or defined sanitization level.

Steam Orifice
A specifically sized hole (e.g., 1/32 or 1/16 inch diameter) to allow condensate or steam to pass through. **[Synonyms:** flow orifice, steam bleed.**]**

Steam Trap
A self-actuating, automatic device that removes condensate and air from the system.

Sterile Boundary
The sterile boundary is the demarcation in a system between the portion of the system that requires sterile contact surfaces (e.g., sterile side of filters and downstream piping) and the rest of the system (e.g., upstream side of filters, condensate drain lines).

Sterility Assurance Level (SAL)
Probability of a single viable microorganism remaining after SIP.

Note: The term SAL uses an assumed quantitative value, generally 10^{-6} or 10^{-3}. When applying this quantitative value to assurance of sterility, an SAL of 10^{-6} has a lower value but provides a greater assurance of sterility than an SAL of 10^{-3} *(10)*.

Sterilization
A process used to render a system free of viable microorganisms with a specified probability.

Superheated Steam
Steam that is at a higher temperature than that indicated by the equilibration curve for the vaporization of water (at a given pressure).

Survivor Curve
Graphical representation of the inactivation of a population of microorganisms with increasing exposure to a microbiocidal agent under stated conditions *(11)*.

System Integrity Test
Any test designed to detect leaks or other breaches in system integrity that might compromise operator safety or system sterility (or sanitary status). **[Synonym:** leak test.]

Mass Flow Integrity Test
A system integrity test that measures the mass flow needed to maintain a given pressure.

System Pressure Hold Test
A system integrity test in which the system is pressurized to a predetermined level with filter sterilized compressed air or other compressed gas, after which the system is isolated and the amount of pressure loss over time is measured.

System Vacuum Hold Test
A system integrity test in which the system under test is evacuated to a predetermined setpoint and the system is isolated from the external environment. The decay in vacuum level over time is measured.

Temperature Probe
A sensor (e.g., thermocouple or resistance temperature detector (RTD)) that has been specifically designed to measure temperature. Temperature probes may be control, resident, surface mounted, validation, mapping, or permanent.

Validation
A documented program that provides a high level of scientific assurance that a manufacturing process will reliably produce acceptable product. The proof of validation is obtained through rational experimental design and the evaluation of data, preferably beginning from the process development phase and continuing through the commercial production phase.

z-value
The number of degrees of temperature change necessary to change the D-value by a factor of 10. The z-value allows integration of the lethal effects of heat over time (i.e., calculation of F_0) as the temperature changes in a cycle.

3.0 Steam in place Science and Technology

Whereas PDA Technical Report No. 1 focuses on essential scientific tools used for the design, development, and qualification of batch (i.e., autoclave) sterilization cycles, this report addresses areas specific to the in situ application of steam for sterilization purposes as well as various types of sanitization processes *(1)*.

3.1 SIP Applications

The overkill design approach is the most common method used for systems that are sterilized by SIP since there is usually not the concern for degradation of product. When claiming sterilization using the overkill approach, bioburden monitoring is reduced or is not required (see below) since a worst-case bioburden assumption is used to determine the delivered lethality needed to achieve a sterility assurance level (SAL) of 10^{-6} on or in the system being sterilized. When using this approach, the qualification program must demonstrate that both the F_{BIO} and F_{PHYS} are equal to or greater than 12 minutes. Examples of calculating an overkill cycle are provided in **Section 6.3.1.**

When the process does not require sterility, the SIP approach is commonly referred to as "sanitization." Depending on the requirements of process control, the sanitization process would typically not have an established SAL requirement.

Sanitization processes may also be used for reducing bioburden levels in systems that show higher than accepted microbial counts or as a prophylactic measure to keep microbial counts "under control". In these cases, temperature is typically monitored at the worst-case location and cycle time is decided based on temperature readings at this location. Cycle end-point is either bioburden removal or reduction, and success is demonstrated by bioburden measurement after each cycle is performed.

Table 3.1-1 Includes some example applications of SIP processes, cycle development considerations, and potential validation approaches.

Purpose	Application	Cycle Development Considerations	Validation Considerations
Sterilization Process			
	SIP process where sterilization is claimed. (Example: Aseptic filler piping for a sterile drug product)	▪ Time ▪ Temperature ▪ Pressure ▪ System and filter integrity ▪ Positive pressure maintenance ▪ Temperature/pressure correlation ▪ Cold spot determination ▪ Temperature mapping ▪ Air and condensate removal	▪ BIs are used ▪ Temperature probes meet defined limits ▪ Demonstrate total kill ▪ Monitor temperature at worst case locations ▪ $F_{BIO} \geq 12$ minutes (Overkill) at each BI location ▪ $F_{PHYS} \geq 12$ minutes (Overkill)* ▪ Sterile hold time
Sanitization Process			
	SIP process that inactivates bioburden (examples: WFI system that is steam sanitized, vessels used in biopharmaceutical manufacturing to control bioburden prior to use for pooling, etc.)	▪ Time ▪ Temperature ▪ Pressure	▪ No BIs/F_{BIO}/F_{PHYS} required ▪ Monitor temperature at worst case locations ▪ Monitor bioburden level before and after sanitization ▪ Demonstrate bioburden removal

* For typical SIP systems, the F_{PHYS} will need to be greater than the F_{BIO}

Because SIP processes can be used for such a wide range of applications, it is very important for the user to define the purpose and the desired outcome of the SIP process before the system is designed. Risk assessments made prior to the design of the system can be used to help define if a sanitization or sterilization process is required.

3.2 Mechanisms of Lethality

The mechanism of microbiological lethality for steam in place systems is the thermal destruction of microorganisms by direct contact with the sterilizing medium (steam). The mechanism of heat transfer is conduction where the transfer of energy occurs from latent heat. As with other saturated steam sterilization methods, the rate of microbial destruction under conditions of constant temperature progresses logarithmically over time.

The kinetics for these complex reactions are best represented as a First Order chemical reaction. This means that there is a linear relationship between the logarithm of the number of surviving microorganisms and the time of exposure (see **Figure 3.2-1**).

[Equation 1]

$$\text{Log } N_F = -F_{(T,z)} / D_T + \text{Log } N_0$$

where,

N_F = Number of microorganisms after exposure of F equivalent minutes

$F_{(T,z)}$ = Equivalent lethality of a cycle calculated as minutes at a reference temperature (T), using a defined temperature coefficient (z)

D_T = Thermal resistance value, in minutes, of the microorganism at a specific temperature (T).

Note: This specific temperature must be the same as the reference temperature used for calculating F-value.

N_0 = Number of microorganisms prior to exposure

In **Figure 3.2-1**, D_T is a measure (the negative reciprocal) of the slope of the semilogarithmic survivor curve; therefore, it describes the relationship between the number of survivors versus equivalent (F-value) exposure time. F-value is a term used in the model to characterize exposure time to moist heat. By definition, the F-value is expressed by a reference temperature so that it truly represents the equivalent exposure time at that reference temperature in terms of lethality. Since routine operational cycles are not square wave cycles (i.e., the system does not come up to temperature instantaneously, remain at the precise set point throughout the exposure phase, and then cool down instantaneously), the z-value, or temperature coefficient, is used in the model to calculate the equivalent lethality at different temperatures. Examples of lethality rates are shown in **Table 3.2-1**.

Figure 3.2-1 Microbial Survivor Curve

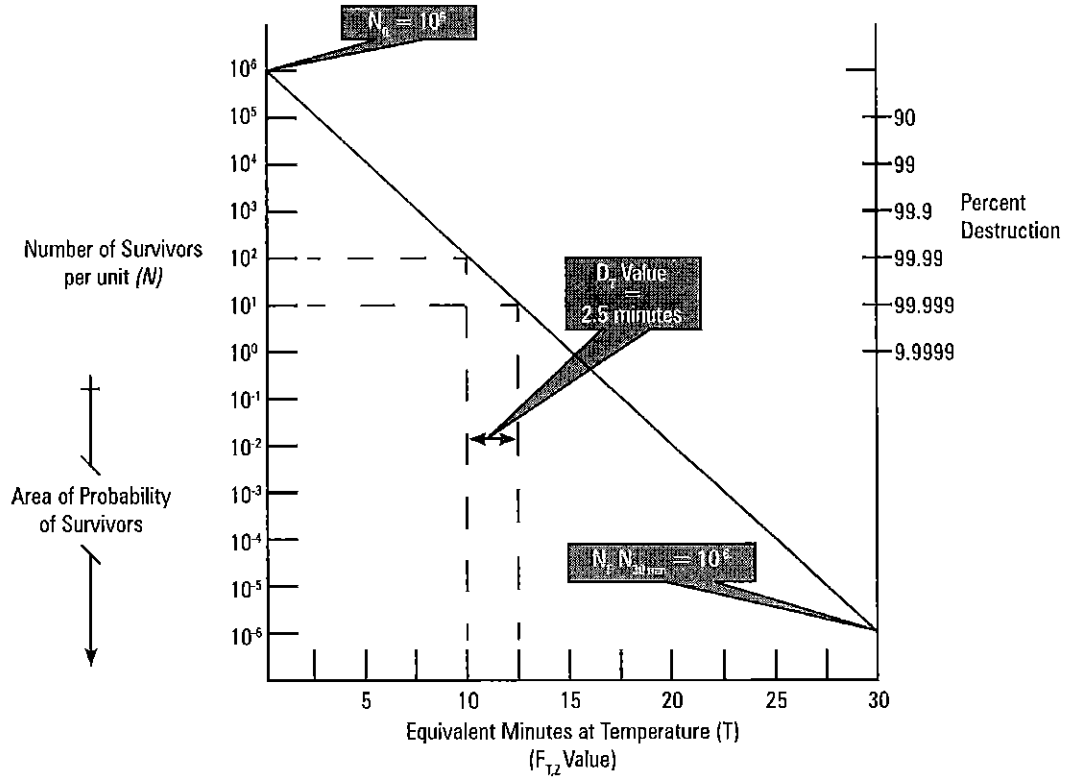


Table 3.2-1 Example Lethality Rates (F0 per Minute) at Various Process Temperatures

°C	F_0 Per Minute
100.0	0.008
105.0	0.025
110.0	0.078
115.0	0.245
120.0	0.776
121.1	1.000
125.0	2.455
130.0	7.762
135.0	24.547

Steam generation and distribution infrastructure should be qualified to demonstrate that the steam is suitable for its intended use (e.g., Pure Steam for product contact surfaces). The semi-logarithmic model of inactivation of microorganisms for saturated steam processes assumes steam is saturated (does not exhibit superheat) and is free from non-condensable gases. Wet steam, superheated steam, and steam containing non-condensable gases have the potential to adversely affect the lethality rate in the sterilization/sanitization processes. The quantities of residual air and condensate that may be present in SIP processes is likely to be in excess of those that could be found in the steam supply. For this reason, point-of-use steam quality testing (e.g., superheat, non-condensable gases, and dryness) is

typically performed on the main steam header branch to the equipment that is being steamed. A risk based assessment should be used to determine the number of point-of-use locations to test in order to best represent the steam supply system.

Figure 3.2-2 illustrates saturated steam conditions (pressure and temperature), which maximize the heat transfer during steaming. Deviation from the saturation line due to air and condensate in the system or superheat in the supplied steam will limit the steam's effectiveness. **Figure 3.2-3** depicts the impact of air trapped inside equipment being steamed in relation to Dalton's Law of Partial Pressure.

Figure 3.2-2 Optimal Heat Transfer Curve

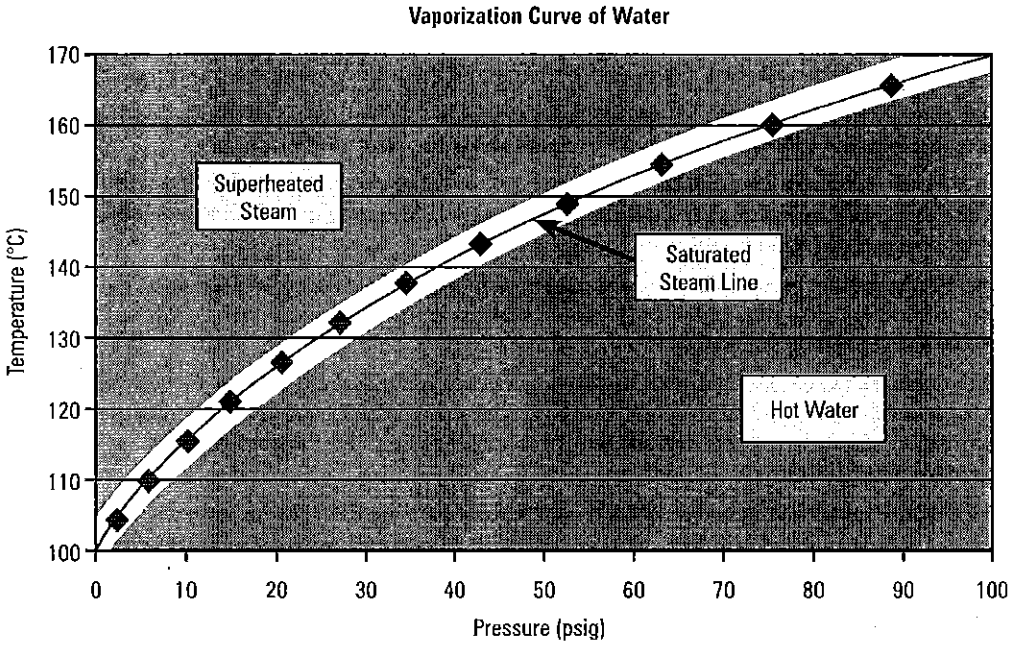
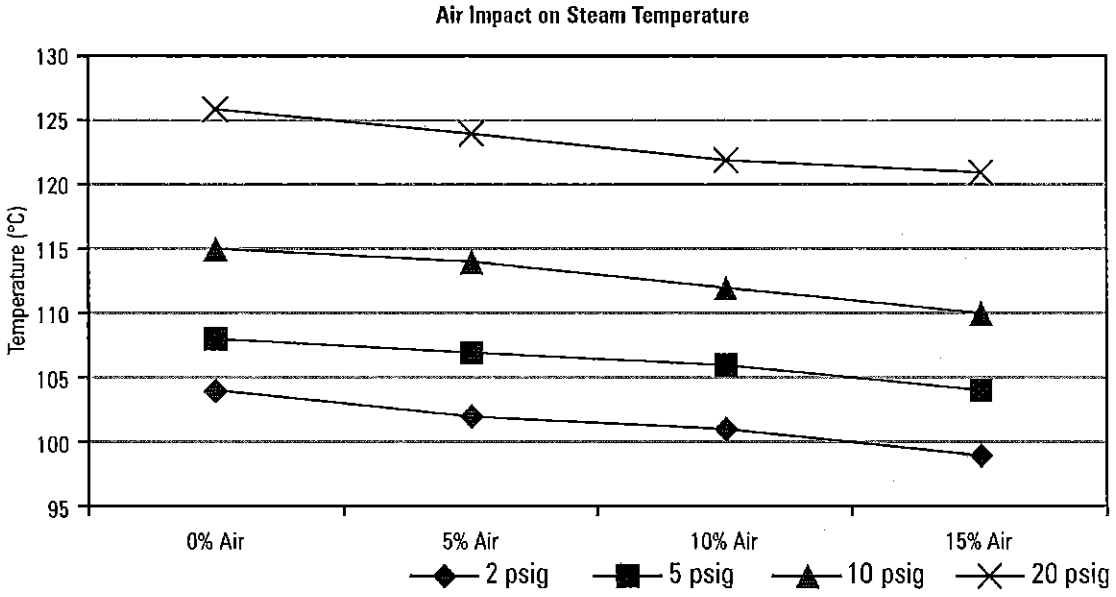


Figure 3.2-3 Effect of Trapped Air on Steam Temperature



4.0 System Design

The design of equipment should take into account SIP to ensure that the system can be steamed effectively and efficiently. This section provides guidance by offering information on what to consider (e.g., equipment design, level of automation, ongoing monitoring) prior to designing an SIP process. A risk assessment may direct effective use of resources during system design.

Overall system SIP sanitization/sterilization can be achieved via two methods. Simple systems inclusive of every possible attachment are typically steamed as a whole in one SIP cycle. Complex systems are frequently steamed via separate SIP cycles. This multi-cycle method can also include the attachment of previously autoclaved equipment to a sterile system.

4.1 Planning for Design of SIP Cycle

The boundary of the system(s) to be steamed (e.g., process equipment, feed and transfer lines) should be defined and documented (such as through process and instrument drawings) to ensure a successful cycle. Consider the following points after establishing the steaming boundary:

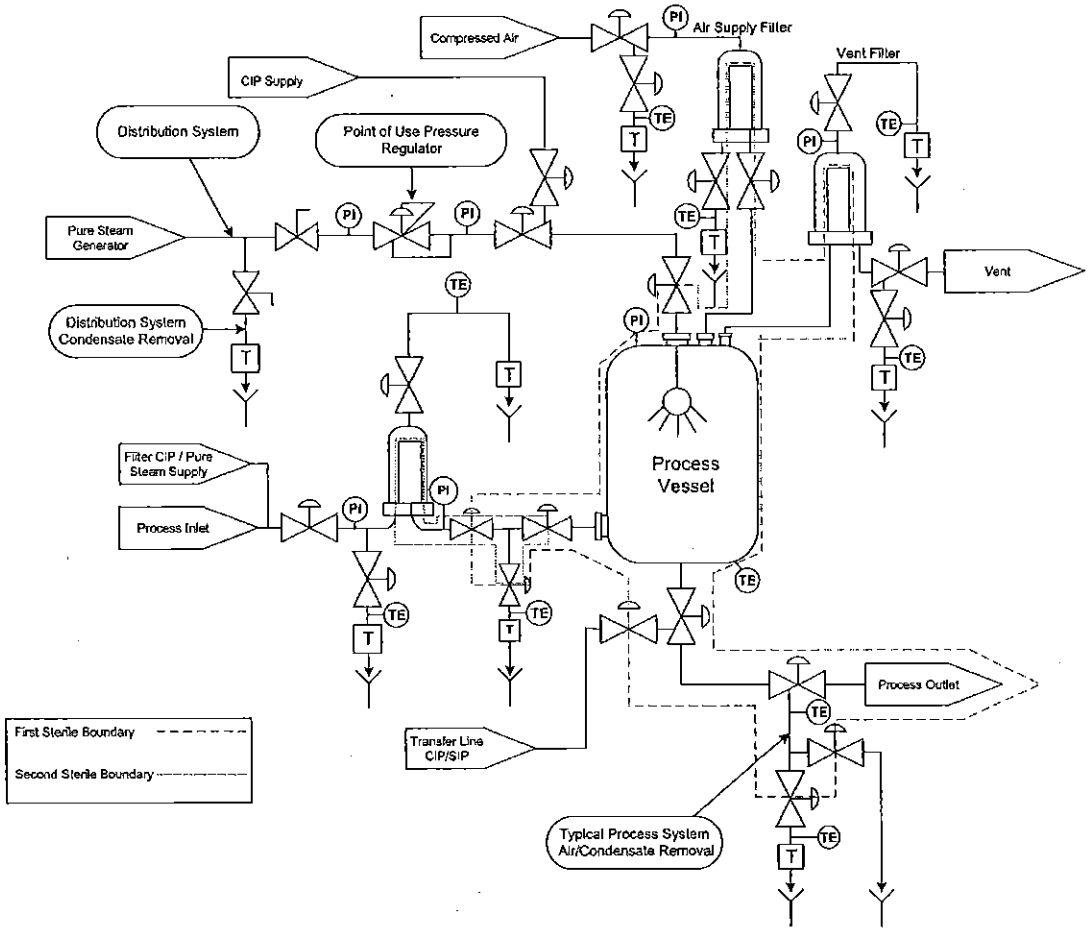
- An SIP cycle design must be capable of steaming all internal surfaces of the system within the boundary for a defined temperature and time. It must also be able to cool down the system within a timeframe which meets the system owners' business needs.
- The SIP cycle must provide delivery and penetration of saturated steam at predefined temperatures to all internal surfaces. This requirement incorporates design aspects associated with heat transfer, steam supply, and air and condensate removal.
- The system should be designed to ensure adequate heat transfer to the system so the heat delivered by steam to the system's internal surfaces (conduction) is greater than the heat lost from the system to the environment (convection). Steam system delivery capacity must match or exceed the system requirement for attaining and maintaining sterilization conditions.
- The system should be designed so air and condensate is not trapped in any location where steam is intended to penetrate. Saturated steam must be in contact with the targeted surface for the duration of the exposure phase, or the efficacy of the cycle will be compromised. Ideally, piping systems and vessels are designed so air and condensate is easily purged out of areas such as deadlegs and filter housings through bleed valves or steam traps. Manually operated SIP cycles will need detailed instructions within procedures to ensure this is accomplished each time in a repeatable manner.
- The SIP cycle must provide measurement, control, and monitoring of system temperature and pressure during the cycle hot phases (heat-up and exposure), and of pressure during the cool-down and hold phases. To enhance condensate removal, pipe slopes should be maximized wherever possible. A typical piping design specification would reference a minimum slope of a 1/8" per foot of pipe.
- Appropriate instrumentation, controls, and monitoring systems must be installed to enable and ensure the delivery and control of saturated steam. Instrumentation should be of the appropriate range and sensitivity to monitor and control temperature and pressure within the targeted value ranges. Critical locations within the sterile boundary should be monitored during each phase of the SIP cycle.
- SIP cycle design should ensure that temperature and/or pressure limitations of the process are not exceeded (such as for filters or elastomers).
- Access points should be provided for insertion of validation biological indicators and temperature sensors at potential worst-case locations (e.g. low points, high points, filter housings).

4.2 Equipment Design Considerations

Similar to other processes, such as clean in place (CIP), SIP equipment design should be integrated into the manufacturing process design and operations *(12)*. Material used for construction and surface finish should be defined and documented for all product contact parts. Accordingly, the surface finish should be specified based on cleaning performance requirements to minimize product or microorganism adhesion.

The system shown in **Figure 4.2-1** is an example of a process tank with a single feed that is delivered aseptically via a sterilizing-grade filter. Two sterile boundaries have been identified in the drawing below as an example. The two boundaries can be steamed separately, or as one SIP circuit. The tank and the filters (feed and vent) are equipped with steam traps to ensure efficient and effective condensate removal during heat up and exposure. The tank trap should be sized to handle the large condensate load as well as temperature control during exposure to ensure a consistent SIP cycle. Temperature elements are placed at SIP boundaries and potential cold spots. The temperature elements provide real time confirmation that the SIP is successful every time it is run.

Figure 4.2-1 Example of Steam Distribution and Process Tank Layout



Legend for Drawing: T = steam trap, TE = Temperature Element, PI= Pressure Indicator

Since systems requiring SIP are often the same as those cleaned via CIP, materials of construction should be selected by considering not only temperature limitations, but potential chemical interactions encountered in both processes. Examples requiring temperature consideration include: process equipment, instruments, and connection gaskets that are in contact with steam. Alternative materials of construction (e.g., high performance alloys, glass, and titanium) may be considered if stainless steel is incompatible with the process.

Equipment design considerations are typically recorded in the user requirements documentation. Risk management tools may be used to facilitate design criteria *(3,6,13,14)*. Consider the following points when designing an SIP system:

Steam Consumption

The steam supply capacity should be designed/evaluated to ensure adequate steam supply for the SIP system(s), with the maximum peak demand during the free-steam heat-up phase. Consideration should also be given to steam supply load to ensure adequate capacity for all uses (e.g., SIP, autoclave, and humidification).

Good engineering practices for steam system design must be followed when selecting the pressure reduction valve. Pressure control should be sufficiently robust to minimize fluctuations in steam pressure delivered to the target SIP system(s).

Note: Steam equipment manufacturers should be consulted for piping design and sizing for steam and condensate requirements as each type of equipment may require different configurations.

Gas Consumption

The gas flow capacity piped to the system should be sufficient to dry and pressurize the system post-SIP to maintain system integrity. Insufficient gas flow may also result in extended cool-down time.

Filter Considerations

Filter and filter housing sizes and materials of construction should be suitable for the intended use and able to withstand SIP *(15)*. The peak airflow demand for air filters usually occurs during the SIP cooling phase.

Filter housing configurations and bleed valves should allow the filter core and housing to drain condensate. Housing options include single or multiple cartridge designs in T- or inline-styles. Vent filter housings may be heated (e.g., via steam jacket or electric blanket) to minimize condensation during use *(16)*.

To ensure condensate and air removal, filter housings frequently have high point vents and should have low point drains and be positioned with the core opening down. Careful consideration should be given to materials of construction for disposable filter capsules, since most capsule shells are not compatible with SIP operating conditions. Consideration should also be given to the selection of filter housings that comply with applicable pressure vessel codes.

Steaming conditions should be reviewed to ensure they are within the filter vendor's recommended parameters. The permissible pressure drop across the filter under SIP temperatures is significantly lower than under ambient temperature conditions and less in the reverse rather than the forward direction. Differential pressure limits are temperature dependent and should be considered when determining the steam path. The pressure differential should be monitored and/or controlled during SIP. Post-SIP filter integrity testing provides assurance that the filter has not been damaged during the SIP cycle, but there are some disadvantages to performing pre-use, post-SIP integrity testing. Potential pros and cons of conducting pre-use integrity tests before or after the SIP process are shown in **Table 4.2-1.**

The timing and use of filter integrity tests should be conducted according to applicable regulatory requirements and through the use of risk assessment tools *(17)*.

Table 4.2-1 Comparison of Pros and Cons for When to Perform the Pre-Use Filter Integrity Test

Pre-SIP/Pre-Use Integrity Test (with filter element installed in the housing to be used for the process)		Post–SIP/Pre-use Integrity Test	
PRO	**CON**	**PRO**	**CON**
Confirms the filter is installed properly and without any damage, for example to the O-ring seal	Filters that may be damaged during SIP would not be detected until the post use integrity test	Reduced business risk for production lot/batch as filter integrity is confirmed after SIP	Typically requires system positive pressure to be relieved to conduct the integrity test (could lead to media and/or sterility failures and extended production down times)
Confirms the filter with the right pore size has been installed	Test is time consuming and redundant to the filter manufacturer's release test	Confirms the right filter has been installed correctly	Significant process down time to do testing and drying of the filter before use to avoid product dilution
Can be performed offline reducing down time of process and it may be easier to test serial filter configurations	Wetting fluid should have low bioburden to avoid additional endotoxin release after steam sterilization	Performed online	Requires downstream manipulation and redesign due to wetting agent removal and venting needs (increase of complexity and ingress points), unless product-wet integrity parameters are used
The filter wetting process removes filter extractables	Serial filter integrity testing is complex and requires sufficient user training	The filter wetting process removes any remaining filter extractables	Can require integrity testing equipment to be introduced into classified areas (e.g., aseptic filling isolator)
Filter can be dried to avoid damage during steam sterilization	Requires specific test area		Serial filter designs may require additional engineering to ensure that sterility between filters is not compromised e.g., sterile gas is required, a valve installed between filters, and a sterile vent on the second housing
Does not infringe on the sterile barrier (does not compromise sterility)	May not meet specific regional regulatory expectations		Does not add to drug safety, since the filter is tested post-use, but potentially increases risks to the sterile filtrate side

In some cases, filters will be wet prior to the SIP cycle but this should be avoided if possible. To prevent excessive pressure differentials when steaming wet filters, the steam may be introduced into both sides of the filter or slowly increased with vent and drain valves fully opened until steam flow is established. This approach may also be used to accommodate very large systems where the filter restricts steam passage to the extent that the system cannot be adequately steamed. An isolation valve

downstream of the liquid service filter housing will allow a second steam supply to operate independently if it remains closed until the pressure on the tank side of the valve equals the pressure on the filter side. There are several other approaches including steaming through the vent filter.

The use of serial sterilizing grade filters may require additional engineering to ensure that the sterility in between the filters is not compromised by:

a) Preventing negative pressure from forming in between the filters, especially if the filters are subjected to SIP in a wetted state, during post SIP cool-down.

(b) Maintaining sterile conditions if the filters are to be integrity tested post-SIP, prior to use.

Condensate Removal via Steam Trap and Flow Orifice Selection *(18-22)*

During the SIP process, it is critical to remove condensate from the system in order to maintain the system temperature. Steam trap and/or flow orifice locations should be designed to facilitate condensate removal during the process. Steam traps may also include the capability of discharging air to reduce system heat up times. Table 4.2-2 may aid in determining adequate air removal in the SIP system and piping systems. The ideal condition is to remove all the air, as shown in the column marked 0% (percentage of air in steam). For example, if the steam pressure is 15 psig, the temperature would be 121°C if all the air is removed.

Table 4.2-2 Temperature Reduction Caused by Air

Pressure bar (psig)	Temp. of Steam Mixed w/ Various Percentages of Air (by volume) °C (°F)			
	0%	10%	20%	30%
0.71 (10.3)	115.6 (240.1)	112.3 (234.3)	108.9 (228.0)	104.9 (220.9)
1.03 (15.0)	121.0 (249.8)	117.7 (243.8)	114.1 (237.3)	110.0 (230.0)
1.74 (25.3)	130.7 (267.3)	127.2 (261.0)	123.4 (254.1)	119.1 (246.4)
2.41 (35.0)	139.9 (283.8)	136.4 (277.5)	132.6 (270.6)	128.3 (262.9)
3.47 (50.3)	147.8 (298.0)	143.9 (291.0)	139.7 (283.5)	135.1 (275.1)

Values derived using Dalton's Law of Partial Pressure

Types of steam trap (as defined by International Standard ISO 6704:1982) *(23)*

There are three primary types of steam traps:

- Mechanical (operated by changes in fluid density) — This range of steam trap operates by sensing the difference in density between steam and condensate. These include 'ball float traps' and 'inverted bucket traps'. In the 'ball float trap', the ball rises in the presence of condensate, opening a valve which allows the denser condensate to pass. With the 'inverted bucket trap', the inverted bucket floats when steam reaches the trap and it rises to shut the valve. Both are essentially 'mechanical' in their method of operation.
- Thermostatic (operated by changes in fluid temperature) — The temperature of saturated steam is determined by its pressure. In the steam space, steam gives up its enthalpy of evaporation (heat), producing condensate at steam temperature. As a result of any further heat loss, the temperature of the condensate will fall. A thermostatic trap will pass condensate when this lower temperature is sensed. As steam reaches the trap, the temperature increases and the trap closes (e.g. bimetallic).
- Thermodynamic (operated by changes in fluid dynamics) — Thermodynamic steam traps rely partly on the formation of flash steam from condensate, e.g. thermodynamic disc.

Table 4.2-3 An example of considerations for steam trap selection

Feature	Mechanical		Thermodynamic	Thermostatic
	Inverted Bucket	Ball or Float w/ without Thermostatic Disc	Disc	Balanced Pressure Disc / Bimetallic
Sanitary Application	No	No	Yes	Yes
Method of Operation	Intermittent	Continuous	Intermittent	Intermittent
Energy Conservation (Time in service)	Excellent	Good	Poor	Fair
Resistance to Wear	Excellent	Good	Poor	Fair
Corrosion Resistance	Excellent	Good	Excellent	Good
Resistance to Hydraulic Shock	Excellent	Poor	Excellent	Fair
Vents Air and CO_2 at Steam Temperature	Yes	No	No	No
Ability to Vent Air at Very Low Pressure	Poor	Excellent	N/R	Good
Ability to Handle Startup Air Loads	Fair	Excellent	Poor	Excellent
Operation Against Back Pressure	Excellent	Excellent	Poor	Excellent
Resistance to Damage from Freezing	Good	Poor	Good	Good
Ability to Purge System	Excellent	Fair	Excellent	Good
Performance on Very Light Loads	Excellent	Excellent	Poor	Excellent
Responsiveness to Pools of Condensate	Immediate	Immediate	Delayed	Delayed
Ability to Handle Dirt	Excellent	Poor	Poor	Fair
Comparative Physical Size	Large	Large	Small	Small
Ability to Handle "Flash Steam"	Fair	Poor	Poor	Poor
Mechanical Failure (Open-Closed)	Open	Closed	Open	Open or Closed

4.3 SIP System Control and Monitoring

4.3.1 Automation

Effective SIP operations may be conducted irrespective of the extent of process automation. The degree of automation for control and monitoring is a decision that should be made based on the criticality of the application and the resources available for commitment to control and monitor tasks. With thoughtful and proper implementation, increased levels of automation can benefit more complex SIP processes. However, if the required SIP operations are not operationally intensive, greater economies may be realized with reduced levels of automation. Organizations should evaluate the level of automation desired while considering the intent, complexity, and scope of the SIP process. Determination of the level of automation desired may be facilitated through the use of risk assessment tools.

A high level of automation allows less operator oversight, repeatable compliance to procedures, less variability post-implementation and may also facilitate use of process analytical technologies (PAT). Fully automated systems involve more work initially to assure that sterilization or sanitization is being performed correctly and as intended each and every time it is run in terms of system design, software development, and qualification. Conversely, with lesser degrees of automation, process monitoring becomes more dependent on operator training, documentation, and procedures which are well known to lead to possible errors.

The implications of three different levels of automation are described in **Table 4.3-1.**

Table 4.3-1 Examples of Levels of Automation

Level	Software Development	Qualification	Process Variability	Resource Requirements
Automated	Extensive	Extensive	Repeatable	Automation
Semi-automated	Variable	Variable	Variable	Automation and Operations
Manual	None	Minimal	Operator dependent	Operations

4.3.2 Controlling the SIP Cycle

The SIP cycle may be controlled using either a time and temperature strategy, or a calculated F_0 value as the process variable. The time and temperature approach relies on durations empirically determined during cycle development, and usually depends upon the successful, uninterrupted exposure of the system to a relatively constant temperature. Should the temperature (monitored at critical locations throughout the system) drop below the designated minimum cycle temperature, the timer may be stopped or reset. Alternatively, controlling based on F_0 allows for determination of an SIP cycle endpoint by taking into account equivalent physical lethality across multiple temperatures.

For saturated steam, there is a direct correlation between temperature and pressure. Either variable can be used for feedback control, but in practice, this information is used to control introduction of steam either through dedicated pressure control valves or through the use of existing diaphragm valves as described in **Section 4.2.**

Two keys to successful control are 1) proper selection and sizing of the steam supply and 2) fixing the pressure via a locked regulator immediately upstream of the equipment. The pressure is typically set slightly above the expected operating pressure to account for systemic pressure losses. Maintenance of steam pressure should be ensured once the cycle is developed.

Another effective method of SIP control is modulating pressure or temperature control valves to control steam during SIP. Depending on the design, either the valve toggle method or control valve method can have economic advantages. If vessel piping and valve placement are designed properly, most or all of the piping associated with the vessel may be steamed along with the vessel, rather than having separate cycles for the piping.

4.3.3 SIP for Portable Vessels

Similar methodologies may be applied to SIP of portable vessels by designing a CIP/SIP station with automatic sanitary diaphragm valves in the CIP/steam supply. A single station can service tanks of various sizes via individual recipes that operate the valves for each tank size or design. Correct timing for each tank is determined during cycle development.

4.3.4 Semi-Manual and Manual SIP Operations

While increasing the level of automation is recommended for enhanced control and cycle reproducibility, it is not uncommon to encounter manually operated systems. The prerequisites and design principles considered for automated systems also apply to manual SIP systems. Reducing the extent of automation ranges from semi-automated systems that are manually initiated but automatically controlled, to manually operated systems controlled through hand operated valves and pressure and temperature indicators, to those with limited to no instrumentation. In such cases, through the use of appropriate documentation and well defined operating procedures, manually operated SIP processes can be conducted with a reasonable level of reliability.

Manually operated SIP cycles require many of the same safety, risk assessment, and validation considerations expected of automated systems. Process monitoring logistics, the physical limitations of operators, and increased process variability should be taken into consideration if a manually operated system and SIP cycle are to be designed and operated. Without automation, process interlocks must be proceduralized through SOPs, signage, and highly visible warnings.

At a minimum, systems should be designed with appropriate isolation valves, pressure regulators at the steam source, and a means of monitoring the pressure within close proximity to the associated manual valves. As with any system, steam traps should be installed at the low points to remove condensate. If internal temperature indicators are not installed, surface mounted probes, melt sticks, or sensors can be used to confirm that the external temperature is greater than the specified minimum. Using a combination of both pressure and temperature measurement in close proximity to each other ensures that saturated steam conditions exist (refer to **Figure 3.2-2**).

For validation of the cycle, supplemental temperature measurement techniques are recommended. Further, since the potential for greater process variability exists with a manually controlled SIP cycle, an overkill sterilization approach is preferred.

5.0 Cycle Development Considerations

The SIP cycle development objective is a robust process that is repeatable and consistently meets user requirement specifications.

The user should assess the intended application and purpose of the system and consider regulatory expectations for systems when claiming sterilization versus sanitization. The overkill method (F_{BIO} and F_{PHYS} ≥12 min) is not required when claiming sanitization of a system.

Operational parameters and their classification (e.g., "critical" or "key") are determined during cycle development. A risk assessment based on intended use may be useful in determining these parameters.

Risk assessments of SIP processes can be implemented at identified control points through a review of the unit operations and various phases of the process. Operations where the opportunity and likelihood of product contamination is low may be focused on the equipment, instrumentation, and procedures supporting microbial control through sanitization.

Each SIP cycle can be divided into a series of steps or phases, depending on the intended use and risk assessment of the SIP cycle. The phases of a typical SIP cycle may include:

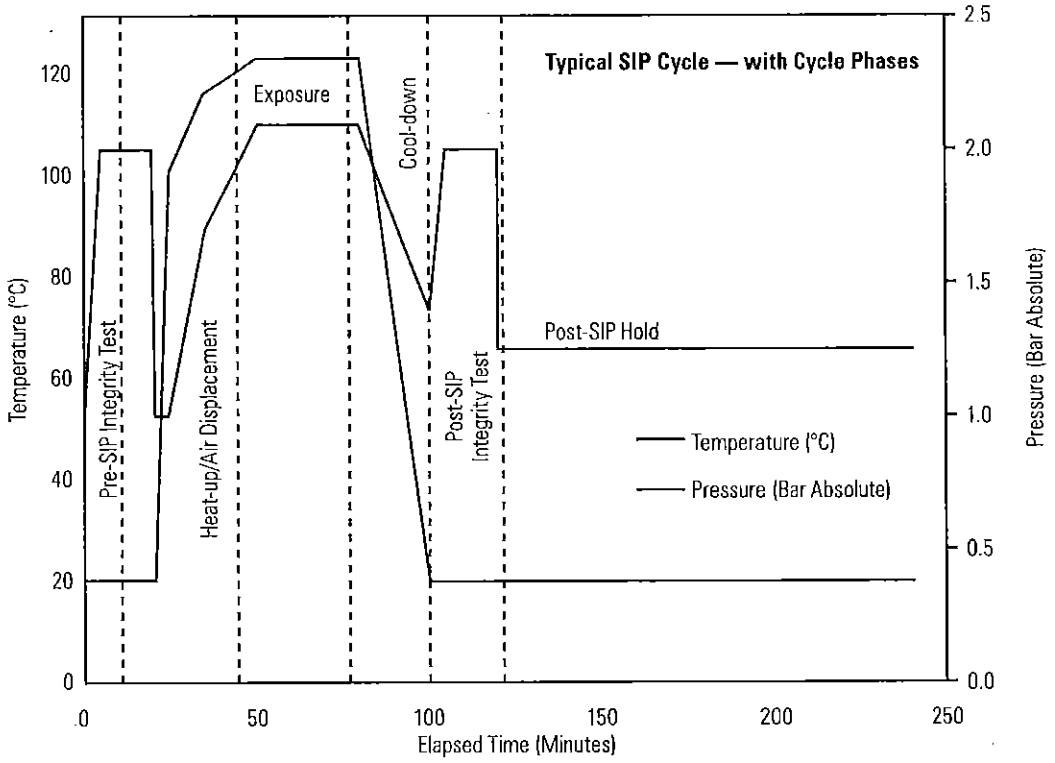
- Pre-SIP system integrity test
- Heat up/Air displacement
- Exposure/Dwell time
- Cooldown/Steam removal/Drying
- Post-SIP system integrity test

Each phase has its own intended objective. **Table 5.0-1** lists the phases of a typical SIP cycle, and briefly describes the objectives of each phase. This table is intended to provide a brief illustration of phases that could be encountered during a typical SIP cycle and is not all-inclusive. Specific parameters that are controlled during each phase are discussed further in **Table 5.2-1. Figure 5.0-1** provides an illustration of the phases of a typical SIP cycle.

Table 5.0-1 Typical Steps and Phases of an SIP Cycle with Relevant Objectives

Phase	Objectives
Pre-SIP System Integrity Test (optional)	Determine if the system will meet user-defined requirements for operator safety and system integrity.
Heat Up/Air Displacement Phase	Displace air and heat up the system via introduction of steam at high points in the system, forcing air out through low points in the system (gravity displacement) or by single or repeated evacuation of the system followed by introduction of steam to a predetermined pressure set point (vacuum/steam pulses).
	Condensate removal
	Keep filter differential pressures within defined limits
Exposure/Dwell Time Phase	Maintain the system at a predefined temperature and pressure for the time required to achieve the desired minimum lethality.
	Condensate removal
	Keep filter differential pressures within defined limits
Cool-down/Steam Removal/ Drying Phase	Cool and dry the system to user-defined levels; typically uses filtered gases (e.g., compressed air or nitrogen) to cool, dry, and maintain pressure on the system.
	Keep filter differential pressures within defined limits
Post-SIP System Integrity Test (optional)	Determine if the system will meet user-defined requirements for operator safety and system integrity.
Post-SIP Hold (optional)	Maintains the system integrity between completion of SIP and use of the system. Post-SIP Hold usually uses compressed gases to pressurize the system. This phase is recommended for critical/ sterile applications.

Figure 5.0-1 Example of SIP Cycle Phases



5.1 Use of Risk Management during Development

Risk management techniques may be used in cycle development to ensure that product risks are appropriately minimized and that a risk-based level of cycle development is performed. Appendix A provides more details on the application of a risk based approach for SIP systems.

5.1.1 Risk Assessment

Risk assessment should look at the overall risk to the product and, if appropriate, provide a detailed risk review of the system to determine potential points of failure and mitigation methods. The detailed risk review, if needed, should build upon the engineering design review. Items to consider as part of the risk assessment may include:

- The processing step in which the system being steamed in place is involved. For example, a system used to prepare buffers or reagents for early manufacturing steps for an API may present a relatively low risk, whereas a system involved in final fill and finish of an aseptically filled product may present a very high risk
- The potential for product impact based on the degree of product contact (non-contact, indirect contact, or direct contact) with the system being steamed-in-place
- The complexity of the system undergoing SIP
- The severity of failure and probability of detection of an SIP failure. These two factors are related to their location in the processing stream, degree of product contact, and system complexity. The severity of failure should always be considered at the highest value for an aseptically filled product (see PDA Technical Report No. 44) *(3)*.

5.1.2 Risk Mitigation

Risk mitigation should be based on the level of risk tolerance determined during the risk assessment. Depending on the level of risk tolerance, mitigation efforts may include:

- Pre-SIP and pre-use system integrity tests
- Additional monitoring points and biological indicators (if any) used during cycle development
- Additional safety factors built into the SIP cycle
- Additional alarm points and/or control limits for temperature and pressure.

5.1.3 Cycle Development Data

The amount of cycle development data needed to determine the key and critical parameter levels should be commensurate with the overall system risk. A high-risk system should have extensive data before proceeding to qualification; Other than routine commissioning tests, a low-risk system may need little or no cycle development data to proceed to qualification.

5.1.4 Testing

Some routine tests that might be performed during cycle development:

- Perform temperature mapping to determine cold and hot spots in the system.
- Verify that excessive condensate is not building up at any points (e.g., low point drains, filter housings) in the system. The use of thermal imaging instruments can be used for this. Adjust/modify steam traps and system bleeds as necessary.
- Verify that valve timing and sequence of operations are as described in the automation control systems' detailed design specification.
- Determine time and temperature required to achieve an appropriate F_{PHYS} value.
- Biological challenge, to ensure that an adequate F_{BIO} is achieved before proceeding to qualification of high-risk systems.

5.2 Cycle Parameter Determination

Critical operating parameters for SIP cycles include time, temperature, and pressure. These parameters are manipulated to achieve the desired degree of sterilization or sanitization. Time, temperature, and pressure parameters should be determined for each phase of the SIP cycle (see **Table 5.0-1** for typical cycle phases). **Table 5.2-1** provides the operating procedure for each phase, as well as a brief description of the time, temperature, and pressure set points.

Table 5.2-1 also describes time, temperature, and pressure parameters for the optional System Integrity Test that may be performed before and/or after SIP of a system. Although there are several System Integrity Test methods (e.g., Pressure Hold, Mass Flow, and Tracer Gas), **Table 5.2-1** only includes the most common System Integrity Test: the Pressure Hold Test.

Table 5.2-1 Example of Typical SIP Cycle Operational Parameters

SIP Step	Parameters	Considerations
Pre-SIP System Integrity Test (optional)		
Pressure Hold Test	Procedure	The system pressure is brought to a predetermined set point. All valves are shut off and the system is held for a predetermined amount of time. Pressure drop over that time is measured.
	Time	There are typically three time periods defined for a pressure hold test: pressurization time, stabilization time, and hold time. Each time period during a pressure hold test should be monitored and controlled. Pressurization Time is the time required to pressurize the system to the operating pressure for the pressure hold test. It is dependent on the volume of the system and the capacity (liters per minute at a given pressure drop) of the compressed gas supply. Most procedures set an upper limit for pressurization time, as extended pressurization time may indicate a significant loss of system integrity. Stabilization Time is a brief period—typically around 5 minutes—allowing the pressure within the system to equilibrate and any temperature increases due to pressurization of the system to stabilize. Hold Time is defined by system size, system pressure, and the size of leak to be detected. Hold times should be minimized to avoid excessive temperature fluctuations.
	Temperature	The system temperature is monitored (typically at ambient temperature) throughout the test to minimize false positives ("virtual leaks") or false negatives caused by temperature fluctuations. **NOTE:** Gay-Lussac's Law: The pressure of a fixed amount of gas at a fixed volume is directly proportional to its temperature in degrees Kelvin. ($P_1/T_1 = P_2/T_2$). When specifying the temperature for a pressure hold test, system characteristics such as jacketing, insulation, measuring instrument accuracy and ambient temperature of the surrounding area and the compressed gas supply should be considered.
	Pressure	The system is typically pressurized to a pressure less than or equal to the system pressure during the SIP cycle. The pressure hold period begins after pressurization and the system pressure is allowed to stabilize for a few minutes. The allowable pressure loss during the pressure hold period is based on several factors including system volume, system design, and system criticality. Except during initial pressurization, system pressure is monitored, but not controlled.
Heat-Up/Air Displacement (Gravity Displacement or Vacuum/Steam Pulses)		
Gravity Displacement Method (typical method used)	Procedure	Steam is introduced into the high points of the system. Density differences cause the heated steam to displace trapped air in the system downward. Steam traps and constant bleeds at low points in the system allow the trapped air and condensate to exit. In large systems, gravity displacement may occur as a two-phase process: Phase 1: Valves in the system (system low points and farthest points from steam inputs) may be opened to allow for a rapid displacement of air and heat-up of the system. This phase typically operates at or near atmospheric pressure. Phase 2: Valves allowing for a rapid steam purge are closed, forcing the steam to exit from the traps and/or constant bleeds. This allows the system to pressurize and heat to the final system temperature and pressure.
	Time	Time is typically monitored. The time required for gravity displacement is determined empirically by temperature mapping (see section 5.4). The endpoint of gravity displacement may be determined by temperature at one or more low points in the system.
	Temperature	Temperature should be monitored during the displacement process. The end point of gravity displacement is typically determined by comparing the temperature at the control probe to the pressure at the same point.
	Pressure	Pressure should be regulated and monitored during the heat-up air displacement phase. By the end of the gravity displacement phase, the system pressure should be approximately equivalent (within predetermined limits) to the pressure of saturated steam at the operating temperature of the system (see **Table 4.2.2**). The accuracy of system instrumentation and the location of the pressure and temperature measurements should be taken into account when setting limits for the system temperature/pressure relationship.

SIP Step	Parameters	Considerations
Vacuum/Steam Pulse Method	Procedure	Air is mechanically evacuated from the system to a predetermined vacuum setpoint. Steam is then injected to a predetermined setpoint to heat the system. The evacuation or series of evacuations (vacuum pulses) followed by steam injections (steam pulses) are used to remove air and heat up the system. Steam traps and trap bypass valves are used to remove condensate from the system. **Note:** trap and bypass valves should only be opened when the system is under positive pressure.
	Time	Time may be monitored or controlled to assure air displacement. The following parameters should be documented: • Pulse times • Proportional valve settings • Multiple vacuum/steam pulses as required
	Temperature	Temperature should be monitored during air displacement and used during cycle development to determine the number of vacuum/steam pulses required.
	Pressure	Pressure is typically controlled during a vacuum/steam pulse cycle. The depth and number of vacuum pulses, steam pulse pressure, and number of steam pulses correlate directly to the amount of air removed during a vacuum/steam pulse air displacement cycle. See PDA Technical Report No. 1, **Section 4.3.1.1** for further discussion of vacuum/steam pulses *(1)*.
Exposure	Procedure	The system temperature is brought to a predetermined temperature set point after completion of the heat-up/air displacement phase. Exposure timing begins once the predetermined temperature set point is reached. Exposure timing continues until the minimum required time is reached.
	Time	Time is monitored and controlled. Exposure time set point should be sufficient to achieve the desired degree of sterilization or sanitization. **Note:** some systems may use F_0 control, in which the exposure period is terminated once a predetermined F_0 is achieved. Time to achieve the desired F_0 should be monitored for F_0 control cycles.
	Temperature	Temperature is monitored and controlled. Exposure temperature is typically ≥121°C, but may be different based on system and cycle design parameters. System time and temperature should be adjusted as necessary to achieve the minimum desired level of sanitization or sterilization.
	Pressure	Pressure should be monitored to evaluate the temperature/pressure relationship related to saturated steam. The accuracy of system instrumentation and the location of the pressure and temperature measurements should be taken into account when setting limits for the system temperature/pressure relationship. See **Table 4.2-2** for more information.
Steam Removal/ Cool-Down/Drying	Procedure	Steam supply to the system is shut off and compressed gas is supplied to the system to cool down and dry the system. Positive pressure is maintained in the system during cool-down and drying. For a description of an alternate method for a system drying under vacuum, see **Section 8.5.11.**
	Time	Time may be monitored and controlled to determine the length of this phase.
	Temperature	Temperature is monitored to ensure system is cooled to a predetermined temperature before use.
	Pressure	Pressure should be monitored to ensure system pressure remains within predefined limits.
Post SIP System Integrity Test (optional)	See Pre-SIP System Integrity Test	

5.3 Filter Cycle Development Considerations

It is important to control temperature and differential pressure as well as minimize condensate build-up during SIP to maintain filter integrity. It is crucial to ensure that filters are used in accordance with the manufacturers' technical specifications and recommendations.

5.3.1 Wet Filters

Maintaining low differential pressure across the filters will be largely dependent on whether the membrane is wet or dry. Wet membranes hold liquid in the pores by capillary forces. Liquid in the pores is a barrier to gas flow. Steam introduced on one side of the filter cannot easily penetrate and pressurize the opposite side of the filter if a membrane is wet, resulting in buildup of differential pressure. A filter may be wetted prior to SIP due to:

- Performance of a pre-use integrity test on the filter, which requires the filter to be completely wetted
- Filter type (some require wetting prior to heat sterilization)

There are three strategies to avoid differential pressure across a filter that has been wetted prior to the SIP cycle:

- Blow-down—Filter drying may be accomplished by applying air pressure in the forward direction above the filter bubble point to establish bulk flow through the filter. Sufficient time for bulk gas flow must be allowed to ensure that the filter is dry enough to readily allow steam passage without excessive pressure differential.
- Controlled introduction of steam to the system—Steam is introduced slowly from the upstream side of the filter during the heat up phase until a rise in pressure is observed downstream of the filter. The time required to heat the liquid in the pores is dependent on steam flow over the surface of the filter. Steam flow over the surface is facilitated by opening vents and drains during steam introduction.
- Use of dual steam sources (upstream/downstream)—Careful consideration should be given to sequencing of valve openings and closings to ensure air displacement/steam penetration of the filter membrane and prevention of vacuum conditions post-SIP.

If steam is introduced to the core side of the wet filter (reverse SIP), steam flow over the surface is greatly reduced because it is not possible to vent the high point of the cartridge core. The system is essentially dead-ended. Under these conditions it can take a long time to heat and vaporize the liquid in the pores. High differential pressure conditions may result during this time. While the reverse SIP of wet filters is not recommended, the reverse steaming of dry, hydrophobic filters is an acceptable practice, as long as the filter manufacturer's maximum differential pressure rating is not exceeded.

Correct valve sequencing and properly located low-point condensate drains can ensure the filter does not become wet unintentionally during SIP. Isolating the filter and heating the piping prior to introducing steam to the filter assembly is an effective strategy for avoiding excess condensate introduction to the filter during the heat up phase.

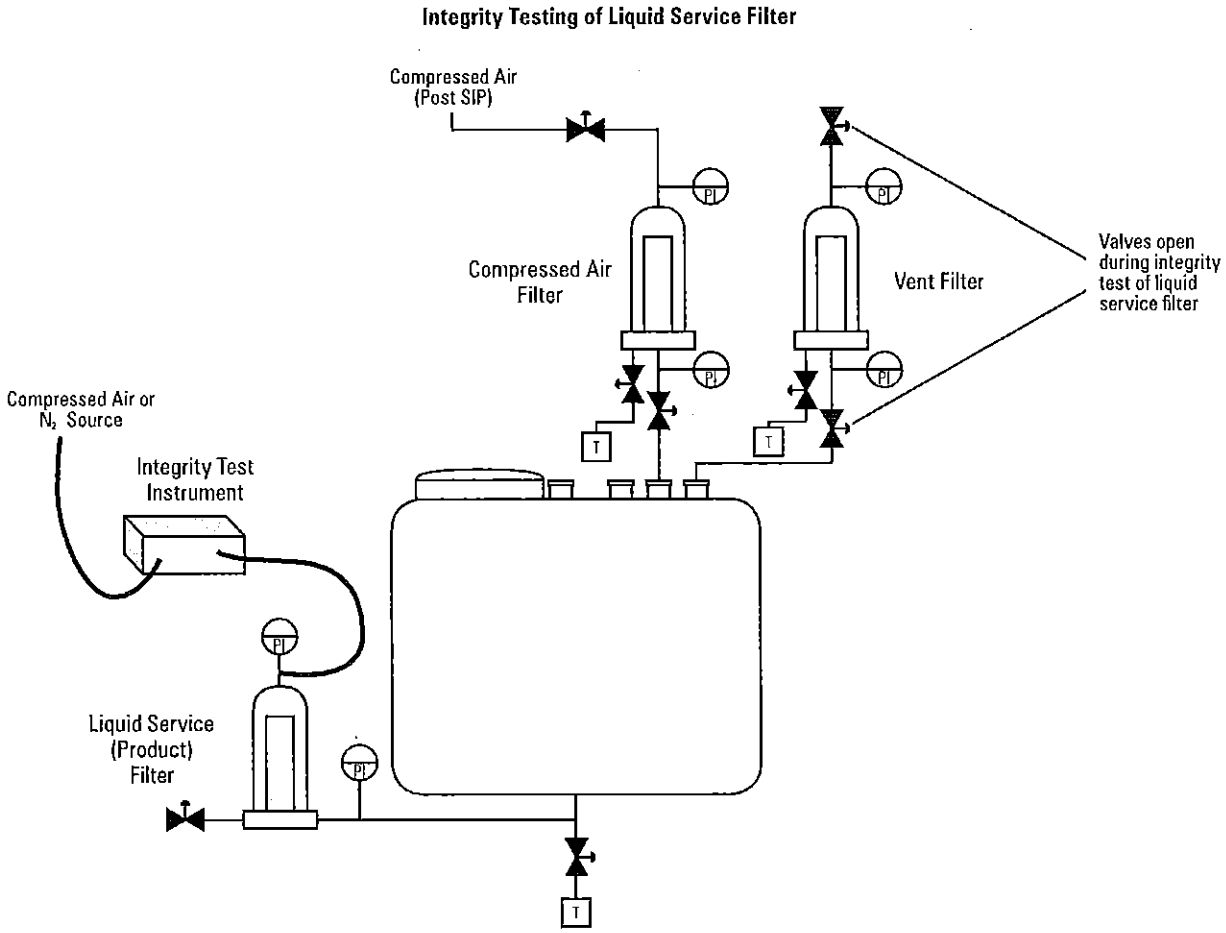
Filters wet with alcohol solutions should be dried via blow-down or oven prior to SIP to avoid potential chemical compatibility issues from hot alcohol exposure and to minimize ingression of alcohol vapor into the SIP system.

5.3.2 Filter Integrity Tests *(16,17)*

Suitable integrity test of the filter cartridge may be performed pre-use and must be performed post-use for sterile applications. Regional expectations vary on the application of pre-use integrity tests. Downstream sterility must be maintained post sterilization. The downstream side of the filters tested by Bubble Point or Forward/Diffusive Flow in situ on a tank or system should be vented to atmosphere through a sterilized vent filter as depicted in **Figure 5.3.2-1.** Off-line integrity testing of filters

pre-SIP and post-use is acceptable, however, the installation integrity check is lost unless the housing with the filter is removed and tested. Refer to **Section 4.2** for more details related to filters and filter integrity testing considerations.

Figure 5.3.2-1 Example of In Situ Filter Integrity Test



5.4 Temperature Mapping

Temperature mapping is an iterative process with physical adjustments to the system or changes in SIP process sequences to optimize temperature distribution. These studies can identify locations for permanent monitoring devices and validation probes in areas of the SIP system that are the most difficult to heat and/or displace air. They are performed by placing temporary probes throughout the SIP system boundary to ensure the control probes effectively capture thermal behavior of the SIP system. Probe locations and the rationale for selecting each location should be documented. The number of temporary probes needed varies according to the complexity, criticality, and size of the system.

Ideally, temperature mapping should not be performed until after the system has been insulated, as applicable. Thermal imaging instruments may be used to identify slower-to-heat surfaces that could benefit from the installation of additional insulation. However, many systems have components such as filter housings or aseptic filling parts that cannot be insulated due to use of the system and or components.

6.0 Performance Qualification

Performance qualification (PQ) is the documented verification that the equipment and ancillary systems, as assembled, can perform effectively and reproducibly based on the approved process method and specifications. When or where these activities (OQ or PQ) are performed is up to the end user. Performance qualification may include the following:

- Physical qualification: will include temperature mapping runs to confirm that the temperature range requirements are met and that the minimum F_0, or time and temperature, is consistently achieved in the system.
- Biological qualification: conducted with appropriate microbiological challenges to confirm that the minimum F_{BIO} is consistently achieved in the system *(24)*.
- Bioburden control or sterile hold time studies onducted as required to demonstrate that the sanitized or sterilized system can be maintained in that condition for the desired length of time before use.
- Documented assessment and rationale for the selection of locations for the following:
  - Permanent temperature monitoring locations
  - Validation temperature monitoring locations
  - Biological indicator locations

Measurements of time, temperature, and pressure may be sufficient for SIP sanitization qualification. For SIP sterilization qualification, measurements of time, temperature, pressure, and biological indicator destruction are required to demonstrate consistency between physical and microbiological results. A safety margin of higher temperatures and/or extended exposure times may be built in for routine operational cycles to account for process, biological, and instrument variability. Temperature and time considerations should be included for heat labile items (e.g., filters, gaskets, tubing).

Multiple runs (typically 3) should be conducted to confirm reproducibility of the steam in place process for initial qualification.

The following activities should be completed and documented in accordance with company policy and current regulatory expectations prior to performance qualification:

- Procedures for the operation of the system(s) being qualified
- Qualification of utilities as needed for the steam in place process (e.g., steam and compressed gases with appropriate quality and capacity testing)
- Qualification of the system (e.g., design qualification, commissioning and/or installation qualification, and operational qualification) and calibration of critical instrumentation (e.g., control systems, monitoring devices, and alarms)
- Development of the SIP cycle parameters, including preliminary temperature mapping
- Training of personnel involved in performance qualification

6.1 Use of Risk Management during Qualification

Risk management tools can be used to determine the level of qualification required, develop specific acceptance criteria, and tactical considerations such as placement of temperature sensors and BIs. The level of qualification required typically parallels the amount of cycle development required. Qualification efforts can generally be divided into System/Equipment Qualification and Cycle Qualification.

6.1.1 System/Equipment Qualification

Depending on system/equipment risk level, qualification may range from documented factory and site acceptance tests (FAT/SAT) to detailed qualification protocols and test scripts. Typical items considered during system/equipment qualification include:

- Operation of traps and steam bleeds
- Operation of manual and automated valves
- Verification of valve sequencing
- Verification of defined system pipe slopes / system drainability
- Verification of calibration for critical instruments
- Verification of temperature within the system (the number of points to be verified depends on system risk)
- Verification of operation of controls, interlocks, and interfaces

Additional testing to consider for high-risk systems includes:

- Detailed temperature mapping throughout the system
- Execution of test scripts to ensure that the control system will maintain key and critical parameters under expected conditions
- Detailed verification of critical alarms
- Verification of system integrity, including filter integrity, after operating at time and temperature extremes (within a predefined operating range determined during cycle development)

6.1.2 Cycle Qualification

The level of qualification required for an SIP cycle should be based on the level of risk presented by the system being steamed in place. Items affected by system risk include:

- Use of grouping or bracketing for systems being steamed in place. Low-risk, low-complexity systems may use a bracketing approach, while high-risk, high-complexity systems may require each system to be qualified *(25)*.
- Temperature probes: High-risk systems will typically use more temperature probes during qualification than low-risk systems
- Biological indicators: High-risk systems will typically use more biological indicators at a higher challenge level than low-risk systems, which may even have no biological indicators.

6.2 Physical Qualification

The primary objective of the physical qualification component of SIP qualification is to obtain physical data confirming that the developed cycle consistently delivers the desired minimum lethality throughout the SIP system. The minimum lethality depends on the kind of SIP cycle used and on the desired degree of sanitization or sterility assurance.

6.2.1 Sanitization vs. Sterilization

The extent of qualification depends on the intent of the SIP cycle. SIP performed for the purpose of system sanitization may only require physical qualification. For systems that are sanitized, bioburden testing (via applicable sampling methods such as rinse water samples and swab sampling) should be performed as part of the physical qualification of the SIP process as applicable.

SIP performed for the purpose of system sterilization will require both physical and biological qualification. Physical and biological qualification should be performed simultaneously. The destruction of a biological challenge alone is not sufficient evidence of the suitability of a cycle. The biological challenge data should support the physical data and vice versa.

Initial qualification runs are typically performed in triplicate to ensure consistency. Fewer runs may be performed if a technical rationale is provided.

6.2.2 Temperature Mapping

The primary purpose of temperature mapping is to verify steam distribution throughout the system. Significant variation of temperatures during this study could indicate the presence of air or condensate at an insufficient temperature.

Temperature probes should be positioned in the cold spots identified during cycle development. The number and location of the temperature sensors depend on the size, layout, and complexity of the SIP system (e.g., a simple SIP system, such as a transfer line, may only have temperature mapping probes adjacent to the controlling temperature device) *(26)*. Placement of the temperature probes and rationale should be documented.

Probe insertion should be performed in a manner that does not impact the SIP process or impair any of the safety equipment/features of the process. For example, care should be taken to ensure that introduction of the probes into the system does not hinder steam flow or removal of condensate nor facilitate air removal. Typical temperature probe locations for an SIP system include:

- Downstream and/or within filter core
- Possible condensate accumulation locations (e.g., low points and the upstream side of filter housings)
- Upstream of steam traps
- Nozzles and high points where air may be difficult to displace
- Surfaces of large mass items (e.g., lyophilization shelves)
- Surfaces of uninsulated portions of the system being steamed
- System boundaries
- Deadlegs (condensate/air entrapment)
- Adjacent to temperature and/or pressure instruments

Care should be taken to ensure that the measurements accurately represent the system being measured when installing thermocouples. One consideration is that probe tips should be oriented to avoid erroneous measurements due to condensate droplet accumulation at the tip of the probe. **Figure 6.2.1-1** shows two adjacent probes with different orientations. The figure shows that condensate does not accumulate on the tip of TC 1 but does accumulate on the tip of TC 2. This insulates the probe and affects the temperature readings. **Figure 6.2.2-2** depicts data resulting from installing thermocouples oriented as illustrated in **Figure 6.2.2-1.**

Figure 6.2.2-1 Example of Probe Orientation

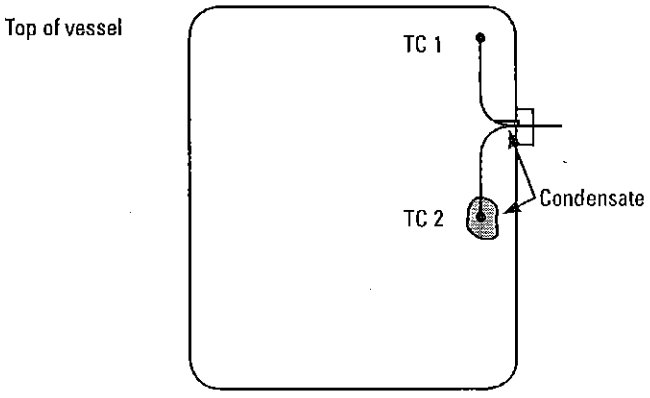
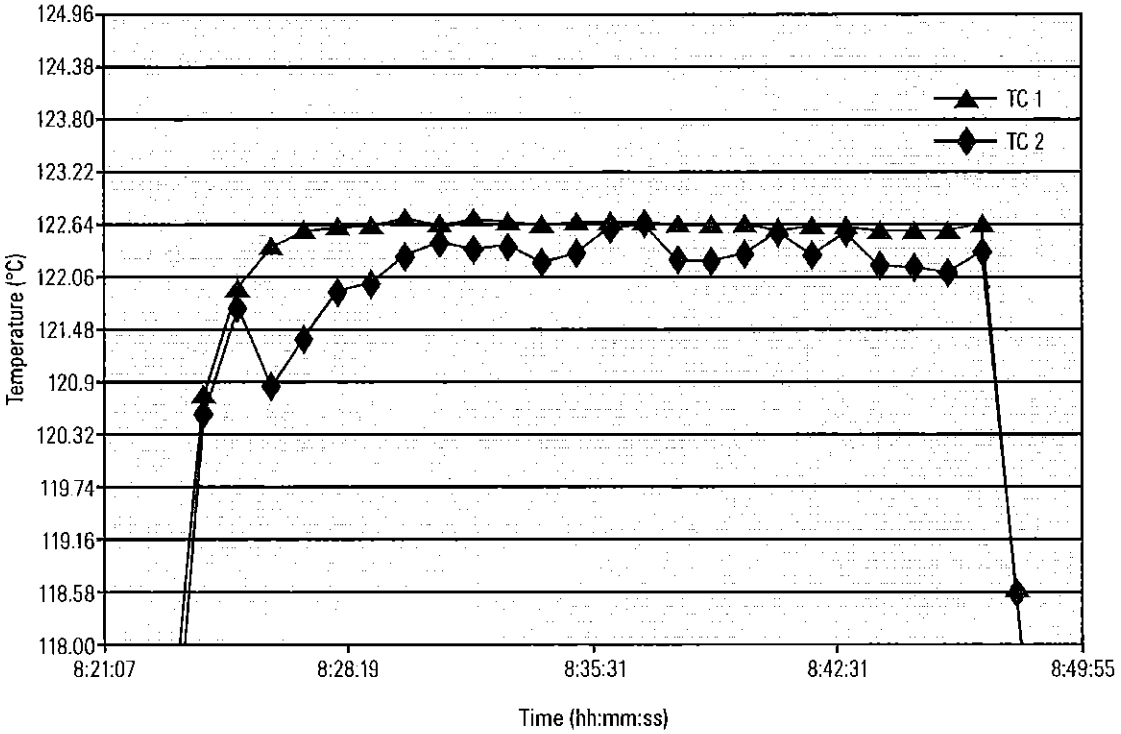


Figure 6.2.2-2 TC 1 vs. TC 2 Temperature Profile



Depending on the system being steamed and the intent of the SIP cycle, the study may require that the temperature locations reach the minimum desired temperature at the end of the heat-up phase. The temperature achieved at the end of the heat-up phase, as measured by a permanent probe, may be used as a routine check (automated or manual) to confirm satisfactory heat up for routine production cycles.

Temperature data should also be collected from permanent temperature probes that are designed into the system to control and/or monitor temperature during the SIP process. This data should be compared to the data from the temperature probes installed during validation to provide a link between the routine production monitoring and the validation study.

6.3 Biological Qualification

The objective of the biological component of cycle qualification is to obtain microbiological data confirming that the developed cycle achieves lethality requirements established during cycle design. Not all SIP systems require biological qualification. If sterility of the SIP system is not claimed, biological qualification may not be required.

6.3.1 Microbial Challenge

In order to assess whether the cycle delivers sufficient lethality to meet design requirements, an appropriate microbiological challenge should be selected to give meaningful results. The microbiological challenge system should have a resistance and challenge level appropriate for its purpose. The biological qualification data is used to calculate the F_{BIO} for the cycle.

Biological qualification using microbiological challenges follows a straightforward sequence:

- An appropriate microbiological challenge system is devised based on the desired lethality (F-value) determined during the design process.
- The SIP system is exposed to minimum acceptable cycle (MAC) conditions (or less depending on the D-value and population of the microbiological system).
- After completion of the cycle, the microbiological challenge systems are retrieved.
- Each microbiological challenge system is individually incubated in appropriate media and conditions for growth of survivors. Directions for use, including data about conditions to be used for recovery of test organisms after exposure to the sterilization process should be obtained from the Biological Indicator manufacturer *(27)*. The length of time that the exposed Biological Indicator is held before incubation should be validated *(28)*.
- The results are evaluated to ensure that the spore log reductions (SLRs) achieved for the microbiological challenge systems meet predetermined acceptance criteria.
- For typical SIP validation runs, all exposed Biological Indicators should show total kill.
- Growth of the microbiological challenge organism is required in positive controls.

When using the overkill approach for "sterile" SIP applications, there are various methods that can be used to demonstrate an F_{BIO} of at least 12 minutes. Following are three qualification examples to demonstrate various methods for obtaining an F_{BIO} of 12 minutes with various BI D-values.

Example 1

For the overkill design approach, the desired lethality, F_{PHYS} and F_{BIO}, is greater than or equal to 12 minutes. If a BI with a resistance of 2.0 minutes and a population of 1.0×10^6 is used in the qualification study, then the F_{BIO} is calculated as follows:

$F_{BIO} = D_T \times SLR$ (spore log reduction)

$F_{BIO} = 2.0 \times 6.0$

$F_{BIO} = 12.0$

Therefore, a minimum acceptable cycle (MAC) that inactivates a BI challenge with an $N_0 = 1.0 \times 10^6$ and a D-value of 2.0 minutes has been biologically qualified as an overkill cycle ($F_{BIO} = 12.0$ minutes).

Example 2

If the design requirement is an F_{BIO} of 12 minutes and the BI has a starting population (N_0) of $1 \times 10^6$and a D-value of 1.5 minutes, then the exposure time has to be reduced. The exposure time factor can be calculated by the following:

Exposure Time Factor = $(D_T \times SLR)$ / Desired F_{BIO}

Exposure Time Factor = $(1.5 \times 6.0) / 12.0$

Exposure Time Factor = 0.75

Therefore, an SIP cycle that is operated at 75% of the minimum production cycle exposure time conditions that inactivates a BI challenge with an $N_0 = 1.0 \times 10^6$ and a D–value of 1.5 minutes has been biologically qualified as an overkill cycle ($F_{BIO} = 12.0$ minutes). In order to calculate the Full Cycle F_{BIO}, the Partial Cycle F_{BIO} ($D_T \times SLR$) is multiplied by the reciprocal of the Exposure Time Factor:

Full Cycle F_{BIO} = 1/Exposure Time Factor × ($D_T \times SLR$)

Full Cycle F_{BIO} = 1/0.75 × (1.5 minutes × 6)

Full Cycle F_{BIO} = 12.0 minutes

Example 3

If the design requirement is an F_{BIO} of 12 minutes and the BI has a starting population (N_0) of 1×10^6 and a D-value of 1.0 minutes, then the exposure time has to be reduced. The exposure time factor can be calculated by the following:

Exposure Time Factor = ($D_T \times SLR$) / Desired F_{BIO}

Exposure Time Factor = (1.0 × 6.0) / 12.0

Exposure Time Factor = 0.5

Therefore, an SIP cycle that is operated at 50% of the minimum production cycle exposure time that inactivates a BI challenge with an $N_0 = 1.0 \times 10^6$ and a D-value of 1.0 minutes has been biologically qualified as an overkill cycle ($F_{BIO} = 12.0$ minutes). In order to calculate the Full Cycle F_{BIO}, the 50% Cycle F_{BIO} ($D_T \times SLR$) is multiplied by the reciprocal of the Exposure Time Factor:

F_{BIO} = 1/Exposure Time Factor × ($D_T \times SLR$)

F_{BIO} = 1/0.5 × (1.0 minutes × 6)

F_{BIO} = 12.0 minutes

Note: The approach used for example 3 is often called the Half-Cycle Qualification approach.

6.3.2 Use and Placement of Biological Indicators

Biological indicators are typically obtained from commercial sources. The BI challenge system is typically spores of *Geobacillus stearothermophilus;* however, other certified BIs may be used. The use of the semi-logarithmic model to determine the inactivation characteristics of the BI challenge system may also be used to calculate the appropriate challenge to biologically qualify a cycle, regardless of the resistance of the challenge organism selected. (See Section 6.3.1 for examples.)

There are several types of biological indicator challenge systems. The different types of BI systems appropriate for SIP validation are discussed in **Table 6.3.2-1.** The table gives the description of the different BI types and the pros and cons of using them to qualify an SIP cycle.

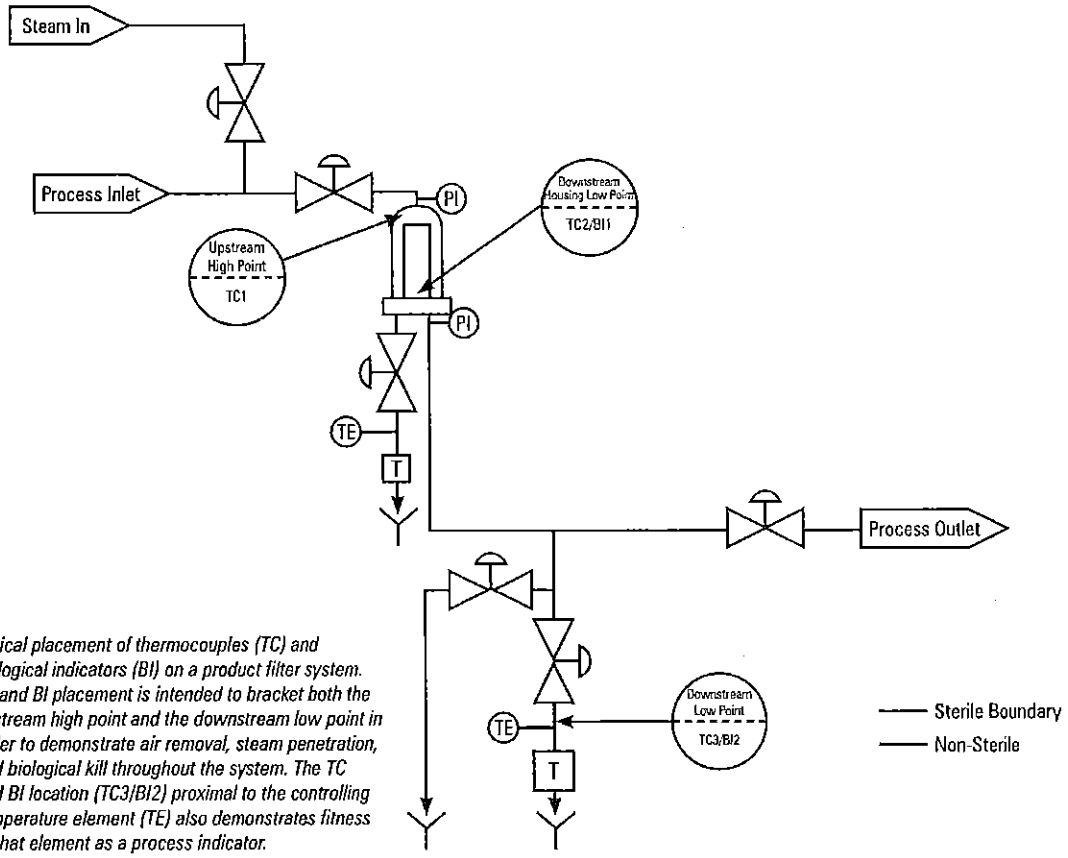
Table 6.3.2-1 Types of Biological Indicators

BI Type	BI Description	Pros	Cons
Spore Suspension	Suspension of spores of known D-value, population, and z-value inoculated onto an item or coupon	• Allows direct inoculation of components (e.g., filters, tubing) being sterilized • Does not obstruct steam, air or condensate flow	• Inoculation and recovery method more difficult • Surviving spores can cause contamination • D-value needs to be measured with the coupon or item • Introduction of open spore suspensions into a manufacturing facility may present significant regulatory, logistical or product safety issues
Self-contained BI*	Growth medium contained inside the primary packaging for the indicator	• Convenient packaging • Eliminates aseptic manipulation of the indicator strip, which can lead to indicator contamination (i.e., false positives) • Simple recovery method • Recovery method and D-value are typically supplied by vendor • Potentially eliminates exposure of the area to the spores	• Indicator is bulky and not suitable for monitoring small diameter systems • Glass media container can break when not anchored properly and exposed to a turbulent steam flow
BI Carrier	Spores added on a carrier (e.g., stainless, paper, plastic, glass, wire) individually packaged to maintain integrity	• Allows versatility in size and rigidity based on the selection of the carrier • Minimizes exposure of the area to the spores • Widely recognized and used for SIP sterilization validation • Recovery method and D-value are typically supplied by vendor	• Depending on the selection of the carrier, may not be suitable for small-diameter systems • In the case of non-packaged / bare BI's, the BI's should be aseptically handled.

* Biological indicators should have direct contact with steam. Some BIs are in sealed ampoules that contain spore suspension in liquid media and therefore may not represent actual system conditions. Therefore, these types of indicators should not be used.

BI challenge systems are placed adjacent to temperature sensors in the cold spots/and hardest-to-sterilize locations within the SIP system. For example, they may be located within cartridge filters, in nozzles, in the highest point in the tanks, in deadlegs where it may be difficult for steam to access or in low point drain/condensate valves. For large filter housings (e.g., > 20 inches or > 1 cartridge), BIs may be placed in the top and bottom of the filter cartridge(s) to evaluate the areas within the housing that could have excessive trapped air and/or excessive condensate pooling. In addition, BIs should be placed in low locations, especially in distal lines where condensate flows to a drain or pools.

Figure 6.3.2-1 Example of BI Placement



Typical placement of thermocouples (TC) and biological indicators (BI) on a product filter system. TC and BI placement is intended to bracket both the upstream high point and the downstream low point in order to demonstrate air removal, steam penetration, and biological kill throughout the system. The TC and BI location (TC3/BI2) proximal to the controlling temperature element (TE) also demonstrates fitness of that element as a process indicator.

To evaluate the correlation between F_{PHYS} and F_{BIO}, biological indicators should be placed near temperature probes. Biological indicators, TCs, and probes should not block the steam path nor hinder the removal of condensate. Materials used for placement or attachment of the biological indicators inside the system should not hinder the inactivation of the BI or operation of steam traps and sanitary valves. Attachment method must be robust enough to prevent the BI being carried away with the turbulent flow and condensate. For paper strip BIs it is recommended that they be held in place with wire mesh or other suitable method to allow steam penetration while containing the wet BI. Placement and location rationale of biological indicators should be documented.

Figures 6.3.2-2 and **6.3.2-3** depict alternative methods of thermocouple and biological indicator placement in pipe. Materials of construction used for installation (e.g., mesh pouch, wire, clamps, connection gland) should be compatible with process requirements.

Figure 6.3.2-2 Example of Pipe with BIs and TC Connection Gland

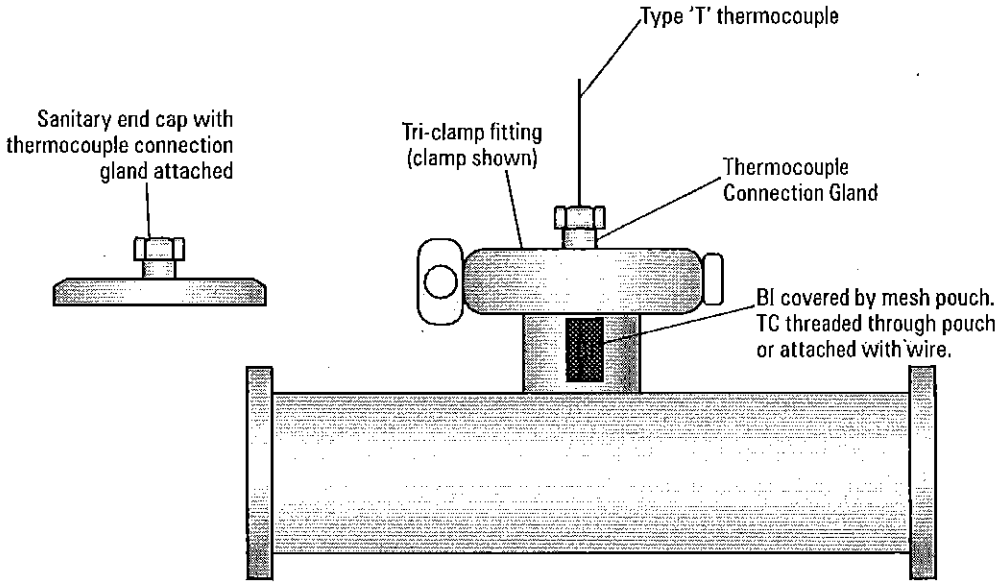
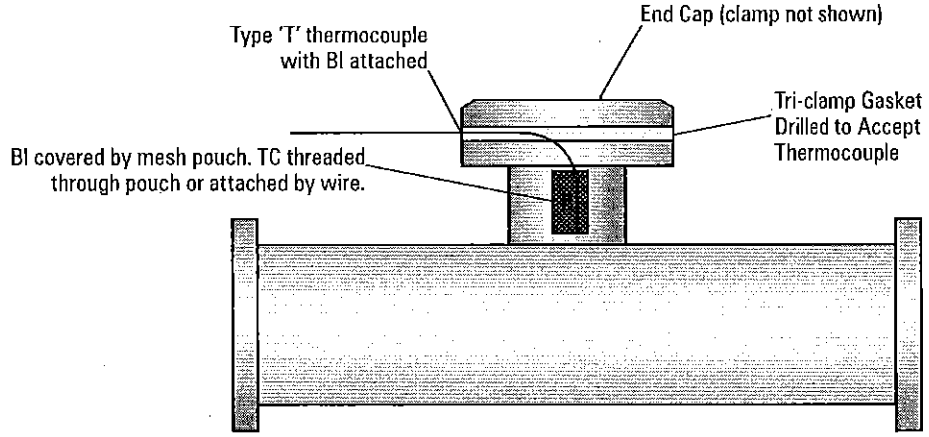


Figure 6.3.2-3 Example of pipe with BIs with Ported Gasket



6.4 Qualification Acceptance Criteria

Acceptance criteria should be clearly defined in the test protocol(s). These criteria should be based on the type of steam in place process, applicable regulatory expectations, and the operating parameters determined in cycle development. Following is a list of typical acceptance criteria that should be considered when qualifying an SIP cycle. This list is not exhaustive and other acceptance criteria may be necessary depending on the specific situation.

SIP system integrity (Pressure/Vacuum test) (optional)

A system integrity test may be performed and results should meet the predefined leak rate. A leak rate may be selected based on the complexity, volume, and process risk assessment. Temperature drift during the pressure hold test should be monitored.

Heat up time
Document the time it takes for all locations to reach the defined process temperature. The time difference (lag time) between the validation probes and the control/monitoring probes to reach minimum exposure temperature should be documented to ensure the cycle has been designed to account for cold spots that are not permanently monitored.

Lethality (F_{PHYS})
Predefined time at temperature and/or F_0 values should be met and documented *(29,30)*.

Exposure/Dwell time
Predefined exposure time should be met and documented.

Lethality (F_{BIO})
The calculated lethality (F_{BIO}) calculated using the D-value and population of the target organism, and applicable exposure time factors should meet the predetermined acceptance criteria.

Minimum and maximum temperature during exposure
Minimum and maximum temperature during exposure should meet predefined criteria and should be documented.

Number of functioning probes
The number of probes that can fail and still maintain a valid run should be defined. Documentation should include rationale for addressing probe failures.

Positive and negative controls function as specified
Positive control biological indicators show growth after incubation at specified temperatures. If applicable, negative controls show no growth after incubation at specified temperatures.

Filter integrity should be verified before use and after SIP by an appropriate method such as bubble point, diffusive flow, pressure hold, or water intrusion. If the filter is directly inoculated with a biological indicator (destructive test), then filter integrity needs to be assured in a separate qualification run.

Additional Monitoring:

Correlation of temperature and pressure for saturated steam
To maximize efficiency of the SIP cycle, adjacent pressure and temperature measurements may be used to evaluate whether saturated steam conditions are within the user-defined range.

Steam pressure
Steam supply pressure, as it relates to the control pressure, should be documented.

Post-SIP hold (optional)
Acceptance criteria (e.g., maintenance of positive pressure) should be defined that ensure system integrity during post-SIP hold.

Steamed equipment may require a hold study if stored. The equipment is steamed-in-place according to the qualified parameters and stored under normal or worst-case operating conditions.

6.5 Validation Approaches
Many operations involve similar or identical process operations (e.g., filtration or sanitization) or equipment (e.g., mixing vessels and bioreactors). In such cases, matrix and/or family validation approaches may be acceptable. It is recommended that these approaches be presented to the appropriate

regulatory agencies prior to protocol development and execution. Further information on validation methodology can be found in PDA Technical Report 60: Process Validation: A Lifecycle Approach *(31)*.

6.5.1 Family Validation

Validation studies may be significantly decreased by grouping equipment or equipment systems into "families." Family validation applies to equipment that is identical or similar, like bioreactors, column housing units, and tanks. A documented approach to the creation of these families is needed to provide the rationale for system equivalence. An example would be a bank of three bioreactors of similar design and size to be used in the manufacture of a single product. One reactor may be validated by three consecutive runs, and the other two may be validated by one confirmatory run each. Justification for using the family approach should be stated in the protocol and report. The degree of similarity should be fully documented in the associated IQ, OQ, and PQ *(32)*.

6.5.2 Matrix Validation

Matrix validation (also known as bracketing) is conducted at the full range or extremes of a process or equipment parameter (i.e., when a group of different-sized vessels of similar configuration are used in a process, the largest and the smallest vessels are validated). Validation of intermediate-sized vessels can be encompassed by this study with adequate justification.

7.0 Ongoing Process Control

Continuous evaluation, control, and maintenance of SIP cycle performance is critical during the commercial production phase due to the operational importance of SIP processes and the potential for adverse consequences to product quality. Evaluation of SIP cycle performance is typically accomplished through data monitoring and periodic requalification. Control is achieved through investigation and resolution of cycle deviations and equipment/process change control. Finally, to ensure maintenance of steaming performance, effective preventative maintenance and calibration programs are essential.

7.1 Use of Risk Management for Ongoing Process Control

On-going process control activities after initial validation ensure that the system and processes supporting SIP continue to operate as intended and achieve the desired levels of sterilization or sanitization as required by the production process requirements. These activities encompass requalification and revalidation, which have traditionally been executed on a periodic basis regardless of historical SIP process performance or potential impact to product quality. Many in industry have begun to concentrate validation efforts through the use of risk management and statistical process control methodologies to identify those systems that pose the greatest risk based on inherent variability or process capability. Depending on the level of automation, ongoing validation activities for very robust processes may be limited to periodic or continual monitoring, with revalidation conducted as an event-driven activity.

7.2 Routine Monitoring

Following completion of the cycle development and performance qualification exercises, monitoring of the routine operational cycles should be performed to ensure an ongoing state of control. Critical parameters should be documented and data recorded (critical data) for each cycle. Routine monitoring data should be analyzed to ensure the system has remained in a state of control as demonstrated by the qualification data. The routine operational cycle is typically controlled to produce additional lethality over the qualified MAC to provide increased sterility assurance. Cycles that have not met minimum defined critical cycle parameters should be rejected. Deviations from key parameters should be investigated and their impact assessed to determine whether the cycle is acceptable or not.

An alarm system for temperature and/or pressure may be used to facilitate the detection of any deviation from the defined process parameters. Alarm conditions should be properly documented.

7.2.1 Operational Parameters

Critical operational parameters may include the following:

Temperature

Temperatureshould be monitored at locations as described in section 4.2 to ensure that the minimum process temperature is achieved throughout the system during routine production cycles.

Temperature and pressure profiles for the SIP cycles should be recorded and assessed on a periodic basis to confirm that no significant change in the qualified state has occurred.

Pressure

The system pressure should be monitored at appropriate locations during key phases of the SIP cycle, including air removal, heat up, exposure, steam removal/cool-down and hold.

Steam pressure and temperature measurements conducted in close proximity to the pressure transducer/gauge should correlate to the corresponding saturation pressure in dry saturated steam tables. Correlation criteria should include measurement uncertainty of the pressure instrumentation and/or control system. Steam tables are useful to determine temperature/pressure correlation.

Pressure monitoring post-SIP is important to ensure that system integrity is maintained. Gas used for pressurization of sterile systems should enter the system via an integral, liquid-rated, hydrophobic sterilizing grade filter(s) *(17)*. Other filters may be considered acceptable for systems not claiming sterility *(16,17)*.

It may be necessary to monitor differential pressure across filters to confirm that the maximum pressure differential is not exceeded during the SIP process.

Pressure or vacuum hold tests may be conducted to confirm system integrity before SIP. These tests are important from both a quality and safety perspective.

Time

Time duration of cycle phases should be monitored to ensure the SIP cycle remains within the qualified state.

F_0

Cumulative F_0 may be used as a process control parameter to end the cycle in lieu of predetermined time and temperature.

Monitoring strategies of SIP parameters and their associated alarms should be designed to provide data appropriate to demonstrate that the SIP process was performed successfully. System monitoring may be automated, manual, or a combination, provided that the data obtained is accurate and easily retrieved. The information recorded for each run should be linked to the validation of the cycle. Resumption of an SIP cycle following resolution of an alarm condition should ensure that the minimum exposure time is achieved.

7.2.2 Filter Testing

Integrity testing of the filter cartridge may be performed pre-use and must be performed post-use for sterile applications (i.e., after the system has been used for its intended use; not after SIP). The test frequency may be defined according to the needs of the application *(17)*. Where possible, the filter should be tested in the housing in which it is to be or was used. Filter testing should be performed with a clear indication of pass/fail. Additional guidance on integrity tests may be found in reference literature.

7.3 Change Control/Revalidation

A robust change control system should be in place to maintain the validated state of the SIP process.

Any proposed changes to the SIP process (including procedures, hardware, software, cycle configuration, supply utilities, filter types/sizes) should be evaluated to determine the potential effects of those changes on the SIP cycle and the extent of requalification/revalidation required to demonstrate that the modified process performs as intended and still meets the applicable acceptance criteria.

7.4 Periodic Requalification/Revalidation

A periodic review of the system should be performed to ensure the state of control is maintained and to evaluate the impact of cumulative "minor changes" over the review period.

This should also include review of performance data from various monitoring sources (e.g., process, engineering, maintenance, and calibration data) to verify that there have been no adverse trends or drifting away from the baseline performance established during validation. A review of change control documentation should be conducted as part of the requalification/revalidation.

Review frequency should be based on the system's intended use and applicable regulatory expectations. Requalification may include supplemental thermal and/or biological indicator testing to verify F_{BIO} and F_{PHY} acceptance criteria for systems claiming sterilization.

7.5 Preventative Maintenance Strategy

In order to ensure consistent system performance, a maintenance strategy should be in place that addresses potential changes in material and component performance due to operation, exposure, and time. In particular, the strategy should take into account how thermal and pressure cycles associated with heat-up, exposure, and cool-down may impact the service life of various components, particularly polymeric (elastomeric) components.

A maintenance strategy should include special considerations toward polymer replacement practices due to their criticality in maintaining system integrity and their limited lifetime. Polymer service life may be affected by various operational stresses such as thermal conditions, process frequency, product chemistry, and cleaning frequency.

Elastomer manufacturer recommendations may be used as a basis for initial determination of replacement frequency. However, manufacturer testing may not sufficiently challenge the elastomer performance under actual usage conditions, so an assessment should be performed to determine adequacy of the replacement frequency..

SIP performance can be directly impacted by improper functioning of other components such as pressure regulators, steam traps, or isolation valves.

The preventative maintenance program should include periodic inspection and/or replacement of components that are critical to SIP performance. The frequency of the preventative maintenance may be determined based on component maintenance history, manufacturer recommendations, or risk evaluation and mitigation. In addition to specific component maintenance requirements, a review of the overall equipment assembly and operation may be performed to identify issues that could impact SIP cycle performance (e.g., altered piping slopes).

7.6 Calibration Strategy

The calibration program should include instruments that are used to control and monitor the cycle. Both the control of the SIP cycle and the confirmation of successful cycle completion are dependent on the proper indication and recording of critical operational parameters. Calibration serves as both the means to maintain instrument performance as well as to document proof of performance.

Calibration tolerances and periodicity is determined by instrument capability, history, manufacturer recommendations, and process risk. The impact of instruments found outside calibration tolerances during periodic recalibration evaluations should be investigated. A risk assessment may be used to establish instrument calibration frequency.

8.0 Appendices

8.1 Appendix A: Risk Assessment of Steam in Place Processes

8.1.1 Introduction

Planning and preparation are crucial when designing a steam in place system. A comprehensive risk assessment conducted proactively can save time, effort, and resources during qualification, validation, and routine monitoring. Other benefits include:

- Improved planning and preparedness for potential failures
- Increased process understanding
- Improved stakeholder relationships through better communication
- Increased quality assurance through documentation of the decision-making process
- Reduced risk to patients through process modifications to eliminate or reduce high risk steps
- Identification of fault conditions that require monitoring
- Optimization and prioritization of validation resources
- Selection of test methods and acceptance criteria that are aligned with critical quality attributes of products
- Compliance with regulatory expectations
- Assistance in maintaining a state of process control *(3)*

Additional guidance on risk assessment tools and performing risk assessments may be found in published literature ***(3,6,30-34).***

This appendix provides a risk assessment example for illustrative purposes only. The example provided focuses on a bioburden-controlled biologic manufacturing process. The overall process is first evaluated to identify process steps with the potential to adversely impact product quality. Those steps are then further analyzed to identify actions that may be taken to reduce the likelihood of failure.

8.2 Risk Assessment Tools

8.2.1 Hazard Analysis and Critical Control Point (HACCP)

HACCP *(6)* analysis is a rigorous system that may be used to analyze processes to identify hazards and establish measures that may be instituted to mitigate risk and ensure product quality. It is a complete system that focuses on preventative measures rather than end product testing. In the context of HACCP analysis, a hazard is defined as any biological, chemical, or physical condition that may adversely impact the safety, efficacy, and quality of the product being analyzed.

A complete HACCP analysis results in the identification of points or process steps that must be controlled to ensure product safety, efficacy, and quality. These steps are referred to as critical control points (CCP). The HACCP system is based on the following preparation steps and seven principles:

Preparation Steps for HACCP

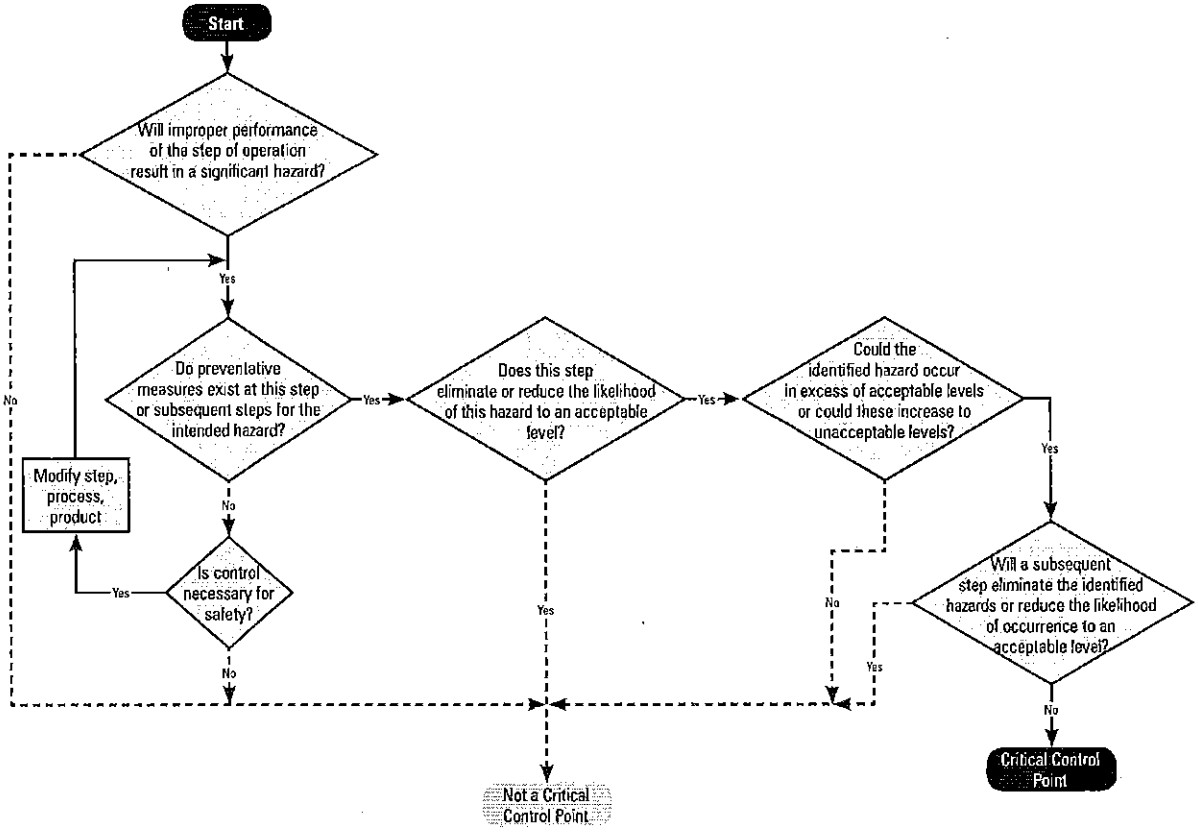
1. Assemble the team
2. Describe the product and process
3. Identify the intended use
4. Develop and verify a process flow diagram (include nonstandard or abnormal conditions)

Seven Principles of HACCP

1. Conduct a hazard analysis
2. Determine the critical control points (CCPs). (see CCP decision tree, **Figure 8.2-1**)

3. Establish target levels and critical limits
4. Establish a system to monitor the CCPs
5. Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control
6. Establish procedures to verify that the HACCP system is working effectively
7. Establish documentation concerning all procedures and keep records appropriate to these principles and their application

Figure 8.2-1 Critical Control Point Decision Tree



8.2.2 Failure Mode and Effects Analysis (FMEA)

FMEA is a detailed tool that may be used to identify potential failure modes and engineer them out of the process, improve reliability, or put controls in place to identify the failure modes before product quality is impacted. An FMEA may be conducted using either a quantitative approach assigning a risk priority number (RPN) or a qualitative approach assigning a level of risk (high, medium, low). Either method results in a risk prioritization rank (RPR) that is calculated based upon the following:

- Severity of the result of the failure
- Occurrence (frequency of occurrence of the failure)
- Detection (how likely the failure is to be detected)

The scales used to quantify severity, occurrence, and detectability must be defined as part of the FMEA setup and training. The scales may be based on available data or they may be subjective and based on professional experience. The process may be assessed and a risk ranking determined using these scales. Risk ranking establishes relative risk and identifies process steps that may benefit from the implementation of mitigating actions.

In this example, a qualitative approach has been selected. Table 8.2.2-1 provides a more detailed example of scales that are typically used in a qualitative FMEA assessment.

Table 8.2.2–1 Risk Ranking Assignment Chart

Risk Category	High	Medium	Low
Severity	The process failure will result in direct and severe impact to patient health and is life threatening.	Process failure is indirect, moderate, or will have a slight impact to patient health; harmful but not life threatening.	Very little or slight impact to patient health.
Occurrence	There is a high probability that process failure will occur and will result in the unwanted event.	Process failure occurs occasionally, but not often; may result in the unwanted event.	Process failure rarely occurs; not likely to result in an unwanted event.
Detection	If the process failure occurs, it will probably not be detected by existing controls.	If the process failure occurs, it may be detected with existing controls.	There is a high likelihood that existing controls will detect the process failure.

8.3 Example of HACCP for a New SIP Process

The example uses two risk management tools. Hazard Analysis and Critical Control Point (HACCP) analysis is used to assess the impact of individual manufacturing steps. Failure Mode and Effect Analysis (FMEA) is used to perform a more detailed risk assessment of individual SIP process steps to identify risks as well as actions that may be taken to preclude or minimize failures.

The example presented here describes a bioburden-controlled, biologic manufacturing process. A flow chart of the manufacturing process from pre-culture to bulk (API) filling is shown in **Figure 8.3-1** and includes typical manufacturing steps such as cell culture, media and buffer preparation, harvest, recovery, purification, and bulk filling.

The process steps highlighted in **Figure 8.3-1,** the Production Bioreactor System and the Purification System, are further detailed below:

Table 8.3-1 documents two process steps identified during the HACCP analysis. The HACCP decision tree, shown in **Figure 8.2-1,** was used to determine if an individual process step was a CCP.

The Production Bioreactor Vessel was identified as a CCP because achievement and maintenance of sterility was critical to ensure continued protein production during the cell culture process. As a result of the CCP being identified, the system was further assessed by establishing critical limits, monitoring procedures, and corrective actions using a standard HACCP analysis table. Then an FMEA assessment was performed on the SIP process to further identify individual failure modes. The entire production bioreactor system was deemed to require steam in place sterilization. Performance Qualification of the SIP sterilization process requires following an overkill sterilization approach using temperature monitoring and biological indicator challenges.

The second chromatography step in the Purification System was also analyzed and no CCPs were identified. This determination means that though the process step is important, there are monitoring

procedures in place (bioburden monitoring being a key one) to ensure that the process does not exceed limits. The amount of qualification and the requirements for any subsequent requalification can be determined and justified based upon this assessment. This portion of the manufacturing process need only undergo a steam in place sanitization procedure since a low bioburden level is acceptable and a filtration procedure is in place after the purification steps and prior to proceeding to the bulk API fill steps.

Figure 8.3-1 Manufacturing Process Flow Diagram Depicting Stages of HACCP Analysis

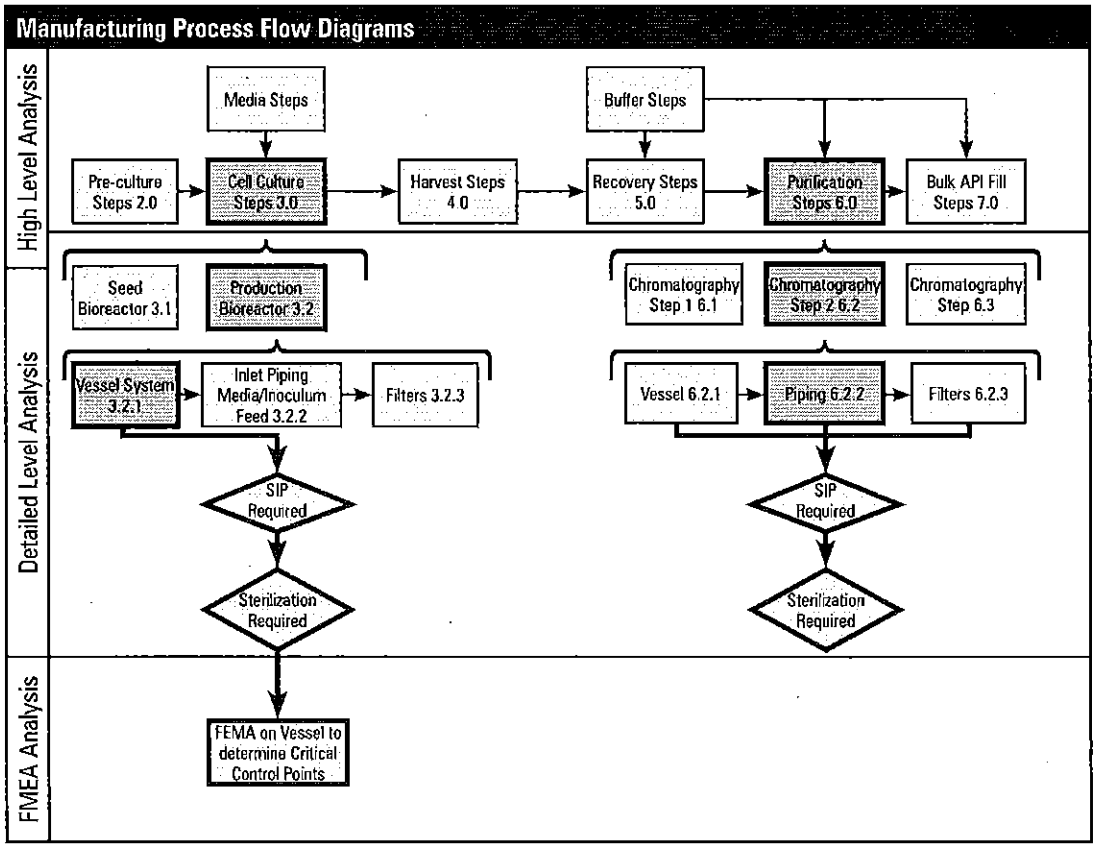


Table 8.3-1 HACCP Analysis Table

Step Ref.	Process Step	Hazard No.	Hazards	CCP Yes/No	CCP Rationale	Critical Limits	Monitoring Procedure	Corrective Actions
3.2.1	Production Bioreactor Vessel	3.2.1-1	Inadequate SIP exposure time and/or temperature	YES	Maintenance of sterility critical in this step to ensure protein production.	Exposure Temperature: 121°C plus measurement uncertainty Exposure Time: 12 minutes minimum plus safety margin for robustness Sterilization Required	Monitor time and temperature during every SIP process Manual check of all steam traps to ensure adequate air removal prior to initiating cycle Periodic calibration of all temperature probes Bioreactor sample bioburden monitoring	Verify calibration of all measuring equipment Shorten calibration cycles as necessary Verify steam trap function Replace as necessary Investigate SIP run and previous runs to ascertain root cause
		3.2.1-2	Microbial contamination	YES	If sterilization is inadequate, then contamination could occur, resulting in termination of production and culture disposal.	Axenic culture (no contamination) Sterilization Required	Verify bioreactor samples do not contain contamination	Perform root cause investigation and implement countermeasures to prevent reoccurrence
		3.2.1-3	No SIP after line breakages for calibration or maintenance	YES	When the system is no longer in a closed condition, system integrity and sterility must be restored through SIP following line breakage.	Exposure Temperature: 121°C plus measurement uncertainty Exposure Time: 12 minutes minimum plus safety margin for robustness Sterilization Required	Monitor time and temperature during every SIP process Manual check of all steam traps to ensure adequate air removal prior to initiating cycle Periodic calibration of all temperature probes Bioreactor sample bioburden monitoring	Develop line-breaking procedure that includes SIP of the line after maintenance completion
6.2.2	Purification System Chromatography Step 2 – Piping	6.2.2-1	Inadequate SIP exposure time and/or temperature	No	Final filtration step prior to bulk API filling in place to reduce or remove bioburden	Bioburden and endotoxin levels meet predetermined criteria Sanitization Only Required	Monitor time and temperature during every SIP process Manual check of all steam traps to ensure adequate air removal prior to initiating cycle Periodic calibration of all temperature probes Product bioburden monitoring	Verify calibration of all measuring equipment Shorten calibration cycles as necessary Verify steam trap function Replace as necessary Investigate SIP run and previous runs to ascertain root cause
		6.2.2-1	Microbial contamination	No	Final filtration step prior to bulk API filling in place to reduce or remove bioburden	Bioburden and endotoxin levels meet predetermined criteria Sanitization Only Required	Purification sample bioburden monitoring procedure in place	Perform root cause investigation and implement counter measures to prevent reoccurrence Extend SIP cycle

8.4 Example of FMEA for a Steam in Place Process

A more detailed risk assessment of the SIP was warranted since the HACCP analysis identified the Production Bioreactor System SIP process as a CCP. In this case, the FMEA works well at the component level to challenge the design and operation of the system and identify potential areas of failure.

8.4.1 Risk analysis

Severity, occurrence, and detection were considered in the estimation of risk for each cause and process failure. Qualitative risk assignments including Low, Medium, and High rankings were used as shown in Table 8.2.2–1.

8.4.2 Risk Evaluation

Risk evaluation was also performed using a qualitative risk prioritization ranking (RPR) approach. Since the occurrence of non-sterile liquid product is considered unacceptable due to patient risk, severity is always rated as high and not included in the risk prioritization ranking chart. Therefore, the RPR was performed based on likelihood of detection and occurrence frequency. Table 8.4.2-1uses color-coding to depict the RPR including Low (green), Medium (yellow) and High (red).

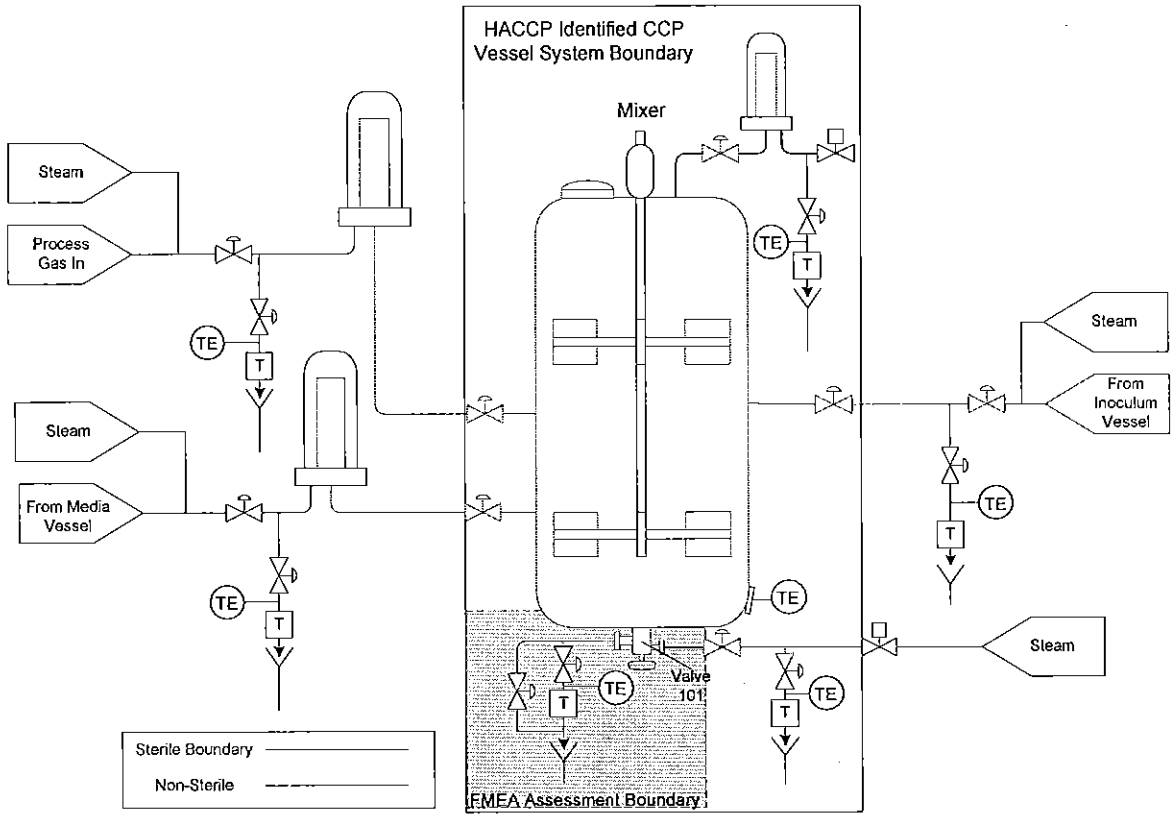
Table 8.4.2-1 Risk Prioritization Ranking Chart

		Detection		
		Low (High likelihood failure will be detected)	Medium	High (It is not likely failure will be detected)
Occurrence	High	This cause is likely to occur, but when it does it will be detected. Risk is Medium	This cause is likely to occur and may be detected. Risk is High	This cause is likely to occur and is not likely to be detected. Risk is High
	Medium	This cause could occur and will be detected. Risk is Low to Medium	This cause could occur and may be detected. Risk is High	The cause may occur and it will not be detected Risk is High
	Low	This cause is not likely to occur and if it does, it will be detected. Risk is Low	The cause is not likely to occur and if it did it, may be detected. Risk is Low or Medium	The cause is not likely to occur and not likely to be detected. Risk is Medium

8.4.3 Risk Assessment

A risk assessment was conducted on the bioreactor vessel system using the FMEA tool. Figure 8.4.3-1depicts the CCP boundary identified during the HACCP analysis. A smaller boundary has been added to show the scope of FMEA used in this example. This figure has been simplified for the purpose of this exercise and is not an accurate representation of a complete bioreactor system.

Figure 8.4.3-1 Example of Bioreactor Vessel System



The assessment of the manufacturing and SIP process is shown in **Table 8.4.4-1.** Qualitative values (Low, Medium, and High) were assigned to each failure for frequency of occurrence and likelihood of detection. The combination of the occurrence and detection values was used in the risk prioritization chart to determine the current RPR. The acceptability of each failure cause was determined using the RPR. High RPRs were generally considered unacceptable and mitigation actions were investigated to reduce the risk. A Medium RPR required investigation for further mitigation and then a final determination was made as to its acceptability. Mitigation may be accomplished either through re-engineering or increased monitoring to reduce the RPR.

8.4.4 Risk Reduction/Post-mitigation RPR

A means of reducing the risk was developed with preference given to engineering controls over procedural controls wherever possible. The occurrence frequency and likelihood of detection were evaluated after each of the mitigation actions was implemented. For all cases in this example, the mitigation actions were able to reduce all RPRs to "Low," which is considered an acceptable level of risk.

Current Controls	DETECTION	Initial Risk Prioritization Number	Risk Accepted (Yes/No)	Recommended Action	SEVERITY Post- mitigation	OCCURRENCE Post-mitigation	DETECTION Post-mitigation	Risk Prioritization Rank Post- mitigation	Risk Accepted Post-mitigation
oe detected by the control system as temure and pressure set points will not be met; SIP will not progress to the exposure phase	M	M	N	Add preventive maintenance schedule for steam traps annually	H	L	M	M	Y
				Add quarterly steam trap evaluation	H	M	L	M	Y
				Add preventive maintenance schedule for steam traps annually and quarterly steam trap evaluation	H	L	L	L	Y
temperature probe above this trap will condensate and therefore will be reading temperature; The control system will not ess SIP into the exposure phase if this temure is low	L	L	Y						
oe detected by the control system as temure and pressure set points will not be met; SIP will not progress to the exposure phase	L	M	N	Further develop engineering standard for steam trap	H	L	L	L	Y
ıment specification standards in place				Check ordering details, model numbers, inventory Check maintenance procedure					

used for aseptic processing, but can be applied to other equipment and systems.

nps, vent and compressed gas filters, CIP spray devices (for some lyophilizers), and chamber isolation valve. An example lyophi-

l and lowered by a large hydraulic piston (ram). The distance between each shelf in the shelf stack is maintained by positioning ;er chamber. When designing an SIP cycle for a lyophilizer, it is important to ensure that the moving components in the lyophig the shelves during the SIP cycle.

ı a lyophilizer: internal condenser, in which the cooling coils are located inside the lyophilizer chamber, and external condenser,

r chamber and condenser. Separate temperature monitoring probes are not required for internal condensers.

difficult because limited access impedes placing temperature sensors. Since external condensers are often cold points, consider-

8.5.3 Heating and Cooling System

Heating and cooling fluid is supplied to each shelf via flexible hoses connected to fluid inlet and fluid outlet manifolds either inside or outside of the lyophilizer chamber. Any penetrations into the system for the heating and cooling system should be of sanitary design. All flexible hoses must be designed to withstand the temperature and pressure/vacuum of a typical steam SIP cycle.

The heating/cooling system should be vented and remain off during SIP to prevent damage to the equipment. The heating/cooling system can be turned on during the cooling phase of the SIP cycle, if shelf temperatures are below temperatures that could damage the equipment.

8.5.4 Chamber Vacuum Pumps

Lyophilizers often use multiple types of vacuum pumps: a liquid ring vacuum pump is typically used for the SIP process, and high-vacuum pumps are used for freeze-drying. These pumps are not compatible with moisture and therefore should be isolated during the SIP cycle.

8.5.5 Vent and Compressed Gas Filters

Lyophilizers contain one or more sterilizing grade vent filters and/or compressed gas filters. Depending on lyophilizer design, these filters may be steamed in place or may be sterilized separately and connected using an aseptic design.

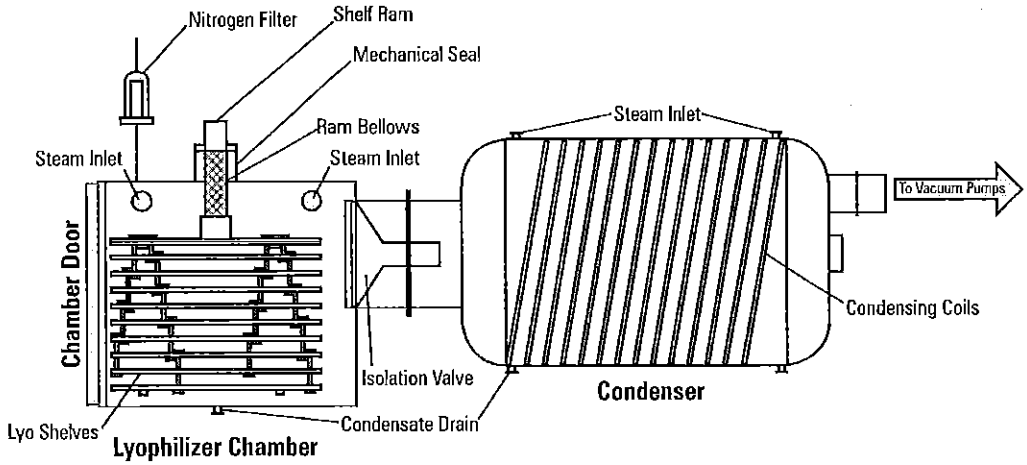
8.5.6 Clean-in-Place (CIP) Spray Devices

If a lyophilizer has spray devices for CIP of the chamber and condenser, it is important that these penetrations are sterilized as part of an SIP cycle. This is typically done by introducing steam into the lyophilizer chamber and condenser through the spray devices during the SIP cycle.

8.5.7 Chamber Isolation Valve

Lyophilizers with external condensers use isolation valves to allow separation of the lyophilizer chamber from the condenser. These valves are generally of two types: butterfly valves or piston valves. The isolation valves should remain open during SIP to ensure sterilization of the valves and the connection between the lyophilizer chamber and the condenser. Special attention should be given to the isolation valve and to the connection between the lyophilizer chamber and the condenser because this can be a cold spot in the lyophilizer system. The flange between the condenser and lyophilizer chamber needs to be designed so that the flange gasket is flush to prevent pooling of condensate.

Figure 8.5-1 Simplified Schematic of a Lyophilizer and Condenser



8.5.8 Post-sterilization Leak Test

Because lyophilizers operate under vacuum, it is important to leak test the lyophilizer after sterilization. Most lyophilizers have vacuum leak test cycles built in that pull a vacuum on the lyophilizer and then seal off all inputs to the lyophilizer. The vacuum leak test passes if the increase in pressure in the chamber is less than a predetermined amount over a pre-determined time. The acceptance criteria for a vacuum leak test will vary based on lyophilizer volume and design, and should be determined with the aid of the lyophilizer manufacturer. It is important to ensure that the chamber is thoroughly dry to prevent "virtual leaks" caused by sublimation of residual moisture. Most post-SIP leak tests are performed after a chamber drying cycle.

8.5.9 Cycle Considerations for Lyophilizers

SIP for lyophilizers differ in a few important considerations from other systems being considered. Following are some of the main considerations for lyophilizers:

- Lyophilizers are rated for deep vacuum (typically £ 100 micrometers Hg) and are fitted with multiple vacuum pumps, including liquid ring pumps that make it possible to evacuate the system before and after SIP. This design allows the use of vacuum/steam pulses to remove air from the system during the SIP cycle.
- Lyophilizers operate under deep vacuum after SIP, requiring the system to have a lower leakage rate after SIP than systems that are maintained under positive pressure after SIP.
- Lyophilizers contain moving parts that must be exposed to steam during SIP (e.g., the lyophilizer shelves, a lyophilizer ram covered with a bellows or other device, and chamber isolation valve). If these moving parts remain stationary during the SIP cycle, they should be in a position to allow surfaces that are in contact with the lyophilizer chamber to be exposed to steam during SIP.
- Lyophilizers are typically divided into a lyophilizer chamber and external condenser that must each be steamed in place. The external condenser is normally defrosted before SIP can begin.
- Lyophilizers require sterilizing grade vent filters to release vacuum at the end of the lyophilization cycle (and at the end of the SIP cycle). While this does not differ from many other systems, sterilization of the vent filters must be considered.

Other considerations, such as thermal stress on lyophilizer components (lyophilizers may operate from <-70°C to >121°C) and the handling of heat transfer fluids in the lyophilizer shelves and condenser are part of the lyophilizer design and need to be considered during SIP cycle development.

8.5.10 Pre-SIP Phases for Lyophilizers

Lyophilizers typically require several pre-SIP preparation phases before performing the actual SIP cycle. These pre-SIP phases may include:

Defrost Phase: The defrost phase removes residual ice from the condensers in preparation for cleaning and SIP. The condenser coils are defrosted by heating them for a predetermined time and temperature. Steam may be injected into the condenser to speed up the defrost process. The condenser should be thoroughly defrosted before SIP.

Cleaning Phase: Cleaning the equipment prior to SIP ensures that soils are reduced to acceptable levels. Most modern lyophilizers are cleaned by a CIP cycle, although some may be cleaned manually. Cleaning and cleaning validation for lyophilizers is outside the scope of this document.

Pre-SIP Leak Test Phase: Sometimes a pre-sterilization leak test (vacuum or pressure hold test) is performed. This test is performed to detect leaks that could compromise personnel or equipment safety before beginning the SIP cycle. If a vacuum hold test is used, the lyophilizer must be thoroughly dried before beginning the test, to prevent "virtual leaks" caused by sublimation or evaporation of residual moisture.

8.5.11 Typical SIP Phases for Lyophilizers

The typical phases for SIP of a lyophilizer include:

Heat-up/Air displacement phase: Remove air from the lyophilizer using one or more vacuum pulses to evacuate air to a predetermined level. The system is pressurized with steam to heat up the system following each vacuum pulse. See **Table 5.2-1** for a more detailed description of the air removal phase.

Heat-up phase: Pressurize chamber with steam to bring chamber/condenser temperature to specified temperature.

Exposure phase: Hold the chamber at the specified temperature for a predetermined amount of time.

Steam removal: Evacuate steam from the chamber/condenser.

Vacuum dry/cool-down phase: Remove excess moisture from the lyophilizer chamber and condenser. Chamber drying is necessary to prevent residual moisture in the system during the Post-SIP test. The order of the vacuum dry and cool-down differ by company (some choose to cool down after drying, since the heat facilitates drying). Allow the chamber to cool down to ambient temperature. Shelves and condenser coils may be used to speed cool-down after the chamber temperature falls below a safe temperature.

Post-SIP leak test phase: Perform a vacuum hold test to ensure the lyophilizer is integral before loading product into the lyophilizer chamber.

8.5.12 Validation Considerations for Steam in Place

Many of the principles that apply to SIP of other systems and equipment apply to SIP of lyophilizers. Lyophilizers used for aseptic processing require evaluation of a few special considerations during validation:

- Definition of sterile boundaries – the design review for a lyophilizer needs to include a clear definition of the sterile boundaries based on an assessment of risk to the product.

This is especially important when considering the SIP cycle for an external condenser. Because the condenser is connected to the lyophilizer by a tube separated by a large piston or butterfly valve, some pharmaceutical manufacturers consider the condenser to be outside the sterile boundary They then assign the separating line at the isolation valve, much in the same way that the sterile boundary for a formulation vessel may be at the bottom valve or steam trap. Other manufacturers include the condensing chamber within the sterile boundary.

It is important that the location of the sterile boundary be clearly defined in the user requirements and/or design specification for the system. This definition should be accompanied by a risk assessment that evaluates factors such as mass flow from the lyophilizer chamber to the condenser, and the effect of failures of vacuum pumps or cooling systems on the sterility of the lyophilizer chamber.

Placement of temperature sensors: Some SIP cycles for lyophilizers may include raising and collapsing the lyophilizer shelves, to ensure moving parts are adequately sterilized. In these cases, temperature sensors should be carefully placed so that they are not damaged by or cause damage to the lyophilizer.

Placement of biological indicators: If a condenser is considered within the sterile boundary for an SIP cycle, careful consideration should be given as to where and how biological indicators should be

placed in the condenser. This is especially important as the condenser, unlike the lyophilizer chamber, is not typically designed for easy access. Addition of access ports to the condenser during construction of the condenser chamber may be worthwhile if the condenser is considered within the sterile boundary. This is especially true for large condensers that may be several meters in length and diameter. The majority of the BIs should be placed on the shelves, in the chamber, and in the chamber drain.

Condensation on lyophilizer shelves and floor: Lyophilizer shelves are large flat surfaces with great thermal mass. This means that condensate may accumulate on lyophilizer shelves, causing problems in sterilization validation. Condensation of steam is, of course, part of the sterilization process, but excessive condensate pooling on shelves and floor should be assessed during SIP cycle design and validation.

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