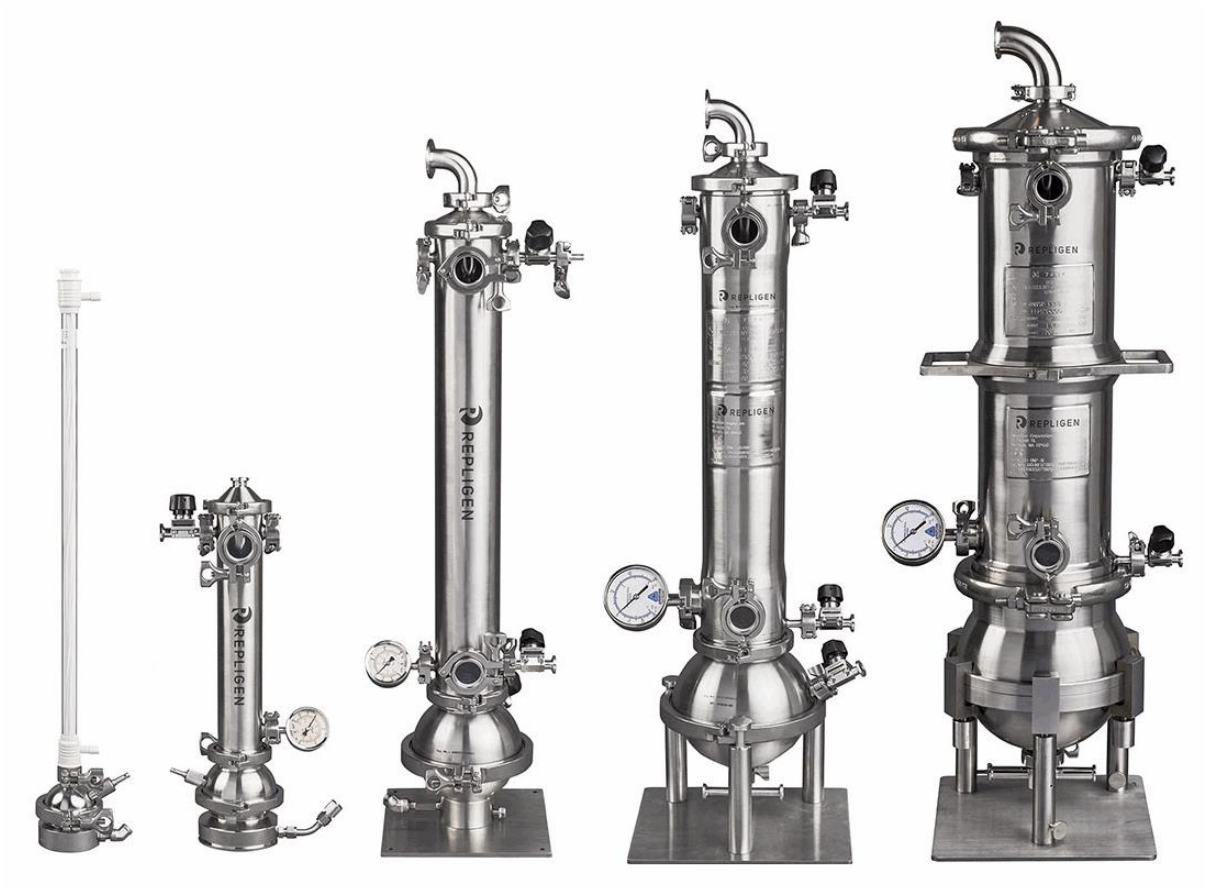
XCell™ ATF Systems
Filter Preparation and Autoclave

User Guide



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1. Introduction

This guide outlines the proper sequence for the assembly, wetting, pressure/integrity testing and autoclaving of the filters used in the Repligen XCell™ ATF stainless steel devices. The procedures outlined in this document are valid for XCell™ ATF2 through XCell™ ATF10. Adherence to these procedures are important to ensure satisfactory results with sterility and to maintain the cartridge integrity and full performance.

2. Scope

This guide is applicable to all hollow fiber filters sold by Repligen for use with the XCell™ ATF System.

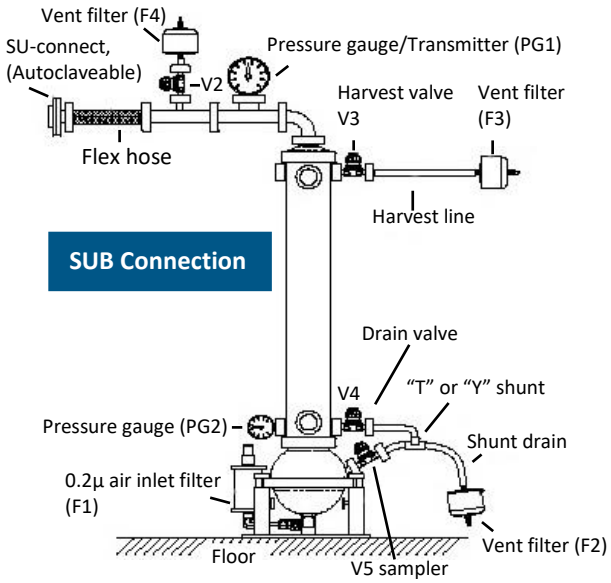
3. Safety Requirements

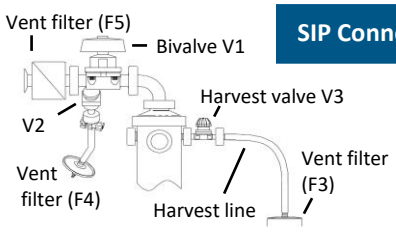
Potential hazards include:

- **Steam** – Use caution and proper PPE when in contact with the systems after autoclaving
- **Pressurized air** – Ensure that all components are suitable for use with high pressure and are properly secured. Use safety glasses/face shield
- **Heavy equipment** – Use assistance for lifting and assembling

4. Assembly of XCell™ ATF System for ATF System pressure testing

Figure 1. XCell™ ATF System filtration assembly prior to pressure testing





Note: *The XCell™ ATF2 system uses a self-contained hollow fiber cartridge and is not suitable for pressure testing. With the XCell™ ATF 4, 6, and 10, the hollow fiber cartridges are sealed within the stainless-steel housing using O-rings that are positioned at either end of the cartridge. Use only the O-rings supplied by Repligen and replace the O-rings after each use to ensure proper sealing. Slight variations in the assembly of the external connections are possible depending on the configuration of the equipment purchased and the connections being made to the bioreactor. For more detailed assembly instructions, refer to the manual or contact Repligen.*

Caution: *The "Y" shunt between pump valve (V5) and filtrate drain valve (V4) is used for rapid pressure equilibration between the permeate and the retentate side of the filter. Use pressure rated hose (2 bar min.)*

5. Pressure Test

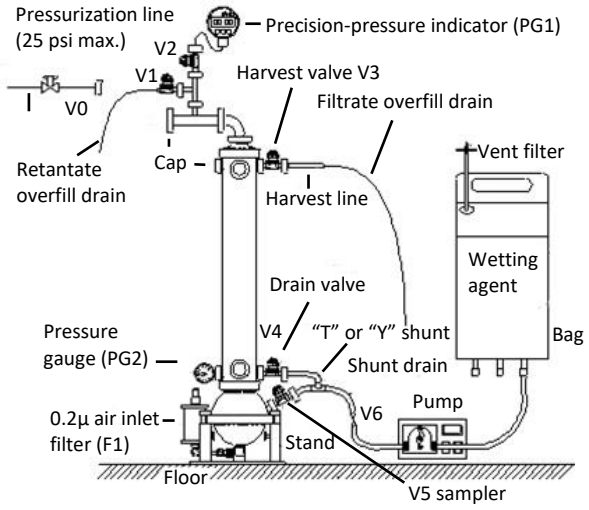
This test ensures that the XCell™ ATF System filtration assembly is free of leaks and can withstand the required operating pressures. The assembly can be pressure tested, with or without a filter module, before or after sterilization. Preferably, the test is performed with a filter prior to autoclaving.

1. Assemble the XCell™ ATF System according to Figure 1, but with the TC after the pressure gauge PG1 as the terminus with an end cap.
2. Prepare a regulated ~22psi/1.5bar, dry, oil-free air source.

3. Close all valves. Attach the pressurization line to the shunt line (V4/V5). Secure all connections.
4. While monitoring the pressurization rate on PG1 and PG2, slowly regulate the opening of V5 to pressurize the retentate side first, then slowly open V4 to equalize the pressure on the filtrate side.
5. Once the XCell™ ATF Filtration unit is pressurized to about 20 psi, close all valves.
6. Wait five minutes for the pressure in the XCell™ ATF system to stabilize.
7. Record the values for both the retentate and filtrate pressure gauges, PG1 and PG2.
8. After 60 minutes, record these pressure values again.
9. Any drop-in pressure should not exceed 1psi.
10. If the Pressure Test has failed, find the leak and repeat steps 4 through 9 again.
11. To depressurize the system, disconnect the pressurization line and slowly open V4 to depressurize the filtrate side first, then open V5 to depressurize the retentate side.

6. Assembly of XCell™ ATF System for Wetting and Integrity Testing

Figure 2. XCell™ ATF System filtration assembly prepared for wetting and integrity testing



Slight variations in assembly are possible depending on the configuration of equipment purchased and its intended use. For more detailed assembly instructions, refer to the manual or contact Repligen.

7. Filter Wetting

After the filter cartridge is installed and the pressure test confirms an integral assembly, the filter must be wetted prior to autoclaving. Rinsing filters prior to use is a standard practice within the industry and the pre-autoclave integrity test requires a fully wetted cartridge. The following wetting liquids should be used with the indicated filters and are intended as single-use-pass through the filter to disposal. It is recommended that the filter be flushed with the appropriate wetting agent until a permeate volume of 2 ml/cm^2 of surface area is reached:

- **Micro-filtration (MF) 0.1-0.65 µm** - use WFI or buffer as a wetting agent.
- **Ultra-filtration (UF) 10-750 KDa** - use 10-25% isopropyl alcohol (IPA).

Table 1. Wetting volume and flow rate

XCell™ ATF	Minimum wetting volume (L)	MF wetting flow rate (L/min)*
2	2.6	0.2
4	15.4	1
6	50	4
10	220	15

*For UF membranes, use 10 psi feed pressure

1. Assemble the XCell™ ATF Device according to Figure 2.
2. Connect the container with the wetting solution volume outlined in Table 1 to V5 and place the tubing into a peristaltic pump. Direct feed from a WFI drop may also be used, provided there is a means to modulate the pressure and flow to satisfy the settings in Table 1.
3. Close V2 and V4, keep V1, V3, V5 open.
4. Pump the liquid into the system from the bottom up. Ensure that no air enters the system. (Flow should occur from bottom to top as this naturally removes the air from the system. Top to bottom can result in trapped air, which may lead to incomplete filter wetting and a failed integrity test result).

5. Continue filling the system until the first sign that the liquid begins to flow from V1. Stop the pump.
6. Close V1. Keep V3 and V5 open.
7. Start the peristaltic pump to wet the filter at the following recommended flow rates noted in Table 1.
8. Once liquid begins to flow from V3, tilt the ATF at 20° away from V3 to facilitate air removal from the top of the filtrate compartment until no more air flows through.
9. After wetting is complete, stop the peristaltic pump.
10. Close V3 and V5 and allow the UF filter to soak in the wetting solution for at least 1 hour (Step not needed for MF filters).
11. Remove the liquid from the system, both the filtrate and retentate chambers: Open V4 and V3 (remove the harvest line) to drain the filtrate side by gravity; then, open V1 and V5, and slowly run the peristaltic pump in reverse to drain the retentate side.

Note: *If IPA is used for wetting, repeat the above procedure at least three times with WFI to thoroughly remove the IPA. Soaking is not required for these additional WFI flushes to maximize removal of liquid from the system, the diaphragm must be pushed up by slowly adding air through the 0.2 µm air inlet filter up to 2 psi, ensuring not to overinflate the diaphragm. Tilt the XCell™ ATF towards V5 by 20° to facilitate drainage. Leaving a small amount of WFI (i.e. milliliters) inside the pump is acceptable for autoclaving.*

8. Integrity Testing (Pre-Autoclave)

Repligen strongly recommends the practice of a pre-autoclave integrity test to ensure a proper assembly prior to autoclaving.

1. Refer to Figure 2 for proper set up. Equipment must be at ambient temperature.
2. Close V1, V4 and V5. Keep open V3.
3. Connect air pressurization line to V1.
4. Open V2 to monitor the pressure on PG1.
5. Open the pressurization line at V0.
6. Carefully regulate V1 to pressurize the retentate chamber to 10 psi for MF membranes and 20 psi for UF membranes. Close V1 rapidly to hold the pressure.
7. Allow for a 1-minute equilibration period prior to taking either pressure or flow readings.
8. Record PG1 and PG2 initial values.
9. Monitor the pressure decay rate on PG1: Record PG1 and PG2 pressure at one minute intervals for 5 minutes.

Note: *PG2 should remain at zero.*

10. Calculate the average pressure decay rate over 5 min. See Table 2.2 below for pressure decay rate criteria. The calculated decay rate should not exceed those in Table 2.2.

Note: *The air diffusion rate may also be used for integrity assessment by monitoring the flow rate from V3 using a digital mass flow meter. Integrity test instruments may also be used to test the integrity of filters.*

Table 2.1 and Table 2.2: Pre-Autoclave Filter Integrity test criteria for XCell™ ATF filters: (Maximum allowable values at 10 psi)

Table 2.1

XCell™ ATF Filter	Fiber spec. (sccm/ m^2)	Filter area (m^2)
2	20	0.13
4	20	0.77
6	20	2.5
10	20	11

Table 2.2

XCell™ ATF Filter	Diffusion rate (ml/min)	Retentate volume (L)	Pressure decay rate (psi/min)
2	2.6	0.16	0.26
4	15.4	0.77	0.29
6	50	2.37	0.31
10	220	10.7	0.30

9. Integrity Testing (Post-Autoclave)

1. Refer to Figure 1 for proper setup. Equipment must be at ambient temperature.
2. Clamp Flex Hose with a C-clamp to create a dead end.

Note: *If Flex Hose is reinforced, a bivalve like V1 must be included in the setup, in-line between pressure gauge PG1 and vent F4.*

3. Close V2 and V4. Open V3 and V5.
4. Pressurize retentate-side by introducing air through sterile vent filter F2 up to 10 psi for MF membranes and 20 psi for UF membranes.
5. Close V5 rapidly to hold the pressure.
6. For Pressure Decay, monitor pressure from pressure gauge PG1 at one-minute intervals for 5 minutes. Calculate the average pressure decay rate over 5 minutes. See Table 3.2 for pressure decay rate criteria. The calculated decay rate should not exceed those on Table 3.2.
7. For diffusion rate, monitor diffusion rate by attaching digital flow meter to sterile vent filter F3. See Table 3.2 below for diffusion rate criteria. The diffusion rate should not exceed those listed in Table 3.2.

Note: *The post-autoclave maximum levels are elevated above the pre-autoclave levels as the membranes may partially dry out during the autoclave cycle.*

Table 3.1 and 3.2: Post-Autoclave Filter Integrity test criteria for XCell™ ATF filters: (Maximum allowable values at 10 psi)

Table 3.1

XCell™ ATF Filter	Fiber spec. (sccm/ m^2)	Filter area (m^2)
2	30	0.13
4	30	0.77
6	30	2.5
10	30	11

Table 3.2

XCell™ ATF Filter	Diffusion rate (ml/min)	Retentate volume (L)	Pressure decay rate (psi/min)
2	3.9	0.16	0.39
4	23.1	0.77	0.44
6	75	2.37	0.47
10	330	10.7	0.45

10. Autoclaving Protocols

The XCell™ ATF 2 uses a small diameter, self-contained hollow fiber cartridge design connected to a small diaphragm pump. This device can be autoclaved using a typical pre-programmed liquid cycle using a temperature setting of 121° C and a duration of 30 minutes. The XCell™ ATF 4, 6, and 10 have stainless steel housings to enclose the hollow fiber cartridge. To ensure both device sterility and avoid thermal stress that may damage the hollow fiber cartridge, these larger designs require the use of a more complex autoclave sequence.

The recommended cycle sequence noted below should be programmed by the customer. Repligen strongly advises adherence to the parameters of the custom program below to ensure satisfactory results with sterility and to maintain the cartridge integrity and performance. In some cases, it may be necessary to consult with the autoclave manufacturer to assist with the autoclave programming. This procedure includes three pre-vac pulses which provided proper chamber evacuation for a faster and more consistent ramp to the required temperature.

CAUTION: Pre-programmed cycles by the autoclave supplier that are based on “liquid or dry cycles“ are not recommended for use on the XCell™ ATF 4 through 10 assemblies. Theses cycles may result in sterility failures and damage to the hollow fiber cartridges.

1. Ensure that the filter has been fully wetted (and IPA removed) as per section 7, Filter Preparation, of this guide and drained of liquid.

2. Prepare the XCell™ ATF Device filtration assembly for autoclaving with appropriate vent filters, as illustrated in Figure 1. See Table 4.1 and 4.2 for recommended vent filter specifications to ensure adequate gas flow in and out of the assembly during the cycle for each vent filter(F#).

Table 4.1 and Table 4.2: Vent Filter Required Surface Area (F 1-5 corresponds to Vent Filter locations noted in Table 1)

Table 4.1

XCell™ ATF Filter	F1	F2	F3
2	0 .002 m^2	NA	0.002 m^2
4	≥ 0.15m^2	≥ 0.05m^2	≥ 0.05m^2
6	≥ 0.15m^2	≥ 0.05m^2	≥ 0.05m^2
10	≥ 0.3m^2	≥ 0.15m^2	≥ 0.15m^2

Table 4.2

XCell™ ATF Filter	F4	F5
2	0.002 m^2	NA
4	≥ 0.05m^2	≥ 0.15m^2
6	≥ 0.05m^2 * ≥ 0.15m^2 **	≥ 0.15m^2
10	≥ 0.3m^2 * ≥ 0.05m^2 **	≥ 0.3m^2
* SUB Connection ** SIP Connection		

3. Keep all valves open.
4. Keep the large clamps slightly loose for autoclave.
5. The system should be placed in the autoclave. A vertical fit is preferred, but an angled fit is acceptable. Do not place in a horizontal position.

Autoclave cycle sequence parameters:

6. Preheat the XCell™ ATF Device 60-90 minutes, using autoclave jacket heat only (no direct steam) with the load probe positioned in the drain. The cartridge should reach a temperature of ~60° C after this exposure due to the convective heat within the chamber.
7. Follow with a purge cycle for 5 minutes- the pressure should not exceed 6 psig.
8. Start first vacuum cycle, using exhaust rate of -1 psig/min, up to a reading of – 6 psig.
9. Hold at maximum vacuum for one minute.
10. Apply steam pressure to 6 psig and hold for 20 minutes.
11. Perform a total of three vacuum/pressure cycles, identical to steps 8, 9 and 10.
12. Apply a fourth vacuum cycle using the exhaust rate of -1 psig/min to -6 psig and hold for 1 minute.
13. Gradually apply steam pressure to reach the sterilization setting of 15 psig / 121-123° C, by limiting temperature rise to 1° C/min. This sequence should take 30-35 minutes.
14. Sterilize for 55-60 minutes.
15. Reduce the autoclave temperature at rate of 0.5-1° C/min to about 0.1 psig or 100° C. A slow rate of cooling is preferred to avoid imposing thermal stress on the filter cartridge and to avoid partially drying out the water in the membrane pores.
16. The autoclave may be opened at this time to allow the XCell™ ATF Device to cool down to ambient temperature. Optionally, continue temperature drop at rate of 0.5-1.0° C/min until safe handling temperature is reached (40-50°C).
17. After opening the autoclave door, tighten all connections especially the large clamps when the ATF temperature reaches 50°C or less. Inspect for any damage or other irregularities.
18. Leave all vents open. Close valves only after complete cool down to ambient room temperature.
19. If the XCell™ ATF Device filtration assembly is to stand unused for more than 12 hours,

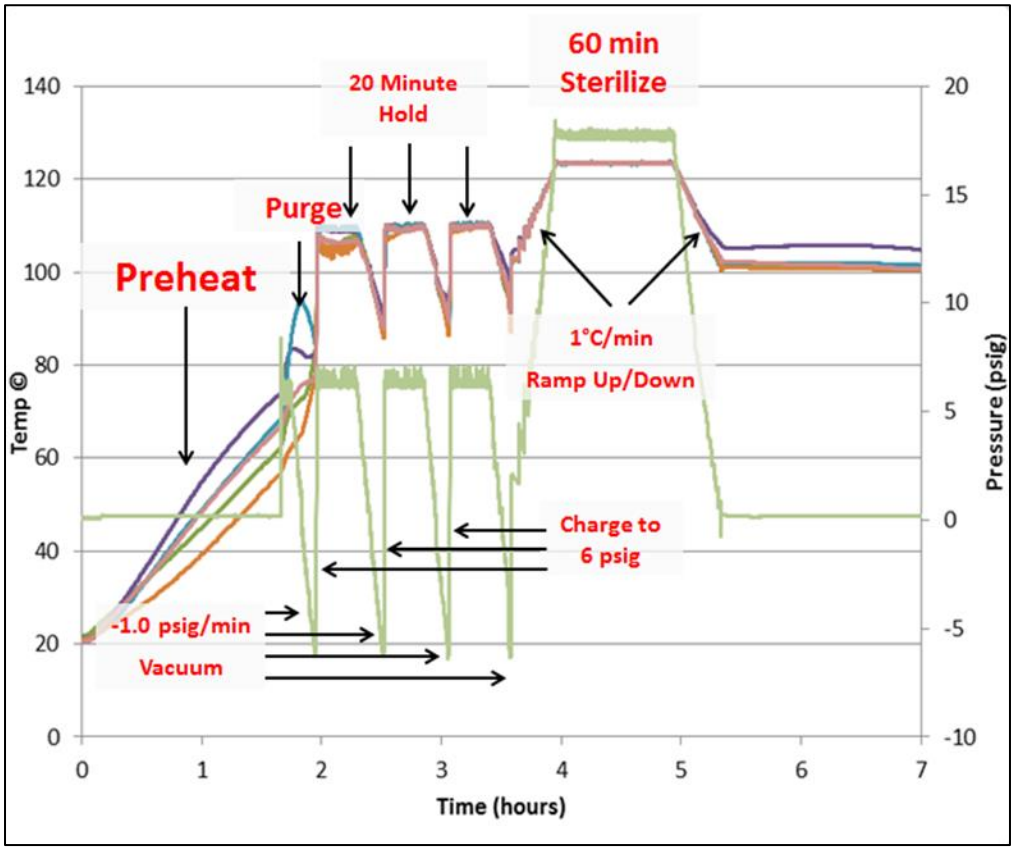
pressurize the system with air through the vent filter of the shunt line (V4/V5) up to 5 psi until use.

Table 5. Autoclave Cycle Program Parameters

Step #	Stage	Time (min)	Set point	Chamber* Temp. (°C)	Chamber * Press (psig)	Notes
6	Pre-heat	60-90	60°C	59	0.1	Convective heat gently pre-warms the assembly
7	Purge	5	6	110	6	Steam flow removes chamber air
8	Vac. #1	12	-1.0 psig/min	85	-6 psig (12“ Hg vacuum)	Remove air/steam mixture at a controlled rate of change
9	Vac. Hold	1	< -6 psig (< 12” Hg vacuum*)	81	-6 psig (12“ Hg vacuum)	
10	Charge	< 1	6 psig	110	6	Steam to a low pressure setting
10	Hold	20	6 psig	110	6	The 20 min hold allows time for the temperature within the assembly to equilibrate
11	Vac. #2	12	-1.0 psig/min	85	-6 psig (12“ Hg vacuum)	Repeated pulses with holds removes air and uniformly warms the assembly
11	Vac. Hold	1	< -6 psig (< 12” Hg vacuum)	85	-6 psig (12“ Hg vacuum)	
12	Charge	<1	6 psig	100	6.0	
12	Hold	20	6 psig	110	6	
14	Vac. #3	12	-1.0 psig/min	85	-6 psig (12“ Hg vacuum)	
15	Vac. Hold	1	< -6 psig (< 12” Hg vacuum)	85	-6 psig (12“ Hg vacuum)	
16	Charge	<1	6 psig	110	6	
17	Hold	20	6 psig	110	6	
18	Vac. #4	12	-1.0 psig/min	85	-6 psig (12“ Hg vacuum)	
19	Vac. Hold	1	< -6 psig (< 12” Hg vacuum	85	-6 psig (12“ Hg vacuum)	
20	Charge	36	1 psig/min	122.6	17.1	Gradually increase the steam pressure to the sterilization settings
21	Sterilize	60	17 psig	122.6	17.1	All areas within the assembly reach the set point. Do not exceed 123°C.
22	Exhaust	17	0.5 psig	100	0.4	Gently cooling the cartridge is equally critical to avoid thermal shock.
23	Cool		50°C			

* Indicates the approximate value at end of stage (absolute vacuum = -14.7 psig or 29.9.

Figure 3. Autoclave Cycle



This graph shows each part of the autoclave cycle for illustrative purposes.