IMPORTANCE OF AIR REMOVAL IN AN AUTOCLAVE STEAM STERILIZATION PROCESS

Introduction
The presence of air in an autoclave sterilization cycle can adversely affect steam penetration and steam contact with the materials being sterilized. Saturated steam condensing onto a surface is essential in killing microorganisms that may be present on the material surface. In prevacuum style autoclaves, air in the chamber is replaced with saturated steam through a series of alternating vacuum and steam injection pulses, as seen in Figure 1.

Figure 1. Simplified Temperature and Pressure Curve for A Prevacuum Sterilizer Cycle.

The number of prevacuum pulses, the prevacuum depth and steam injection pressure must be tested and confirmed to be adequate, given the different equipment and load configurations in the autoclave.

The air removal process in a prevacuum autoclave is different than the process for gravity displacement autoclaves, where steam enters the top of the chamber and compresses the air and forces it out of the autoclave drain at the bottom of the chamber. To verify air does not impede the air removal process in a prevacuum steam sterilizer, STERIS recommends routinely performing an air removal test in the autoclave.

This Technical Tip addresses air removal tests, regulatory guidelines for usage and the microbiological risk mitigation.

AIR REMOVAL TEST

Air removal tests challenge the autoclave prevacuum cycle for sufficient air removal, as well as indicate leaks in the chamber and/or associated piping. It is not only important to remove air prior to steam sterilization, but also to ensure air is not being drawn into the autoclave during the prevacuum process. An air removal test package is placed at the bottom of the autoclave, close to the drain, as this location is where air is most likely to become entrained in the chamber. It is important that the sterilizer jacket and the associated steam supply are at full operating temperature before performing an air removal test. Therefore, a warm up cycle is conducted prior to the test cycle to ensure the sterilizer jacket is at the correct temperature and to also help purge the steam supply of any excessive moisture and non-condensable gases (air, carbon dioxide, etc) which may build up when the sterilizer is not in use for long intervals, e.g. overnight.

Air removal test results can be affected by the number of prevacuum pulses, the prevacuum depth and the steam injection pressure. Other cycle parameters that might affect the test include purge times and hold times at various vacuum and/or pressure setpoints. It is important that the same prevacuum parameters are used for the air removal verification cycle and the production autoclave cycle, as this is the only way the test accurately measures performance of the autoclave air removal cycle.

An air removal test package can be in one of many forms, including Bowie-Dick Test Packs, Bowie-Dick Test Cards and Dart® Testing Apparatus (to determine the effectiveness of removing air from a chamber).

Bowie-Dick Type Test Pack

Bowie-Dick Test Packs consists of a wrapped stack of steam-penetration (air removal) barriers with a chemical indicator sheet in the center. The test pack is placed directly in an otherwise empty steam sterilizer chamber, with no retaining device required. During processing, the cycle must remove or displace the air from the barrier material and replace it with steam throughout the pack. A color change on the indicator (from yellow to a uniform blue/purple) indicates adequate steam penetration. The thermochromic ink formulation can also indicate problems with steam quality (presence of non-condensable gasses, wet steam and/or superheated steam) and is free of lead and other heavy metals. Bowie-Dick Test Packs are preassembled single-use test packs designed to evaluate the performance of the air removal system of a pre-vacuum equipped sterilizer at 121°-124°C (250°-255°F) or 132°-135°C (270°-275°F). The test conforms to ISO11140-5 and EN 867-4 Class 2/B.
Dart® Testing Apparatus:

The Dart® Testing Apparatus consists of a clear plastic enclosure containing insulator material and a paper indicators strip with six bars of chemical indicator ink printed on one side. The enclosure has a hole in one end to allow air removal and steam penetration. This preassembled card is used to evaluate the effectiveness of air removal from the steam sterilizer chamber during a single or multiple prevacuum steam sterilizer cycle. The test is designed for use in a 132°-134°C (270°-274°F) prevacuum cycle with an exposure time of 3-1/2 to 4 minutes. Following the test cycle, the chemical bars of the Bowie-Dick Test Card uniformly darken, indicating that air has been sufficiently removed to allow complete steam penetration into the test card. If air is trapped in the card during the exposure phase of the cycle, the bars will not darken to an equal degree. Thus, the card can provide an immediate indication of inadequate removal of air during a cycle.

The Following Technical Data Sheets are available for applicable Test Cards:

- 450-100-5581-VERIFY® S3095 Bowie-Dick Test Card for Multiple Prevacuum Steam Sterilizers
- 450-100-5583-VERIFY® S3098 Bowie-Dick Test Card for Single Prevacuum Steam Sterilizers

Daily Air Removal Test

The Dart® Testing Apparatus is functionally equivalent to the Bowie-Dick Test package recommended by the Association for Advancement of Medical Instrumentation (AAMI) in determining the air removal efficiency of prevacuum sterilizers. It is a preassembled test to evaluate the effectiveness of air removal from the sterilizer chamber during a prevacuum steam sterilizer cycle at 132°-134°C (270°-274°F) and conforms to ISO 11140-5 and EN 867-4. The Following Technical Data Sheet is available for the STERIS Dart® Testing Apparatus:

- 450-100-5621-DART® Daily Air Removal Test for Prevacuum Steam Sterilizers

Regulatory Considerations

The pharmaceutical and biotech industry is required to meet regulatory guidelines regarding the use of air removal tests in prevacuum autoclave sterilizers. The International Organization for Standardization (ISO) along with the Association for the Advancement of Medical Instrumentation (AAMI), British Standards (BS) and European Standards (EN) and the American National Standards Institute (ANSI) all provide guidance regarding the use of air removal tests. Excerpts and references defining the use of daily air removal tests are provided. European and British guidance BS EN 554: Validation and Routine control of sterilization by moist heat has been harmonized with ISO and is superseded by ISO 17665-1: 2006. The following documents provide guidance on the use of the air removal verification tests:

1. ISO 17665-1:2006, section 12.1.6 states:

"If the sterilization process relies on the removal of air from the sterilizer chamber in order to achieve rapid and even penetration of steam into the sterilizer load, a steam penetration test shall be carried out each day before the sterilizer is used.

The steam penetration test is carried out using a device having a defined challenge to air removal and steam penetration for the process. For industrial applications, if the saturated steam process uses consistent, defined sterilization loads known not to inhibit the penetration of steam, alternative methods may be used based on specified physical measurements and a risk assessment of the likelihood of process failure."

While the second paragraph may suggest that a daily steam penetration test (air removal test) is not mandatory for some load types, not routinely performing the test involves risk and needs to be evaluated. Alternative steam penetration verification methods may not be reliable, reproducible or as readily accepted by regulatory agencies.

ISO/TS 17665-2 provides guidance on the application of ISO 17665-1 and defines the use of daily air removal tests, as follows:

The Following Technical Data Sheets are available for Air Removal Test Packs:

- 450-100-5581-VERIFY® S3095 Bowie-Dick Test Card for Multipulse Prevacuum Steam Sterilizers
- 450-100-5583-VERIFY® S3098 Bowie-Dick Test Card for Single-Pulse Prevacuum Steam Sterilizers
- 450-100-5609-TST Single Use Bowie-Dick Type Test Pack for 121°C (250°F)
- 450-100-5610- Steraffirm® Bowie-Dick Test Pack For Exposure Temperature 121°-124°C (250°-255°F)
- 450-100-5672-TST Bowie-Dick Test Pack For Exposure Temperature 134°-137°C (273°-279°F)

Section A.5.1 Bowie and Dick Test. "This test is a steam test, similar to the small load test and intended for daily use."

Annex A (Evaluation of a sterilizer process primarily based on the measurement of physical parameters), Table A.3. Suggests the daily use of Bowie and Dick Tests.

Annex B (Evaluation of a sterilization process primarily based on biological inactivation and an accompanying mechanical air removal procedure), Table B.1. Suggests routine air removal tests.

Although applicable mainly to healthcare facilities, ANSI/AAMI also recommends the daily use of Bowie-Dick Tests for air removal verification.

3. ANSI/AAMI ST79, section 10.7.6.1 "The Bowie- Dick Test should be carried out each day the sterilizer is used, before the first processed load."

4. FDA Guidance, September 2004 "It is important to remove air from the autoclave chamber as part of a steam sterilization cycle....careful consideration should be given during sterilization validation to the nature or type of material chosen as the carrier of the biological indicator to ensure an appropriately representative study."

Current Good Manufacturing Practices (cGMPs) require manufacturers to demonstrate and document that the sterilization process is effective, controlled and reproducible. The daily use of air removal tests helps meet cGMPs, by demonstrating the effectiveness of prevacuum cycles in stream sterilizers. The chemical indicator sheet (taken from the air removal tests) provides evidence for documentation purposes.

Risk Mitigation

Frequency of performing the air removal test is determined by evaluating the quality, business and regulatory risk. The impact of not detecting sufficient air removal during the autoclave prevacuum cycle must be taken into consideration, as the sterility of all materials processed since the last passing test would be called into question.

Inadequate air removal from an autoclave has direct impact on product quality; therefore failure to comply leads to a lengthy Quality Assurance investigation. Depending on investigation results, the worst case scenario is non-sterile product and possible product recall. Equipment remediation requires downtime and lost production, which impacts overall business. Non-compliance with regulatory expectations and cGMPs may result in citations and other negative repercussions.

In routine production, the probability of not adequately removing air from the autoclave chamber may be low. However, the impact of not detecting inadequate air removal is quite severe, as this has direct impact on sterility assurance of the materials. Therefore, daily air removal verification using a Bowie-Dick Test package or Dart® Testing Apparatus is the simplest and most robust form of detection.