Understanding Steam Sterilization Monitoring Devices for Load and Pack Control: *Biological Indicators, Class 5 Integrating Indicators and Class 6 Emulating Indicators*

**Background:**

In December, 2005 the International Organization for Standardization (ISO) published a new document, ISO 11140-1: 2005 *Sterilization of healthcare products – Chemical Indicators – Part 1: general requirements.* The AAMI organization has approved this standard *without* US deviations, which means this document is now a recognized AAMI Standard.

When compared to ANSI/AAMI ST60:1996, there are two key changes you should be aware of:

1. Class 5 Integrating Indicator requirements were upgraded.
2. Class 6 Emulating Indicators, which were already part of an earlier ISO standard, are now included in the AAMI Standard. Additionally, the 2008 update to AAMI ST79 will include a definition of Class 6 Emulating Indicators. Given this, it is important to understand the difference between Biological Indicators, Class 5 Integrating Indicators, and Class 6 Emulating Indicators.

The remainder of this tutorial will discuss the differences between Biological Indicators, Class 5 Integrating Indicators, and Class 6 Emulating Indicators. By understanding the differences between these monitoring devices, Sterile Processing professionals can make informed decisions about which monitoring devices are most appropriate for load or pack control.

**Key Definitions:**

Understanding the key terms is critical in understanding the differences in Biological Indicators, Class 5 Integrating Indicators and Class 6 Emulating Indicators.

- **Critical variable:** "parameters identified as being essential to the sterilization process (and requiring monitoring)."1
• **Endpoint**: “point of the observed change as defined by the manufacturer occurring after the indicator has been exposed to specified stated values.”¹ For example, the endpoint for a successful cycle for a moving-front style chemical indicator would be the color bar moving into the “accept” area (see Figure 1).

• **Resistometer**: a specialized test vessel capable of reproducible cycles and used by manufacturers to characterize the performance of chemical and biological indicators.

• **Chemical Indicators (CI)**: “Devices used to detect the presence or attainment of one or more of the parameters required for a satisfactory sterilization process, or are used in specific tests of sterilization equipment.”³ (see Figure 1).

• **Stated value (SV)**: “value or values of a critical variable at which the indicator is designed to reach its endpoint as defined by the manufacturer.”¹ For example, one of the three stated values for a Class 5 Integrating Indicator with a stated value of 3.5 minutes at 134°C should reach its endpoint when tested at 134°C for 3.5 minutes in a resistometer (specialized test vessel).

• **Come-Up Time**: The time it takes for the sterilizer to achieve the selected exposure temperature. *(This would be similar to the preheating of a conventional oven.)*

• **Biological Indicator (BI)**: “Test system containing viable microorganisms providing a defined resistance to a specified sterilization process.”³ Hospitals typically use a self contained BI (as shown in Figure 2 with the 3M™ Attest™ 1292 Rapid Readout Biological Indicator). Biological indicators provide the only direct measure of the lethality of the sterilization process.

---

1. U.S. Pat.No. 4,448.548

2. U.S. Pat.No. 4,448.548

3. U.S. Pat.No. 4,448.548

---

3M Comply™
Steam
Chemical
Integrator
Class 5

Figure 1: Illustration of endpoint moving-front style chemical indicators
Source: 3M internal data

Figure 2: 3M™ Attest™ 1292 Rapid Readout Biological Indicator
Frequently Asked Questions:

1. What are the standard recommended practices for monitoring and releasing loads with biological indicators, Class 5 integrating indicators, and Class 6 emulating indicators?

**Load:** AAMI ST79 recommends weekly, preferably daily routine sterilizer efficacy testing and sterilizer qualification testing with a biological indicator process challenge device (BI PCD) (10.5.3.2).³ Additionally, AAMI ST79 recommends all loads containing implants be monitored with a PCD that contains both a BI and a Class 5 integrating indicator (10.6.1). Loads containing implants should be quarantined until the results of the BI are known unless a documented emergency situation exists (10.6.3). Biological indicators also should be used for periodic quality assurance testing of representative samples of actual products being sterilized (10.5.3.2).³

The 2008 AORN Recommended Practices for Sterilization recommends that flash sterilization should not be used for implantable devices except in cases of emergency when no other option is available (12(PNDS: I85, I138). In an emergency, when flash sterilization of an implant is unavoidable, a rapid-action BI with a Class 5 integrating indicator (or enzyme only indicator) should be run with the load 3,12(PNDS: I70, I98)IV.h.1. The implant should be quarantined on the back table and should not be released until the rapid-action BI provides a negative result.⁴

**Pack:** According to AAMI ST79* recommended practice a Class 3, Class 4 or Class 5 CI should be used inside each package, tray or rigid sterilization container system. (Section 10.5.2.2.2 Internal Chemical Indicators)

The 2008 AORN Recommended Practices for Sterilization recommends use of Class 5 chemical integrating indicators within each sterilizer container or tray. A sterilization chemical indicator should be used inside and outside each package and load sterilized. Internal chemical indicators do not establish whether the item is sterile, but they do demonstrate that the contents were exposed to the sterilant. Chemical indicators should be reviewed for a proper endpoint response (eg. color, migration or other change).

* The 2008 update will only include the definition for Class 6 Emulating Indicators. No use or application will be provided in this update.

2. What are the performance differences between a biological indicator, Class 5 integrating indicator, and a Class 6 emulating indicator and how do those differences affect my monitoring processes?

The design differences are outlined in the table on the following page:
**DESIGN OF**

<table>
<thead>
<tr>
<th>Biological Indicators</th>
<th>Class 5 Integrating Indicators</th>
<th>Class 6 Emulating Indicators</th>
<th>How does this affect my monitoring processes?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological indicators are designed to demonstrate whether the conditions were adequate to achieve sterilization. Biological indicators contain a known number of resistant spore-forming microorganisms to monitor adequacy of the sterilization process.</td>
<td>Class 5 integrating indicators are designed to react to all critical variables (time, temperature, and the presence of steam) and have Stated Values that correlate to a BI at three time/temperature relationships.</td>
<td>Class 6 emulating indicators are designed to react to all critical variables (time, temperature, and the presence of steam) for a specified sterilization cycle. You may hear Class 6 indicators referred to as cycle specific indicators.</td>
<td>Biological indicators continue to be the highest level of sterilization cycle monitoring and are widely recommended as the preferred monitoring device for releasing loads and monitoring sterilization cycles.</td>
</tr>
</tbody>
</table>

**IMPLICATIONS**

| Biological indicators are the only true measure of the sterilization process lethality, they are considered to be the highest level of sterilization cycle monitoring. BI's are widely recommended as the preferred monitoring device for releasing loads and monitoring sterilization cycles. | Class 5 integrating indicators must have three Stated Values at 121°C, 135°C, and at one temperature in between that correlate to a BI. Additionally, the Stated Value at 121°C must not be less than 16.5 minutes. This guarantees the time/temperature response for a Class 5 integrating indicator will respond like a BI when exposed to ideal, saturated steam (see Figure 4). Therefore, if the exposure temperature was not achieved where the Class 5 CI is located in the sterilizer and the BI result was positive (indicating a sterilization failure), the Class 5 CI will respond like the BI and also indicate that a failure had occurred as it mirrors the thermal death rate curve of *G. stearothermophilus* in the BI. | Class 6 emulating indicators have one Stated Value for time and temperature for the specific cycle it is designed for. There is no requirement for three Stated Values for time and temperature and therefore the response may not correlate to a BI (see Figure 5). Note that at lower temperatures the Class 6 response can fall below that of the BI performance (thermal death rate curve of *G. stearothermophilus*) (see Figure 5). | BI's provide the only direct measure of sterilization process lethality. Chemical indicators and emulating indicators seek to mirror the death curve of a BI or response at a specific temperature, respectively. Class 5 integrating indicator and Class 6 emulating indicator performance is not tested in poor steam conditions such as superheat or air/steam mixtures. Additionally, CI's performance compared to a BI is also not tested in these conditions. |

Because the Class 5 integrating indicator response at lower temperatures parallels the biological response, Class 5 CIs are able to detect the failure condition where the desired exposure temperature is not achieved. This condition is likely to occur when there is:

- incorrect packaging
- incorrect loading
- air/steam mixtures
- an incorrect cycle for load contents

Because the Class 6 response at lower temperatures can fall below the thermal death curve of *G. stearothermophilus*, the Class 6 emulating indicator can reveal a pass when the BI would indicate a failure.
3. Does the Class number have any significance? For example, is a Class 6 CI better than a Class 5, and do biological indicators have a class?

Today, there are six classes of chemical indicators. The current ISO document classifies chemical indicators by their intended use and these classifications have no hierarchical significance. For example, the Bowie-Dick Test is a Class 2 chemical indicator and probably provides more information about the steam sterilizer performance than any other class of chemical indicators. ISO provides performance requirements for each classification.

Biological indicators are not subject to a classification system. Biological indicators required for a sterilization process contain resistant spore-forming microorganisms to respond to the conditions within the cycle. Chemical indicators can be made with a multitude of different formats and chemistries. Thus, how they respond to the sterilization process can differ depending on the design. A process or single variable chemical indicator is only required to respond to one of the critical variables, while another type of indicator (i.e., a Class 5 integrating indicator must respond to all of the critical variables).

4. Has 3M completed performance testing of biological indicators, Class 5 integrating indicators, and Class 6 emulating indicators in failure conditions?

Yes, 3M has tested its biological indicators and Class 5 integrating indicator as well as two commercially available Class 6 emulating indicators in several different steam sterilization conditions.

The data from these studies was used to generate the graphs in Figures 4 and 5. The tests were done in a steam resistometer, the special test vessel that generates ideal saturated steam and is the vessel required for ANSI/AAMI/ISO Compliance Testing.

What this testing revealed is that the 3M Class 5 integrating indicators do, in fact, correlate to the biological response or thermal death rate curve of *G. stearothermophilus*. Even though the Stated Values of the Class 6 emulating indicators were 3.5 minutes at 134°C, their time/temperature response compared to the biological response was faster at lower temperatures.

This 3M study also included testing 15 of each type of Class 5 and Class 6 indicator in a shortened, hospital type cycle (see Figure 3). All the Class 6 products gave a PASS result which means they failed to detect the shortened exposure time even though their Stated Values at 134°C were greater than that of the Class 5 product. This data shows that the Class 6 products responded during the come-up time and progressed to their endpoint too soon to detect the steam sterilization process failure that occurred as a result of not remaining at the exposure temperature for an adequate time.

<table>
<thead>
<tr>
<th>Indicator Type</th>
<th># Showing FAIL/# Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 5 (SV: 2.2 Min. @ 134°C)</td>
<td>15/15</td>
</tr>
<tr>
<td>Class 6 (SV: 3.5 Min. @ 134°C)</td>
<td>0/15</td>
</tr>
</tbody>
</table>

Figure 3: Class 5 vs Class 6 CI Results in a Failure Condition
Source: 3M internal test result

**Hospital-Type Gravity Steam Cycle:**
1-Minute Exposure
at 134°C; 4-Minute Come-up Time

**Figure 3: Class 5 vs Class 6 CI Results in a Failure Condition**
Source: 3M internal test result
These monitoring products are tested under ideal saturated steam conditions to satisfy both FDA guidance document and ISO performance testing requirements. However, a scientific study investigating performance of these monitoring products in suboptimal steam sterilization conditions, e.g., superheated steam and incomplete air removal, reveal that only biological indicators are capable of consistently detecting these types of failure modes. Thus, biological indicators will respond correctly by showing positive results under common failure modes, whereas chemical indicators may not.

5. Why are resistometers used for testing chemical and biological indicators?

Resistometers are specialized vessels that can create reproducible test cycles. They were chosen by the Standard Committees so manufacturers utilize common reproducible cycles to verify CI and BI performance. There are hundreds of possible hospital cycles where the depth of vacuum, the number of steam/vacuum pulses, the sterilizer come-up time, and the steam quality differ. These differences would make it impossible to provide consistent CI performance results for in hospital sterilizers.

Steam resistometers must reach the desired temperature in < 10 seconds (see Figure 6). Hospital sterilizers, however, may have up to 10 minutes of pre-conditioning or come-up time (see Figure 7). BI inactivation and CI progression toward endpoint begin to occur during this pre-conditioning or come-up time in hospital sterilizers.

6. So what does this mean in a hospital sterilizer?

The cycle specific data for Class 6 emulating indicators is generated in a resistometer as well, not in a hospital sterilizer. Therefore, in a hospital sterilizer, Class 6 emulating indicators could have significant progression toward their endpoint during the come-up time and reach their endpoint much sooner than their stated value time and temperature.
7. What does 3M recommend regarding load and pack monitoring with biological indicators, Class 5 integrating indicators, and Class 6 emulating indicators?

3M recommendations for load monitoring:

- **To monitor to the highest level of assurance, 3M considers Every Load Monitoring with a BI PCD to be a best practice.**
- 3M recommends daily and implants, preferably every load monitoring using a BI PCD.
- Daily plus implant loads, preferably every load monitoring using a BI PCD.
- A class 5 integrating indicator may be used within a PCD to monitor non-implant sterilizer loads.

3M recommendations for pack monitoring:

- **To monitor to the highest level of assurance use one or more Class 5 integrating indicators inside each package or container.**
- Follow AAMI ST79 recommended practice by using a Class 3, Class 4 or Class 5 CI inside each package. (Section 10.5.2.2.2 Internal Chemical Indicators)
- A Class 6 emulating indicator could be used as an internal indicator at the pack/tray level in cycles for which it is labeled.

![Profile of Steam Resistometer Cycle](image1)
![Profile of Hospital Gravity Cycle](image2)
Key Take Aways:

- Biological Indicators are the only true measure of lethality. They are recommended as the preferred monitoring device for releasing loads and monitoring sterilization cycles by AAMI and AORN.

- A Class 5 integrating indicator reacts to all critical variables and has three Stated Values that correlate to a BI at three time/temperature relationships (see Figure 3). This means a Class 5 integrating indicator will detect failure conditions where the selected exposure temperature is not achieved.

- The Class 5 integrating indicator Stated Value at 121°C must not be less than 16.5 minutes. A CI with a Stated Value at 121°C of less than 16.5 minutes can reach its endpoint too quickly at lower temperatures and miss a sterilization process failure.

- If a load is being released on the results of a Class 5 integrating indicator, it is critical the CI be able to show correct results at lower temperatures i.e. that the CI correlate to a BI over a wide temperature range, similar with BI performance (see Figure 1). Because emulating indicators only require one stated value for time and temperature, at lower temperatures the response of a Class 6 emulating indicator can fall below the BI thermal death curve (see Figure 4).

For more information, call the 3M Help Line: 1-800-228-3957

---


2. ISO 11138-1:2006 Sterilization of health care products -- Biological Indicators -- Part 1: General requirements
   ISO 11138-3:2006 Sterilization of health care products -- Biological indicators -- Part 3: Biological indicators for moist heat sterilization processes

