# How Does An Extended Steam Sterilization Cycle Affect The Early Enzyme Readout Capability Of The 3M™ Attest™ Rapid Readout Biological Indicators?

## —Scientific study results

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## Introduction

In the past few years standard steam sterilization exposure times used by healthcare facilities have increased because of the complexity of instruments and containment devices (i.e., trays, cassettes, rigid containers). What effect do these increased exposure times have on the monitoring tools and packaging used in these cycles? More specifically, what impact do these cycles have on the media contained within self-contained biological indicators (BIs). This scientific study demonstrates whether the 3M™ Attest™ 1292 Rapid Readout Biological Indicator's media ampoules will function properly after exposure to extended steam sterilization cycles and detect surviving spores.[1]


## Scientific Method

3M Attest Rapid Readout Biological Indicator (BI) performance in extended cycles was assessed by comparing BIs assembled with media ampoules exposed to an extended cycle with controls (BIs with media ampoules unexposed to an extended cycle). Several critical BI performance tests were compared. The BIs were evaluated using the survival/kill test and fractional survival method. The fractional survival method is referred to as "fraction negative analysis" and is commonly used to determine the resistance of BIs. In these cycles a fraction of the test samples will show no growth, and the number of surviving organisms can be calculated from this data. The fraction negative analysis establishes a statistically based calculation of surviving test organisms below 50 organisms.[2]

Exposure times in which some of the BIs are growth positive and some growth negative ensures that a very low number of sterilant injured spores survive the cycle, and therefore provides a rigorous challenge to the recovery media and readout system of the self-contained biological indicator. The use of the fractional cycles is the methodology required by FDA to document a reduced incubation time.[3] The study utilized 3M Attest 1292 Rapid Readout BIs taken from three different lots. The BIs from each lot were segmented into two groups of BIs, each labeled "exposed" and "unexposed." The unexposed BIs (not exposed to the extended cycle) were used as controls.

The media ampoules were removed from the "exposed" group of BIs and sterilized in a prevacuum sterilizer for an extended cycle of 20 minutes at 134°C with a 20 minute dry time. Following the extended cycle exposure, the ampoules were reassembled into BIs per 3M assembly specifications. Both the exposed and unexposed BIs were tested in a H&W Resistometer at 134°C using a .65 psia prevacuum. The BIs were exposed using the following times:

- Survival/Kill: 1:00 and 4:00 (min:sec)
- Fractional Cycles: 2:20 and 2:30 (min:sec)

The "exposed" and "unexposed" BIs were activated and incubated in a 3M™ Attest™ 290 Auto-reader for 3-hour fluorescence readout and further incubated for visual growth in humidified incubators for 7 days. The 3-hour, 48-hour and 7-day results were compared.

## Results

The three lots of 3M Attest Rapid Readout BIs containing the "exposed" and "unexposed" (control) media ampoules were all fluorescent and growth positive after the 1.0 minute survival test, and all fluorescent and growth negative after the 4.0 minute kill test. There was no statistical difference in the survival/kill results (see Table 1).

There was no statistical difference in the number of fluorescent and growth positives between the "exposed" and "unexposed" BIs when tested in the fractional survival cycles. In the fractional survival cycles, all of the growth positives were detected within the 3-hour fluorescent readout time. No fluorescent false negatives occurred in the "exposed" or "unexposed" groups (see Table 2).

## Conclusion

The ability of 3M Attest Rapid Readout Biological Indicators with the 3-hour enzyme-based early readout to detect surviving

**Table 1. Survival/Kill Test Summary: Results of Exposed and Unexposed Indicators**

**BIs With Media Exposed To Extended Cycles 134°C 20 min. exposure/20 min. dry time**

| | | No. Positive/24 Tested | | | |
|---|---|---|---|---|---|
| | | Exposure Min:Sec | 3 Hour Fl. | 48 HR Growth | 7 Day Growth |
| Lot A | Exposed | 1:00 | 24 | 24 | 24 |
| | Ampoules | 4:00 | 0 | 0 | 0 |
| Lot B | Exposed | 1:00 | 24 | 24 | 24 |
| | Ampoules | 4:00 | 0 | 0 | 0 |
| Lot C | Exposed | 1:00 | 24 | 24 | 24 |
| | Ampoules | 4:00 | 0 | 0 | 0 |
| **Totals** | **Exposed** | **1:00** | **72** | **72** | **72** |
| | | **4:00** | **0** | **0** | **0** |

**BIs With Media Unexposed to Extended Cycles (Controls)**

| | No. Positive/24 Tested | | | |
|---|---|---|---|---|
| | Exposure Min:Sec | 3 Hour Fl. | 48 HR Growth | 7 Day Growth |
| Unexposed | 1:00 | 24 | 24 | 24 |
| Ampoules | 4:00 | 0 | 0 | 0 |
| Unexposed | 1:00 | 24 | 24 | 24 |
| Ampoules | 4:00 | 0 | 0 | 0 |
| Unexposed | 1:00 | 24 | 24 | 24 |
| Ampoules | 4:00 | 0 | 0 | 0 |
| **Unexposed** | **1:00** | **72** | **72** | **72** |
| | **4:00** | **0** | **0** | **0** |

**Table 2. Fractional Cycle Summary: Results of Exposed and Unexposed Indicators**

**BIs With Media Exposed To Extended Cycles 134°C 20 min. exposure/20 min. dry time**

| | | Exposure Min:Sec | 3 Hour Fl. | 48 HR Growth | 7 Day Growth |
|---|---|---|---|---|---|
| Lot A | Exposed | 2:20 | 17 | 17 | 17 |
| | Ampoules | 2:30 | 10 | 6 | 6 |
| Lot B | Exposed | 2:20 | 17 | 14 | 14 |
| | Ampoules | 2:30 | 14 | 12 | 12 |
| Lot C | Exposed | 2:20 | 17 | 17 | 17 |
| | Ampoules | 2:30 | 16 | 12 | 12 |
| **Total No. Positives** | | | | | |
| **Exposed** | | | **91** | **78** | **78** |

**BIs With Media Unexposed to Extended Cycles (Controls)**

| | Exposure Min:Sec | 3 Hour Fl. | 48 HR Growth | 7 Day Growth |
|---|---|---|---|---|
| Unexposed | 2:20 | 20 | 15 | 15 |
| Ampoules | 2:30 | 12 | 6 | 6 |
| Unexposed | 2:20 | 19 | 16 | 16 |
| Ampoules | 2:30 | 15 | 12 | 12 |
| Unexposed | 2:20 | 20 | 19 | 19 |
| Ampoules | 2:30 | 9 | 7 | 7 |
| **Unexposed** | | **95** | **75** | **75** |

spores is not affected by exposure of the media ampoule to extended cycle conditions (134ºC for 20 minutes and 20-minute dry time). There was no statistically significant difference in the test results between the BIs assembled with "exposed" and "unexposed" media ampoules in the survival/kill and fractional survival method cycles. This scientific study demonstrates that the 3M™ Attest™ 1292 Rapid Readout Biological Indicator's media ampoule will function properly after exposure to extended steam sterilization cycles and detect surviving spores.[1] There will not be any false negative results at 3 hours when used in extended cycles. ✛

## References


1. Michelle et al. User Alert. *The Canadian Journal of Infection Control*, Fall 2006.
2. ANSI/AAMI/ISO 11138-1 *Sterilization of health care products—Biological indicators*—Part 1: General requirements, © ISO 2006.
3. CDRH, *Guide for Validation of Biological Indicator Incubation Time*, Food and Drug Administration, 1985.


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