Effect of Extended Steam Sterilization Cycles on Self Contained Biological Indicators with Enzyme-based Early Readout

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Introduction:

Standard steam sterilization exposure times have increased in recent years due to the increase in weight and complexity of instrument trays used in health care facilities. With the increased use of extended sterilization cycle times, there have been concerns expressed about the impact of these cycles on media contained within self-contained biological indicators (BIs) and the need for BI manufacturers to confirm that their BIs function properly after exposure to the extended cycles.[1]

Objective:

The purpose of this study is to demonstrate whether the 3M™ Attest™ 1292 Rapid Readout Biological Indicator's ampoulized media will function properly after exposure to extended steam sterilization cycles.

Method:

3M Attest Rapid Readout Biological Indicator (BI) performance was assessed by comparing BIs assembled with ampoules exposed in extended cycles to controls (BIs with 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 cycles. 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 method 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 insures 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 ampoules were removed from the "exposed" group of BIs and sterilized in a pre-vacuum 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 re-assembled 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 pre-vacuum. The BIs were exposed using the following times:

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

The "exposed" and "unexposed" BIs were activated and incubated in the 3M™ Attest™ 290 Auto-reader for 3 hour fluorescence 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) ampoules were all fluorescent and growth positive after the 1.0 minute survival test and all fluorescent negative and growth negative after the 4.0 minute kill test. There was no statistical difference in the survival/kill results (refer to the summary "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 cycles. In the fractional 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 (refer to the summary "Table 2").

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

BIs With Media Unexposed to Extended Cycles (Controls)

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

BIs With Media Unexposed to Extended Cycles (Controls)

		No. Positive/24 Tested					No. Positive/24 Tested		
		Exposure Min:Sec	3 Hour Fl.	48 HR/7 Day Growth			Exposure Min:Sec	3 Hour Fl.	48 HR/7 Day Growth
Lot A	**Exposed**	2:20	17	17		**Unexposed**	2:20	20	15
	Ampoules	2:30	10	6		Ampoules	2:30	12	6
Lot B	**Exposed**	2:20	17	14		**Unexposed**	2:20	19	16
	Ampoules	2:30	14	12		Ampoules	2:30	15	12
Lot C	**Exposed**	2:20	17	17		**Unexposed**	2:20	20	19
	Ampoules	2:30	16	12		Ampoules	2:30	9	7
Total No. Positives		**Exposed**	**91**	**78**			**Unexposed**	**95**	**75**

Conclusion:

The 3M Attest Rapid Readout Biological Indicators with the 3 hour enzymatic readout were not affected by exposure of the ampoulized media 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" ampoulized media in the survival/kill and fractional cycles. This study demonstrates that the ampoulized media in the 3M Attest Rapid Readout Biological Indicator functions properly after exposure to extended cycle conditions.

References:

1. Alfa, 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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