

EVALUATION OF A RAPID READOUT BIOLOGICAL INDICATOR FOR FLASH STERILIZATION WITH THREE BIOLOGICAL INDICATORS AND THREE CHEMICAL INDICATORS

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ABSTRACT

OBJECTIVE: Flash sterilization is most commonly used for emergency sterilization of unwrapped items in a gravity displacement sterilizer for three minutes. Sterilization quality assurance is monitored by biological indicators that require a 24-hour incubation prior to reading. In this study, we compared a new biological indicator that provides results within 60 minutes with three conventional, 24-hour biological indicators for monitoring flash sterilization and three chemical indicators.

DESIGN: Conventional biological indicators tested included the conventional Attest 1261, Proof Flash and Assert, while the rapid readout indicator tested was Attest 1291. Attest Rapid Readout detects the presence of a *Bacillus stearothermophilus* enzyme by reading a fluorescent product that is produced by the enzymatic breakdown of a nonfluorescent substrate. Chemical indicators tested included Comply, Incheque, and Thermalog S. Survival at 132°C in a gravity displacement sterilizer was measured by media color change after incubation for 24 hours at 56°C for the three conventional biological indicators, fluorescence at 60 minutes for the Attest

Rapid Readout biological indicator, and color change for the chemical indicators. Each exposure time was replicated four times with 10 of each biological and chemical indicator per run.

RESULTS: The conventional biological indicators (Attest, Proof Flash, and Assert) had 90%, 48%, and 40% spore survival at two minutes exposure; 23%, 3%, and 0% at three minutes exposure; and 3%, 0%, and 0% at four minutes exposure respectively. The Attest Rapid Readout biological indicator had 88%, 33%, and 0% enzyme activity detectable at 2, 3, and 4 minutes exposure. The chemical indicators Comply, Incheque, and Thermalog S revealed sterilization failure rates of 100%, 100%, and 100% at 0 minutes exposure; 100%, 100%, and 45% at one minute; 0%, 0%, and 28% at two minutes exposure; 0%, 0%, and 18% at three minutes exposure; and 0%, 0%, and 0% at four minutes exposure, respectively.

CONCLUSION: The sensitivity of the Attest Rapid Readout parallels the conventional biological indicators. These data suggest that a 60-minute rapid readout biological indicator is equivalent to the 24-hour biological indicators. If

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This study was supported in part by the 3M Company, St. Paul, Minnesota.

Presented in part at the Association of Practitioners in Infection Control's 19th Annual Conference and International Meeting, May 31-June 4, 1992, San Francisco, California.

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Rutala WA, Gergen MF, Weber DJ. Evaluation of a rapid readout biological indicator for flash sterilization with three biological indicators and three chemical indicators. Infect Control Hosp Epidemiol 1993;14:390-394.

further studies demonstrate that a four-minute flash sterilization cycle provides a needed safety margin to ensure sterilization, then consideration should be given to requiring a four-minute flash sterilization cycle. Chemical indicators were too

sensitive to the processing conditions (eg, steam) and are inadequate to ensure adequate sterilization. (*Infect Control Hosp Epidemiol* 1993;14:390-394.)

INTRODUCTION

Sterilization, which is defined as the complete elimination or destruction of all forms of microbial life, is recommended for all "critical" medical items.¹ Items in this category include surgical instruments, cardiac and urinary catheters, implantable devices such as heart valves, and needles. In the hospital, the principal means of sterilization is steam under pressure, although dry heat, ethylene oxide gas, and liquid chemicals may be used in certain settings. Because it is essential to ensure sterilization of critical items, monitoring of the sterilization process is advised. Three types of monitors may be used: mechanical, chemical, and biological. Biological monitors are recognized as the closest to being ideal monitors of the sterilization process.² For this reason, the Association of Operating Room Nurses,³ the Centers for Disease Control and Prevention,⁴ the Association for the Advancement of Medical Instrumentation,⁵ and the Joint Commission on Accreditation of Healthcare Organizations⁶ all recommend at least weekly biological monitoring of steam sterilizers.

Flash sterilization is used for the emergency sterilization of unwrapped, nonporous metal items in a gravity displacement sterilizer for three minutes at 132°C.⁷⁻⁹ Flash sterilization is commonly used in the operating room for emergency sterilization of a dropped or otherwise contaminated instrument, an instrument unintentionally left out of a surgical tray or, inappropriately, to compensate for inadequate inventories of instruments or implantable devices.¹⁰ Conventional biological indicators require 24 hours of incubation prior to reading. However, since a 24-hour quarantine is not practical, patients may be placed at risk. Thus, a biological indicator that provides results within 60 minutes would allow for detection, and possibly prevent the use, of inadequately sterilized instruments.

For this reason, we studied the Attest Rapid Readout biological indicator, which is based on the fluorimetric detection of a spore-bound enzyme, alpha-D-glucosidase, rather than on the observation of spore growth. In this article, we present data comparing this rapid readout biological indicator to three conventional biological indicators and three chemical indicators.

METHODS

Biological Indicators

Our methods were similar to those reported by Vesley et al.⁸ The Attest Rapid Readout indicator employs a dry spore strip containing at least 10⁵ spores of *Bacillus stearothermophilus* derived from ATCC strain 7953. The growth media is a modified tryptic soy broth contained in a crushable glass ampule. The broth contains a nonfluorescent substrate, 4-methylumbelliferyl-alpha-D-glucoside, which is converted to a fluorescent substrate by reactions with alpha-D-glucosidase. The reaction is improved by temperature elevation to 60°C. Because the enzyme is slightly more resistant than spores, it is possible for the enzyme to be detected for a brief period after all spores are killed. The Attest auto-reader provides optimal incubation conditions and contains a fluorescent reader. The reader was used to incubate the indicators and to test for the presence of fluorescence using the Attest Rapid Readout at the standard 60 minutes and at shorter times. In addition, the growth medium contains a pH-sensitive dye (bromocresol purple), which turns yellow within 24 hours at 56°C to indicate the presence of viable spores. If there is a failure of sterilization, both the spore and the enzyme remain active. This failure of sterilization will be indicated both by a fluorescent positive (red light on auto-reader) within 60 minutes of incubation and by a media color change (purple to yellow) due to spore growth by 24 hours of incubation. If the sterilization process is acceptable, both the enzyme and the spore are inactivated. This acceptable sterilization process is indicated by no fluorescence (green light on auto-reader) at 60 minutes of incubation and no visual color change at 24 hours. The auto-reader was calibrated daily.

Three conventional biological indicators were tested: Standard Attest 1261 (3M Company, St. Paul, MN), Proof Flash (American Sterilizer Co., Erie, PA), and Assert (Weck, Inc., Research Triangle Park, NC). These indicators all employ dry spore strips or disks containing *B stearothermophilus* spores and growth medium with a colorimetric pH indicator in crushable ampules. Following 24 hours of incubation at 56°C, the indicators are evaluated for spore growth. All biological indicators for all trials were from the same lot (Attest 1291 lot no. 11; Attest 1261 lot no. 303; Assert

lot no. 0305; Flash Proof lot no. 079002PF) to ensure consistency of spore populations. A positive control (unexposed to sterilization) and negative control (a four-minute sterilization cycle at 132°C) were employed each test day. The control indicators were Attest 1261 and Attest 1291. In every trial, the positive control yielded growth of *Bacillus* and the negative control indicator was sterile. We followed the manufacturers' use instructions for all biological indicators.

Chemical Indicators

The following chemical indicators were used: Comply (3M Co., St. Paul, MN), Incheque (3M Co., St. Paul, MN), and Thermalog S (PyMaH Corp, Somerville, NJ). The Thermalog S lot number was S370-A. The Comply and Incheque chemical indicators consist of thermochromic inks printed on paper. When exposed to saturated steam, the ink undergoes a chemical reaction that results in a color change. The color change associated with the chemical indicator Comply was evaluated using the color match block on the indicator strip. The Thermalog S is a thermosensitive chemical pellet with a capillary scale display. These pellets gradually liquefy as the temperature increases. The melted fluid then wicks along the chromatography paper to form a band whose length is dependent upon time and temperature.² This latter indicator is particularly easy to interpret because it uses a sliding-bar scale.

Flash Sterilizer

All runs were conducted using the same AMSCO Eagle model 2021 gravity-displacement steam sterilizer (American Sterilizer Co., Erie, PA) in the Microbiology Laboratory at UNC Hospitals. Prior to our evaluations, the sterilizer was inspected by an American Sterilizer Co. technician and found to be functioning properly. The autoclave was manually operated during the pilot runs and automatically operated during the comparative trials. All trials were conducted at 132°C with a jacket pressure of 30 lb/in². The accuracy of the sterilizer temperature gauge was monitored by a thermocouple connected to a Doric 400A digital potentiometer (Doric Scientific, San Diego, CA). On each test day, two five-minute cycles were run to condition the autoclave.

For the pilot study, the autoclave was set for manual operation so the cycle time could be varied as required by the protocol. For each trial, a one-minute conditioning cycle was run to raise the temperature to approximately 121°C. The dial was then turned to "sterilize." The "come up" time, or the time to reach 132°C, averaged 27 seconds. The exposure time was started when 132°C was achieved. After the timed sterilization cycle, the dial was turned immediately to

"fast exhaust." The "come down" time, or the time for the chamber pressure to reach 0 lb/in² and the temperature to reach 100°C, averaged 40 seconds.

For the comparative trials, the autoclave was placed on automatic mode (except the 3.5-minute cycle, which was conducted using manual operation) and the cycle time was set at the experimental cycle times. On average, the time to reach 132°C was 1 minute, 33 seconds; however, the conditioning time took 1 minute. Following the sterilization cycle, the autoclave required an average of 32 seconds for the chamber pressure to drop to 0 lb/in² and the temperature to drop below 100°C.

Comparative Trials

Mesh-bottom surgical trays, 23.5 cm by 27.25 cm, were used for all trials. All biological and chemical indicators were placed horizontally in the center of the tray. The tray was placed on the bottom shelf of the empty sterilizer in such a manner that the indicators were positioned in the front, near the sterilizer drain.

A pilot study was done to identify the critical cycle time when there would be positive and negative results indicating marginal sterilization conditions. The nine exposure times tested were: 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 minutes. The preliminary data demonstrated that viable spores were still present at 3 and 3.5 minutes but absent at 4 minutes. Based on this preliminary data, six exposure times were used in the comparative trials: 0, 1, 2, 3, 3.5, and 4 minutes. Ten replicates of each type of biological and chemical indicator were exposed per cycle with the exception of five replicates per cycle for the 3.5-minute exposure time for Proof Flash and Assert. Four replicate cycles were performed for each exposure time. Assert and Proof Flash were not tested at 0- and 1-minute exposure times.

RESULTS

Biological Indicators

All biological indicators had 100% survival at 0- and 1-minute exposure times. The biological indicators revealed significant spore growth after 24 hours following a 2-minute exposure time and nearly all of the Attest Rapid Readout biological indicators were enzyme positive within 60 minutes (Table 1).

At the standard flash sterilization exposure time of three minutes, 0 and 1 of 40 replicates of the Proof Flash and Assert, respectively, were positive based on spore growth at 24 hours. Enzyme activity was detected using the Attest Rapid Readout in 13 (33%) of 40 replicates, although spore growth was noted at 24 hours in only 2 (5%) of 40 replicates. In comparison, the conventional Attest more nearly paralleled the results of the 60-minute enzyme detection with spore

TABLE 1
COMPARISON OF FOUR BIOLOGICAL INDICATORS USING 132°C FLASH STERILIZATION AT VARIOUS CYCLE TIMES

Biological Indicator	Number Positive Indicators/Total in Cycle			
	2.0 min	3.0 min	3.5 min	4.0 min
Attest Rapid Readout 1291				
15 min*	30/40 (75%)	3/40 (8%)	1/40 (3%)	0/40 (0%)
30 min*	34/40 (85%)	7/40 (18%)	1/40 (3%)	0/40 (0%)
45 min*	34/40 (85%)	12/40 (30%)	1/40 (3%)	0/40 (0%)
60 min*	35/40 (88%)	13/40 (33%)	1/40 (3%)	0/40 (0%)
24 hr†	29/40 (73%)	2/40 (5%)‡	0/40 (0%)	0/40 (0%)
Standard Attest 1261				
24 hr†	36/40 (90%)	9/40 (23%)	4/40 (10%)	1/40 (3%)
Proof Flash				
24 hr†	19/40 (48%)‡	1/40 (3%)‡	0/20 (0%)	0/40 (0%)
Assert				
24 hr†	16/40 (40%)‡	0/40 (0%)‡	0/20 (0%)	0/40 (0%)

* Fluorescent detection of enzyme.

† Observation of spore growth via color.

‡ Significantly different compared to 60 minutes with Attest Rapid Readout using a two-tailed Fisher's exact test at $P < 0.05$.

TABLE 2
COMPARISON OF THREE CHEMICAL INDICATORS USING 132°C FLASH STERILIZATION AT VARIOUS CYCLE TIMES

Chemical Indicator	Number Positive Indicators/Total in Cycle					
	0 min	1.0 min	2.0 min	3.0 min	3.5 min	4.0 min
Comply	40/40 (100%)	40/40 (100%)	0/40 (0%)	0/40 (0%)	0/40 (0%)	0/40 (0%)
Incheque	40/40 (100%)	40/40 (100%)	0/40 (0%)	0/40 (0%)	0/40 (0%)	0/40 (0%)
Thermalog S	40/40 (100%)	18/40 (45%)	11/40 (28%)	7/40 (18%)	3/40 (8%)	0/40 (0%)

growth occurring in 9 (23%) of 40 replicates. In no instance was spore growth observed in an indicator that was enzyme-negative. This supports the hypothesis that the enzyme persisted after spore destruction.

At a 3.5-minute exposure time, the conventional Attest yielded spore growth in 4 of 40 replicates and the Attest Rapid Readout enzyme could be detected in 1 of 40 replicates.

With one exception (ie, conventional Attest) there was no spore growth after a four-minute exposure time and the Attest Rapid Readout was enzyme negative.

Chemical Indicators

The chemical indicators Comply and Incheque revealed incomplete processing at 0 and 1 minute, and adequate processing from 2 through 4 minutes (Table 2). The chemical indicator Thermalog S also demonstrated inadequate processing at 0 minutes but commonly demonstrated sterilization at 1- and 2-minute exposure times. At 2 minutes, the conventional Attest demonstrated spore growth and Attest Rapid Readout

enzyme was detected significantly more frequently (88% to 90%) than the Thermalog S (28%) or Comply and Incheque (0%) ($P < 0.001$). Compared with the 24-hour spore growth results of Attest Rapid Readout, Proof Flash, and Assert, Thermalog S yielded more frequent positive tests at 3- and 3.5-minute exposure cycles. At 4 minutes, Thermalog S and all biological indicators, with one exception, yielded no positive results.

DISCUSSION

Biological monitors are recognized by most authorities as being closest to the ideal monitors of the sterilization process² because unlike chemical indicators, they measure the sterilization process directly by using the most resistant microorganism (ie, *Bacillus* spores), not merely testing the physical and chemical conditions necessary for sterilization. Since the *Bacillus* spores used in biological indicators are more resistant and present in greater numbers than the common microbial contaminants found on patient care equipment, the demonstration that the

biological indicator has been inactivated strongly implies that other potential pathogens have been killed during the sterilization cycle. At least weekly monitoring of steam sterilizers with a biological indicator is recommended.³⁻⁶ In addition, most institutions use a chemical indicator to monitor each load.

In the evaluation of biological indicators for hospital use, one must consider the factors that would define an ideal monitor of the sterilization process. The monitor should be easy to use, inexpensive, not subject to exogenous contamination, provide positive results as soon as possible after the cycle so that corrective action may be accomplished, and provide positive results only when the sterilization parameters (eg, time and temperature) are inadequate to kill microbial contaminants. However, the biological indicator should not be so resistant as to cause needless recall and overprocessing. The Attest Rapid Readout has potential for significantly improving the assessment of flash sterilization cycles. The enzyme, alpha-D-glucosidase, was always detected whenever viable spores were present. This was expected because the enzyme is heat resistant and is inactivated at 132°C in a slightly longer time than the spore. At exposure times of 2.0 to 3.5 minutes, the sensitivity of the Attest Rapid Readout was similar to conventional Attest and more sensitive when compared with Proof Flash and Assert. Possible explanations for more frequent spore growth with the conventional Attest than found with other biological indicators may be a more resistant spore associated with this indicator or a packaging design that retards steam permeation. Differences in spore burden are not a factor since the conventional Attest 1261 (1.4×10^5) had fewer *B. stearothermophilus* spores than the Assert (1.74×10^5) and the Attest Rapid Readout 1291 (3.3×10^6).

At the standard flash sterilization time of three minutes, our data showed positive spore growth for several biological indicators. Vesley et al also found the conventional Attest biological indicators to provide positive results after a three-minute exposure cycle.⁹ These data support the concerns expressed by the Centers for Disease Control and Prevention about the marginality of a three-minute flash sterilization cycle at 132°C⁵ and suggest that the standard cycle time should be lengthened to four minutes.¹¹ On the basis of these data, a four-minute flash sterilization cycle is now used at our institution. Ideally, the choice of a standard flash sterilization cycle time should depend on scientifically obtained evidence as to how well these biological indicators ensure sterilization of patient care items even if heavily contaminated, improv-

erly cleaned, or if the sterilizer is improperly loaded.

Chemical indicators are used in conjunction with biological and mechanical indicators to monitor the sterilization process. They are convenient, inexpensive, and indicate that the item has been exposed to the sterilization process. All of the chemical indicators were more likely than biological indicators to indicate sterilization at marginal sterilization times (eg, two minutes). Chemical indicators could be used in conjunction with biological indicators, but should not replace them, because of inadequacies at marginal sterilization times and because only a biological indicator consisting of resistant spores can measure the microbial killing power of the sterilization process.

The ability to monitor flash cycles in a surgical suite and to have results within 60 minutes should enable the operating room staff to intercept improperly sterilized items either prior to use or before the end of the surgery. New indicator technologies such as the Attest Rapid Readout biological indicators are likely to improve patient safety. Additional studies should be undertaken to develop a scientific basis for determining consensus guidelines of sterilization process monitoring.

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