COMPARISON OF A RAPID READOUT BIOLOGICAL INDICATOR FOR STEAM STERILIZATION WITH FOUR CONVENTIONAL BIOLOGICAL INDICATORS AND FIVE CHEMICAL INDICATORS

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ABSTRACT

OBJECTIVE: In this study, we compare a new biological indicator that provides results within 3 hours with four conventional, 48-hour biological indicators and five chemical indicators.

DESIGN: Biological indicators tested included the conventional Attest 1262, Proof Plus, Assert, and Biosign, and the new Attest 1292 Rapid Readout biological indicator. Chemical indicators tested included Comply, Propper, Chemdi, Sterigage, and Thermalog S. Spore survival following 121°C in a gravity displacement sterilizer was measured by media color change after incubation for 24 and 48 hours at 56°C for the conventional biological indicators, fluorescence at 3 hours for the Attest 1292 Rapid Readout biological indicator, and color change for the chemical indicators. Each exposure time was replicated 12 times with five samples of each indicator per run (ie, 60 replicates per indicator).

RESULTS: At 48 hours, the conventional biological indicators Attest 1262, Proof Plus, Assert, and Biosign showed 100%, 95%, 88%, and 93% spore survival at 5 minutes' exposure; 0%, 0%, 0%, and 8% at 10 minutes; and all showed 0% survival at 15 minutes' exposure. Following a 3-hour incubation, the Attest 1292 Rapid Readout biological indicator showed fluorescence at 100%, 72%, and 0% at 5, 10, and 15 minutes, respectively. The chemical indicators Comply, Propper, Chemdi, Sterigage, and Thermalog S revealed sterilization failure rates of 100%, 100%, 100%, 100%, and 100% at 5 minutes' exposure; 0%, 0%, 0%, 92%, and 100% at 10 minutes; and, 0%, 0%, 0%, 3%, and 27% at 15 minutes' exposure, respectively.

CONCLUSIONS: The sensitivity of the Attest 1292 Rapid Readout biological indicator parallels that of conventional biological indicators. These data suggest that a 3-hour rapid readout biological indicator is equivalent to a standard 48-hour biological indicator. Some chemical indicators (eg, Thermalog S) failed to indicate adequate sterilization at 15 minutes' exposure. These chemical indicators have the potential of causing unnecessary recall of adequately sterilized items (*Infect Control Hosp Epidemiol* 1996;17:423-428).

INTRODUCTION

Sterilization, which is defined as the complete elimination or destruction of living microorganisms, is recommended for all "critical" medical devices. Items in this category include surgical instruments, cardiac catheters, implantable devices, and needles. Because it is essential to ensure sterilization of critical items, monitoring of the sterilization process is advised. Selection of the proper monitor should provide this assurance without producing misleading information. That is, monitors should not indicate that items are sterile when they are not, which could result in nosocomial infections, and monitors should not indicate that items are not sterile when they are, which leads to recall and reprocessing of sterile items. Three types of monitors may be used: mechanical, chemical, and biological. Mechanical monitors can verify time and temperature in a sterilizer. Chemical indicators have been used to verify that a desired temperature was reached at a particular spot in the sterilizer. However, biological monitors are recognized as being the closest to ideal monitors of the sterilization process.[1-4] For this reason, the Association of Operating Room Nurses,[5] the Centers for Disease Control and Prevention,[6] the Association for the Advancement of Medical Instrumentation,[7] and the Joint Commission on Accreditation of Healthcare Organizations (JCAHO) all have recommended that biological monitoring of steam sterilizers be performed at least weekly[8] and with each load

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containing implantable objects.[5-7] Recently, JCAHO changed its standards to recommend that each hospital department performing decontamination and sterilization have a policy defining the "use and frequency of appropriate chemical indicator or bacteriological spore tests for all sterilizers."[9]

Biological indicators use high numbers of bacterial spores (*Bacillus stearothermophilus)* to ensure steam sterilization efficacy. *Bacillus* spores are used because they are more resistant than the normal microbial contaminants present on contaminated surgical instruments, and the spores are present in greater numbers than contaminating pathogens. The current procedure of monitoring steam sterilizers using spores is the best that is currently available to ensure the sterilization of surgical instruments.

In the past 30 years, biological indicators have evolved through three generations. Prior to 1970, paper strips inoculated with spores of *B stearothermophilus* were placed in envelopes, aseptically transferred following sterilization to a bacteriologic broth in the laboratory, and then incubated for 7 days prior to being read. Sterilization failure was indicated by visually observing turbidity in the growth broth. Disadvantages of this system included the requirement for an extended incubation time and the need to mechanically transfer spore strips to growth broth, which resulted in possible contamination. In the 1970s, the second generation of biological indicators was introduced. These were self-contained systems in which the spore strip and medium were contained in a single plastic vial. Following sterilization, the inner crushable glass vial was broken, allowing the media to come into contact with the spore strip. A pH indicator (bromcresol purple) was included, which changed color when exposed to the acid byproducts of replicating organisms. Advantages of these indicators included improved readability, reduction of incubation time to 24 to 48 hours, and the ability to accomplish incubation within the department performing sterilization (eg, operating room). Recently, a third-generation biological indicator, the Attest Rapid Readout biological indicator (Attest 1291, 3M Co, St Paul, MN), became commercially available for monitoring flash sterilization. This indicator detects the presence of a spore-associated enzyme, α-D-glucosidase and permits an assessment of sterilization effectiveness within 60 minutes in the 132°C gravity-displacement sterilization cycle.[2,4]

This study was undertaken to compare four second-generation (ie, self-contained) biological indicators to a new Attest Rapid Readout biological indicator (Attest 1292, 3M Co) for monitoring sterilization effectiveness during a 121°C gravity-displacement steam-sterilization cycle. These biological indicators also were compared with five chemical indicators to assess whether chemical indicators provide equivalent results to biological indicators.

METHODS

Biological Indicators

Our methods are similar to those reported by Vesley et al[2,3] and Rutala et al.[4] The Attest Rapid Readout indicator employs a dry spore strip containing at least 10^5 spores of *B stearothermophilus* derived from American Tissue and Culture Collection strain 7953. The growth medium is a modified tryptic soy broth contained in a crushable glass ampule. The broth contains a nonfluorescent substrate, 4-methylumbelliferyl-α-D-glucoside, which is converted to a fluorescent substrate by reaction with α-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 of time after all spores are killed. The Attest auto-reader provides optimal incubation conditions and contains a fluorescent reader. The auto-reader was used to incubate the indicators and to test for the presence of fluorescence using the Attest 1292 Rapid Readout biological indicator at the standard 3 hours and at shorter times. In addition, the growth medium contains a pH-sensitive dye (bromcresol purple) that turns yellow within 48 hours at 56°C to 60°C to indicate the presence of viable spores. If there is a sterilization failure, both the spore and the enzyme remain active. This failure of sterilization will be indicated by both a fluorescence (red light on auto-reader) within 3 hours of incubation and by a medium color change (purple to yellow), due to spore growth, by 48 hours of incubation. If the sterilization process is successful, both the enzyme and the spore are inactivated. This acceptable sterilization process is indicated by no fluorescence (green light on auto-reader) at 3 hours of incubation and no visual color change at 48 hours. The auto-reader was calibrated daily.

Four conventional biological indicators were tested: Attest 1262 (3M Co, St Paul, MN); Proof Plus (American Sterilizer Co, Erie, PA), Assert (Surgicot, Inc, Research Triangle Park, NC), and Biosign (MDT Corp, Rochester, NY). 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 and 48 hours of incubation at 56°C, the indicators were evaluated for spore growth. All biological indicators for all trials were from the same lot (Attest 1262 lot no. Aug. 93-315, Proof Plus lot no. PI083N,

TABLE 1
COMPARISON OF FIVE BIOLOGICAL INDICATORS USING 121°C GRAVITY DISPLACEMENT STEAM STERILIZATION AT VARIOUS CYCLE TIMES

Biological Indicator	Incubation Time	Number Positive Indicators/Total Tested		
		5 minutes	10 minutes	15 minutes
Attest 1292 Rapid Readout				
Fluorescence*	30 min	60/60 (100%)	ND	ND
	1 hr	60/60 (100%)	16/60 (27%)	0/60 (0%)
	2 hr	60/60 (100%)	38/60 (63%)	0/60 (0%)
	3 hr	60/60 (100%)	43/60 (72%)	0/60 (0%)
Spore Growth†	24 hr	32/60 (53%)	0/60 (0%)	0/60 (0%)
	48 hr	60/60 (100%)	1/60 (2%)	0/60 (0%)
Attest 1262	24 hr	58/60 (97%)	0/60 (0%)	0/60 (0%)
	48 hr	60/60 (100%)	0/60 (0%)	0/60 (0%)
Proof Plus	24 hr	55/60 (92%)	0/60 (0%)	0/60 (0%)
	48 hr	57/60 (95%)	0/60 (0%)	0/60 (0%)
Assert	24 hr	51/60 (85%)	0/60 (0%)	0/60 (0%)
	48 hr	53/60 (88%)	0/60 (0%)	0/60 (0%)
Biosign	24 hr	53/58 (91%)	4/60 (7%)	0/60 (0%)
	48 hr	54/58 (93%)	5/60 (8%)	0/60 (0%)

* Fluorescent detection of enzyme.
† Observation of spore growth using color changes.

Assert lot no. 3319, Biosign lot no. 121493) to ensure consistency of spore populations. All products were used prior to the manufacturer's expiration date. A positive control (unexposed to sterilization) and negative control (a 20-minute sterilization cycle at 121°C) were employed each day.

Chemical Indicators

The following chemical indicators were used: Comply 1250 (3M Co), Propper (Propper, Long Island City, NY), Chemdi (AMSCO, Erie, PA), Sterigage (PhMaH Corp, Somerville, NJ), and Thermalog S (PyMaH Corp, Somerville, NJ). All chemical indicators for all trials were from the same lot: (Comply lot no. May 93-053, Propper lot no. US 3091, Chemdi lot no. CSI 059303, Sterigage lot no. B 463A, and Thermalog S lot no. S213-B). The Comply, Propper, and Chemdi 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 changes associated with the Comply and Propper chemical indicators were evaluated using the color match block on the indicator strip. As per manufacturer instructions, Chemdi was evaluated by the development of a black square. The Sterigage and Thermalog S indicators each provide 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 on time and temperature.[1] These latter indicators were easy to interpret because they use a pass-fail scale.

Steam Sterilizer

All runs used 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 Company technician and found to be functioning properly. The autoclave was automatically operated during the comparative trials. All trials were conducted at 121±1°C with a jacket pressure of 20 psi. 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, a 20-minute cycle was run to condition the autoclave.

For the pilot study and comparative trials, the autoclave was placed on automatic mode, and the cycle time was set at the desired experimental cycle times. The "come up" time, or the time to reach 121°C, averaged 78 seconds. The "come down" time, or the time for chamber pressure to reach 0 psi and the temperature to reach 100°C, averaged 32 seconds.

TABLE 2
COMPARISON OF FIVE CHEMICAL INDICATORS USING 121°C GRAVITY DISPLACEMENT STEAM STERILIZATION AT VARIOUS CYCLE TIMES

Chemical Indicator	Number Positive Indicators/Total Tested		
	5 minutes	10 minutes	15 minutes
Comply	60/60 (100%)	0/60 (0%)	0/60 (0%)
Propper	60/60 (100%)	0/60 (0%)	0/60 (0%)
Chemdi	60/60 (100%)	0/60 (0%)	0/60 (0%)
Sterigage	60/60 (100%)	55/60 (92%)	2/60 (3%)
Thermalog S	60/60 (100%)	60/60 (100%)	16/60 (27%)

Comparative Trials

A single mesh-bottom surgical tray, 10.5 in × 15.0 in × 3 in, was used for all trials. To ensure uniform exposure conditions, all biological and chemical indicators were placed horizontally, evenly, and without overlap throughout 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. All runs were conducted with only the experimental tray present in the sterilizer.

A pilot study was done to identify the critical cycle time when there would be both positive and negative results, indicating marginal sterilization conditions. The five exposure times tested were 5, 8, 10, 12, and 15 minutes. Based on preliminary data, only three exposure times (5, 10, and 15 minutes) were included in the comparative trials. Five replicates of each type of indicator were exposed per cycle. Twelve replicate cycles were performed for each exposure time.

RESULTS

Biological Indicators

The results of five biological indicators undergoing sterilization for various cycle times in a 121°C gravity displacement steam sterilizer are summarized in Table 1. After a 15-minute sterilization cycle, all biological indicators were negative (no spore growth) at 24 and 48 hours incubation. The Attest 1292 Rapid Readout indicator (enzyme detection) demonstrated no fluorescence following 1, 2, or 3 hours of incubation.

After a 5-minute exposure time, the biological indicators generally were positive, demonstrating spore growth at 48 hours. Spore growth at 24 hours incubation provided results within 3% of the 48-hour data, with the exception of Attest 1292 Rapid Readout, where substantially less frequent growth was apparent at 24 hours. The Attest biological indicators (1292 and 1262) demonstrated spore growth 100% of the time at 48 hours. The other indicators demonstrated spore growth between 88% and 95% of the time at 48 hours. The Attest 1292 Rapid Readout indicator demonstrated fluorescence in 100% of cycles following 30 minutes, 1 hour, 2 hours, and 3 hours.

After a 10-minute exposure time, the biological indicators showed either no growth (Attest 1262, Proof Plus, Assert) or infrequent growth (Attest 1292, Biosign) at 48 hours' incubation. Spore growth at 24 hours again was within 3% of the 48-hour data. The Attest 1292 Rapid Readout indicator demonstrated variable fluorescence, ranging from 27% after 1 hour to 63% after 2 hours and 72% after 3 hours of incubation.

Chemical Indicators

The results of five chemical indicators undergoing sterilization for various cycle times in a 121°C gravity displacement steam sterilizer are summarized in Table 2. After a 15-minute sterilization cycle, three chemical indicators (Comply, Propper, Chemdi) indicated successful processing following each experimental run. Two chemical indicators, Sterigage and Thermalog S, failed to demonstrate processing 3% and 27% of the time, respectively.

After a 5-minute sterilization cycle, all chemical indicators revealed incomplete processing following all experimental runs.

Large differences in the demonstration of processing were noted following a 10-minute sterilization cycle. Three chemical indicators (Comply, Propper, Chemdi) demonstrated processing following all runs. Two chemical indicators, Sterigage and Thermalog S, failed to demonstrate processing 92% and 100% of the time, respectively.

DISCUSSION

Biological indicators are recognized by most authorities as being the closest to ideal monitors of sterilization process, because, unlike chemical indicators, they measure the sterilization process direct-

TABLE 3
COMPARISON OF FIVE BIOLOGICAL INDICATORS AND FIVE CHEMICAL INDICATORS USING 121°C STEAM STERILIZATION AT VARIOUS CYCLE TIMES

Indicator Type	Percent Positive Indicators (Sterilization Failure)*		
	5 minutes	10 minutes	15 minutes
Biological indicators (incubation time)			
Attest 1292 RR (3 hr)	100	72	0
Attest 1262 (48 hr)	100	0	0
Proof Plus (48 hr)	95	0	0
Assert (48 hr)	88	0	0
Biosign (48 hr)	93	8	0
Chemical indicators			
Comply	100	0	0
Propper	100	0	0
Chemdi	100	0	0
Sterigage	100	92	3
Thermalog S	100	100	27

* Sixty replicates/indicators.

ly by using the most resistant microorganism (*Bacillus* spores), not merely testing the physical and chemical conditions necessary for sterilization. Because 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.[1,4]

Our data confirm the report by Vesley et al[3] who evaluated six biological indicators following steam sterilization in a gravity displacement sterilizer at 121°C. After 48 hours' incubation, most biological indicators demonstrated spore growth after 5 minutes of sterilization and none after 15 minutes of sterilization. Spore growth following 10 minutes of sterilization ranged from 0% to 8% in our study and 0% to 40% in Vesley's study. Few differences were noted in the frequency of spore growth when the 24- and 48-hour incubation intervals were compared. Vesley and coworkers also tested the Attest 1292 Rapid Readout biological indicator when incorporated into test packs placed in a 132°C vacuum-assisted sterilization cycle that was fully loaded with surgical linen packs. The 1292 Rapid Readout biological indicator (at 3 hours) was a more sensitive indicator of marginal steam sterilization cycles than the conventional biological indicators (at 48 hours).

The Attest 1292 Rapid Readout fluorescent indicator, when read at 3 hours, paralleled the conventional 48-hour biological indicators following 5-minute and 15-minute sterilization cycles. This indicator was the most sensitive indicator of sterilization failure, as noted by the 72% failure rate for a suboptimal sterilization cycle (ie, 10 minutes). In no case was spore growth noted in the absence of fluorescence. These results further confirm that the spore-associated enzyme, α-D-glucosidase, is slightly more heat resistant than the spore itself. Thus, fluorescence associated with enzymatic activity can be detected for a slightly longer time than spore viability, as measured by color change after 48 hours of incubation. Our data demonstrate that the less effective the sterilization cycle (eg, 5 minutes), the more spores remain viable, and the more rapid the development of spore growth, as indicated by color change for second-generation biological indicators and the development of fluorescence for third-generation biological indicators. With clearly inadequate sterilization times, the Attest 1292 Rapid Readout indicator may demonstrate fluorescence as early as 30 minutes.

Chemical indicators undergo a chemical or physical change in response to one or more sterilization process parameters. Chemical indicators have been used in conjunction with biological and mechanical indicators to monitor the sterilization process. They can be used on the outside and inside of each package to be sterilized and in multiple locations within a load. They are convenient, inexpensive, and immediately indicate that the item has been exposed to the sterilization process.

The chemical indicators used in this study may be classified as temperature-specific indicators

(Propper, Chemdi) or multiple parameter process indicators (Comply, Sterigage, Thermalog S). These latter indicators also are termed chemical integrators. Temperature-specific indicators demonstrate whether or not a specific temperature was attained, but do not reveal for how long this temperature was maintained. Multiparameter process indicators respond to the combined action of different components of the lethal process. For example, they may indicate both heat and duration of treatment.

Table 3 provides an overall summary of our data. The temperature-specific chemical indicators and one multiple parameter process indicator (Comply) closely matched the conventional biological indicators in this study. Two multiple-parameter indicators (ie, Sterigage and Thermalog S) inappropriately rejected adequately sterilized loads (ie, 15 minutes). Thermalog S was more likely than Sterigage to incorrectly indicate inadequate sterilization. Inappropriate rejection of adequately sterilized loads would result in added costs, but without improved patient safety.

Conventional biological indicators are excellent monitors of sterilization effectiveness. Our data demonstrate great consistency across manufacturers. The Attest 1292 Rapid Readout indicator is an excellent monitor that ensures sterilization without inappropriately indicating failure. The ability to monitor sterilization effectiveness within 3 hours should enhance the ability of hospital staff to intercept improperly sterilized items prior to use. Introduction of some multiple-parameter chemical indicators (eg, Thermalog S) as currently designed would result in recalling and resterilizing some adequately sterilized loads. The temperature-specific chemical indicators and one multiple-parameter process indicator performed well in this comparative trial. Chemical indicators could be used in conjunction with biological indicators, but should not replace biological indicators because only a biological indicator consisting of resistant spores can measure the microbial killing power of the sterilization process. Our current data suggest that chemical indicators do not consistently perform as well as biological indicators in appropriately monitoring sterilization. In a previous study, we demonstrated that some chemical indicators also passed clearly inadequate sterilization cycle times during 132°C gravity displacement sterilization cycles.[4] We believe there is insufficient scientific information to support the current JCAHO standard permitting the use of "appropriate chemical indicators" without routine use of a biological indicator. Additional studies should be undertaken to develop a scientific basis for determining consensus guidelines of sterilization process monitoring.

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