Sterilization is a process that cannot be inspected or tested in a practical manner to assure that all microorganisms have been inactivated. The process must therefore be validated for all of the specific items processed or monitored on a per cycle basis. Consequently, the Association for the Advancement of Medical Instrumentation (AAMI), the Association of periOperative Registered Nurses (AORN), and the Centers for Disease Control and Prevention (CDC) all recommend routine monitoring of the sterilization process in health care facilities with physical monitors, chemical indicators (CIs), and biological indicators (BIs).1-3 Whereas all of these indicators (CIs), and biological indicators (BIs).1-3 Whereas all of these monitoring types are integral components of a sterility assurance program, BIs are considered by most authorities as being the closest practical monitors of the sterilization process.3,4

BIs, however, have a practical time-related limitation; viable microorganisms require time under suitable conditions of temperature, moisture, nutrients, etc, to multiply and produce visible indications of growth. A postexposure incubation period is therefore necessary to allow for growth and visualization of any test organisms that may have survived the sterilization process. Prior to the 1990s, BIs used in the steam sterilization process typically required 24 to 48 hours of incubation before a negative result could be accepted. Although it is desirable to quarantine processed loads until BI results become available, this 24- to 48-hour quarantine time was impractical for most health care facilities. Additionally, the AAMI, AORN, and CDC guidelines strongly recommend that all loads containing an implantable device be monitored with a BI and that the load be quarantined until the BI results are available.1,3

First-generation rapid readout BIs (RRBIs) with significantly reduced incubation times were introduced in the early 1990s. The technology used to provide the rapid response of these BIs is based on detection of an enzyme: α-glucosidase. This enzyme has been shown to be present in the coat of Geobacillus stearothermophilus spores but is also newly synthesized during the germination and outgrowth of spores that survive the sterilization process.5 The α-glucosidase enzyme not destroyed by the sterilization process combined with that produced by the surviving spores converts a
nonfluorescent substrate present in the self-contained BI growth medium to a fluorescent product that is subsequently measured using a specialized incubator/reader. Optional growth results based on a pH color change of the growth medium can also be obtained by extending the incubation time of the RRBIs. Studies comparing these RRBIs with conventional BIs concluded that the RRBIs were a more sensitive indicator of marginal steam sterilization cycles than the conventional self-contained BIs.\textsuperscript{4,7}

A new second-generation RRBI with a shortened incubation time based on the technology described above but optimized for faster readout has been developed and has recently received Food and Drug Administration 510(k) clearance.\textsuperscript{8} This new RRBI is indicated for use in select 132°C (270°F) and 135°C (275°F) vacuum-assisted steam sterilization cycles. The new RRBI has a 1-hour final fluorescent readout time when used in conjunction with the companion automated reader and has optional 48-hour final growth readout based on a visual pH color change of the growth medium.\textsuperscript{9}

**METHODS AND MATERIALS**

**Biological indicators**

1. Attest Rapid Readout Biological Indicators 1292 (3M, St Paul, MN): self-contained BI containing \textit{G. stearothermophilus} spores and requiring incubation at 60°C ± 2°C with a 3-hour fluorescent readout when used in conjunction with the Attest 290 Auto-reader (3M) and an optional 48-hour media pH color change result (subsequently referred to as 3-hour RRBI).

2. Attest Super Rapid Readout Biological Indicators 1492V (3M): self-contained BI containing \textit{G. stearothermophilus} spores and requiring incubation at 56°C ± 2°C with 1-hour fluorescent readout when used in conjunction with the Attest Auto-reader 490 and an optional 48-hour media pH color change result (subsequently referred to as 1-hour RRBI).

**Population determination**

The BI population counts were verified by removing spore strips (3-hour RRBI) or spore carriers (1-hour RRBI) from the self-contained BI units and homogenizing them in a Waring blender (Waring Commercial, Torrington, CT) using a potassium phosphate buffer diluent. Subsequent serial dilutions were plated with Tryptic Soy Agar (Difco, Becton, Dickinson and Co, Sparks, MD) and the plates incubated at 56°C ± 2°C for 24 to 48 hours.

**D value determination**

D values were calculated from the 7-day fractional growth data at 132°C for each lot of both RRBIs and at 135°C for each lot of the 1-hour RRBI using the Stumb-Murphy-Cochran procedure.\textsuperscript{10}

**Test vessel**

The test vessel used for both of the test cycles was a steam resistometer, H&W Technology, LLC (Rochester, NY), meeting the requirements of the American National Standards Institute/AAMI/International Organization for Standards standard for BI and CI testing.\textsuperscript{11}

**Testing at 132°C**

BIIs from 3 lots of the 3-hour RRBI (135 BIs from each lot) and 3 lots of the 1-hour RRBI (135 BIs from each lot) were exposed side by side in steam resistometer cycles at 132°C. The resistometer cycles consisted of a single vacuum pulse with an average 2- to 3-second come-up time. The set point for the exposure temperatures was 132.2°C ± 0.5°C for all cycles. Cycle times were selected to produce at least 1 exposure with all positive results, at least 3 exposures with fractional, ie, both negative and positive results, and at least 1 exposure with all negative results. Each exposure time was replicated a minimum of 3 times. Three 3-hour RRBIIs from each of 3 lots and three 1-hour RRBIIs from each of 3 lots (18 total BIs/cycle) were placed on a perforated metal rack with a perforated metal cover for each exposure. An unexposed BI (positive control) from alternating lots of both BI types was incubated with the test units for each cycle.

Following exposure, 1-hour fluorescent results were determined for the 1-hour RRBIIs using the Auto-reader specified for this product, and 3-hour fluorescent results were determined for the 3-hour RRBIIs using the Auto-reader specified for that product. After recording the respective fluorescent results, the 1-hour RRBIIs were transferred to a 56°C ± 2°C standard convection incubator, and the 3-hour RRBIIs were transferred to a 60°C ± 1°C dry block incubator fitted with cooling fans to prevent media evaporation. Growth results for both products were determined at 48 hours and at 7 days based on a pH-initiated color change of the growth media.

**RESULTS**

The results of the 3-hour and 1-hour RRBI side-by-side testing at 132°C using 8 incremental exposure times ranging from 2.0 minutes (2:00) to 4.5 minutes (4:30) are presented in Table 1. The readout data represent 3 combined lots of each of the 2 BI types and are expressed as fluorescent positive (3 hours and 1 hour, respectively) and growth positive (48 hours and 7 days) for both BIs.

Based on the intended use and utility of these 2 BIs, the fluorescent rapid readout responses are considered to be the most relevant component of the test data. The number and percentage of fluorescent-positive 1-hour RRBIIs after 1 hour of incubation were nearly identical to the number and percentage of fluorescent-positive 3-hour RRBIIs after 3 hours of incubation (Fig 1). For both RRBIIs, the number and the percentage of fluorescent-positive readouts were greater than the corresponding number of growth-positive results. This difference is consistent with the manufacturer’s information relative to the Rapid Readout BI technology. In addition, a number of other investigators who previously evaluated RRBIIs noted this same difference and concluded that the enzyme (α-glucosidase) was slightly more resistant to the steam sterilization process than the \textit{G. stearothermophilus} spore (formerly \textit{Bacillus stearothermophilus}).\textsuperscript{4,7,12,13}

The number of fluorescent-positive BIs that showed growth positives, ie, media pH color change, after extended incubation was greater for the 3-hour RRBIIs than for the 1-hour RRBIIs. A possible explanation for this difference may be that the growth medium of the 1-hour RRBI is a slightly modified formulation optimized to facilitate the faster fluorescent readout of this BI.

All growth positives for both of the RRBIIs had initial fluorescent-positive results, ie, no false fluorescent-negative responses were observed. With 1 exception (3-hour RRBI), the total number of
growth positives at 48 hours was identical to the number of growth positives at 7 days, providing a 99.5% correlation between 48-hour and 7-day results for the 3-hour RRBI and 100% correlation for the 1-hour RRBI. Both RRBIIs demonstrated 100% positive results for fluorescence and growth at the 2-minute exposure time and 100% negative results for both fluorescence and growth after 4.5 minutes of exposure.

The combined results of the 3 lots of the 1-hour RRBI tested at 135°C, expressed as 1-hour fluorescent positive, 48-hour and 7-day growth positive are shown in Table 2. Eight incremental exposure times ranging from 1.5 minutes (1:30) to 3.75 minutes (3:45) were used to evaluate the performance of the 1-hour RRBI at this temperature. In general, the number of both fluorescent-positive and growth-positive responses decreased with a corresponding increase in incremental exposure time. All of the D value calculations for the 3-hour RRBI were also calculated using the 7-day growth results from fractional cycles for each lot of the 3-hour and 1-hour RRBIIs.

Table 3 contains both the experimental and BI manufacturer’s values for population, D_{132}^C, D_{135}^C, survival/kill (S/K) times at 132°C and 135°C as applicable for each of the 3 lots of the 3-hour and 1-hour RRBIIs used in the study (BI manufacturer’s values in parentheses).

Population

The verified population counts for the 3 lots of the 3-hour RRBIIs shown in Table 3 were very close to the manufacturer’s certification counts (~10% difference). The verified population counts of the 1-hour RRBIIs were slightly lower than the manufacturer’s counts, resulting in a greater variation between these 2 sets of counts than that observed for the 3-hour RRBIIs. Overall, however, the verified population counts of the 1-hour RRBIIs were approximately 1 to 1.5 log_{10} higher than the verified population counts for the 3-hour RRBIIs.

D value

D values at 132°C were calculated using the 7-day growth results from fractional cycles for each lot of the 3-hour and 1-hour RRBIIs, respectively. D values at 135°C for each lot of the 1-hour RRBI were also calculated using the 7-day growth results from fractional cycles at this temperature. All of the D value calculations were based on data sets having at least 3 exposures with fractional results per BI standards recommendations.\textsuperscript{10} The D_{132}^C values for the 3-hour RRBIIs ranged from 0.5 to 0.52 minutes, whereas the D_{132}^C values for the 1-hour RRBIIs were approximately 0.1 minute lower, ranging from 0.39 to 0.41 minutes. The comparatively lower D values observed for the 1-hour RRBIIs are attributed at least in part to the higher populations of these BIs. Because the manufacturer D value certifications for the 3-hour RRBIIs were performed at 121°C, a comparison of the D_{132}^C values to the manufacturer’s D values was not possible for this product. However, the calculated D values for the 1-hour RRBIIs were nearly identical to the D values supplied by the manufacturer (Table 3).
and to provide assurance when the cycle performed as intended per indicator are its ability to detect sterilization failures when they occur resistance of the release of steam sterilized loads in health care facilities for the 3-hour RRBIs in 132 fl

**DISCUSSION**

Three-hour RRBIs have been used on a global basis for 3-hour sterilization processes from the current 3 hours to 1 hour is believed to be significant relative to actual health care facility practice. In situations where extended quarantine is not practical, the 1-hour readout may provide a means to detect earlier and therefore possibly prevent the use of inadequately processed items because loads are generally cooled for about 1 hour as standard practice. The need for load recalls would likely be reduced while operating room scheduling and instrument inventory control could be improved.

**REFERENCES**


