Evaluation of a Rapid Biological Spore Test for Dental Instrument Sterilization

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ORIGINAL RESEARCH

Evaluation of a Rapid Biological Spore Test for Dental Instrument Sterilization

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Abstract

Aim: This study evaluated the reliability of a new rapid biological spore test (BST) for determining the sterilization efficacy of dental steam autoclaves within 20 minutes, as compared to a conventional BST requiring 2 days of incubation after autoclave exposure.

Materials and methods: A total of 177 pairs of BST, each composed of a rapid test (Celerity™ 20 Steam Biologic Indicator, Steris) and a conventional BST (Attest™ 1262 Biological Indicator, 3M), both containing *Geobacillus stearothermophilus* spores, were placed into steam autoclaves loaded with instruments, and subjected to either sterilizing (157 pairs) or non-sterilizing conditions (20 pairs). Celerity™ BST was then incubated for 20 minutes at 57°C, with the growth medium evaluated spectrophotometrically for fluorescent α-glucosidase signal changes (no change with successful sterilization; increased fluorescence after failed sterilization). Attest™ BST was incubated for 48 hours at 57°C, after which a pH-based color change in the culture broth was visually assessed (no change in purple color with successful sterilization; change to yellow color with failed sterilization).

Results: Celerity™ and Attest™ BST both accurately identified successful sterilization, with no *G. stearothermophilus* spore growth from either BST after exposure to sterilizing steam autoclave conditions (100% agreement between 157 pairs of each BST). Both BST also accurately detected unsuccessful sterilization, with all tested ampoules positive for *G. stearothermophilus* spore germination after non-sterilizing steam autoclave time periods. Both BST exhibited 100% sensitivity, specificity, and accuracy for detection of sterilizing steam autoclave conditions.

Conclusion: Celerity™ BST, after only 20 minutes incubation, performed equally as well as a BST requiring 48 hours incubation in determining the sterilization efficacy of dental steam autoclaves.

Clinical significance: Rapid BST offer earlier detection of sterilization failure before potentially contaminated dental instruments are used in clinical patient care.

Keywords: Bacterial spores, Dental infection control, Dental instruments, Steam autoclave, Sterilization.

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Introduction

Sterilization of dental instruments is critical to maintaining infection control standards in dental practices and preventing dental patient-to-dental patient transmission of potentially pathogenic bacteria, viruses, and fungi.

Various methods exist for sterilizing dental instruments. Most frequently used is a steam autoclave, which applies moist heat under increased air pressure. Steam provides more latent heat transfer onto dental instrument surfaces than water at the same temperature, and increased air pressure raises the temperature of steam markedly above 100°C to kill bacterial spores.[1] Less frequently employed dental sterilizing methods include the use of dry heat ovens, unsaturated alcohol and formaldehyde vapor pressure, ethylene oxide gas, peroxide vapor, ultraviolet light, ozone, and prolonged immersion in glutaraldehyde.[2] Interestingly, boiling water was regularly used up to the 1960s to disinfect dental instruments, but not sterilize them, since bacterial spores are not killed by water heated to only 100°C.[1] It was recently pointed out that "in some developing countries, boiling instruments persists as a method of reprocessing (dental instruments)".[3]

Inadequate dental instrument sterilization may result in the spread of infectious agents to patients. The first documented case of patient-to-patient transmission of a blood-borne pathogen in a dental setting in the United States was reported in 2007.[4] The case involved the spread of a specific hepatitis B virus strain, verified by DNA sequencing of hepatitis B surface antigens, between two adults treated within 3 hours of each other in the same dental operatory in an oral surgery practice, which occurred despite no identifiable infection control deficiencies noted at the dental office.[4] In 2014, two adults treated in an oral surgery practice developed hepatitis C infections from genetically identical strains, with contaminated surgical instruments suspected as the vector of transmission.[5] Contaminated dental instruments were also implicated in a 2015 outbreak of hepatitis C infections among five adult patients in a general dental practice in England.[6]

Steam autoclave sterilization failure most frequently occurs from operator error involving improper pre-autoclave instrument cleaning, incorrect positioning or wrapping of instrument loads, or improper autoclave settings relative to temperature, cycle time, or

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air pressure.[7,8] Mechanical malfunction may also occur among older or inadequately serviced autoclaves.[7]

Because sterilization is not directly measurable,[6] BST was developed as an indirect measure of sterility, based on the concept that if highly thermoresistant bacterial spores are killed in a sterilization cycle, then all other forms of microbial life are also killed. Biological spore test for steam autoclaves employs spores of *Geobacillus stearothermophilus*, a non-pathogenic, gram-positive, thermophilic bacillus found in soil, hot springs, and ocean sediment.[9] *G. stearothermophilus* spores resist death by moist heat more than all frequently encountered pathogenic vegetative bacteria and viruses.[10] The first reported use of BST to assess dental steam autoclaves was in Germany in 1976, and in the United States in 1979.[7] BST is today considered the gold standard for steam autoclave sterilization quality assurance.[11] In the United States, BST is recommended by the American Dental Association and the Centers for Disease Control and Prevention, and required by many state dental laws, to be performed at least weekly, and every time a dental implant is sterilized.[8,12–14] BST is also used worldwide in hospitals and other healthcare facilities to validate the efficacy of steam autoclave sterilization of reusable medical instruments and devices.[15]

Biological spore test has undergone three phases of commercial development.[16] First-generation BST employ paper strips coated with *G. stearothermophilus* spores, which are incubated in a broth for 7 days after autoclave exposure to detect turbidity changes indicative of spore germination.[16] Second-generation BST have *G. stearothermophilus* spores in self-contained ampoules with a culture medium, which are incubated for 24–48 hours and assessed for pH-induced color changes resulting from post-autoclave spore survival and germination.[16] Third-generation BST similarly employs self-contained ampoules but detects spore germination via increased levels of a specific bacterial enzyme after 60 minutes of incubation.[16]

The most widely used dental BST, largely because of its low cost and visual scoring without special equipment, is a second-generation brand that requires incubation over a 2-day time period, which limits swift identification of sterilization failure.[17] A recently-introduced third-generation BST, with only a 20-minute incubation time, provides a more rapid assessment of autoclave sterilization but has only manufacturer data available on its reliability.[18] The purpose of this study was to compare the reliability of these two types of BST in determining the sterilization efficacy of dental steam autoclaves.

Materials and Methods

This study was carried out in the Oral Microbiology Testing Service (OMTS) Laboratory at Temple University School of Dentistry in Philadelphia, Pennsylvania. The OMTS Laboratory is licensed by the Pennsylvania Department of Health and CLIA-certified by the United States Centers for Medicare and Medicaid Services for high-complexity bacteriological analysis.

Two commercial brands of BST were evaluated: a recently-introduced rapid BST test (Celerity™ 20 Steam Biological Indicator, Steris Corporation, Mentor, Ohio, USA),[18] and a widely used conventional BST (Attest™ 1262 Biological Indicator, 3M Corporation, St Paul, Minnesota, USA).[16] Both of these BST indicators contained thermoresistant *G. stearothermophilus* spores, with a mean of 1.0-4.0 $\times$ 10^6 colony-forming units of the organism in each BST ampoule.

One of each of the BST brands was placed weekly in pairs over a 9-month period into 14 dental school steam autoclaves loaded with dental instrument cassettes, and subjected to manufacturer-recommended sterilizing settings, providing 157 pairs of BST brands exposed to autoclave sterilization conditions. The dental school steam autoclaves were composed of gravity displacement and vacuum-assisted models, including two Getinge 533HC autoclaves, two Getinge 733HC autoclaves, two Midmark UltraClave autoclaves, two Tuttnauer Elara 11 autoclaves, two Tuttnauer 3870EA autoclaves, one SciCan Statim 2000 autoclave, and three SciCan Statim 5000 autoclaves. All of the steam autoclaves were professionally serviced and maintained, with trained dental school staff operating them at manufacturer-recommended sterilizing settings. The gravity displacement steam autoclaves were operated at a sterilizing temperature setting of 121°C at 15 pounds of force per square inches of air pressure (psi) for at least 15 minutes, with vacuum-assisted models employing a sterilizing temperature of 135°C at 30.8 psi for holding times of 3.5 (Statim 2000) or 6 minutes (Statim 5000). Additional pairs of Celerity™ and Attest™ BST (20 ampoules each) were placed into a single gravity displacement steam autoclave for a non-sterilizing aborted time of 5 minutes, instead of the manufacturer-recommended sterilizing time of 15 minutes.

Aseptic processing and laboratory incubation of each of the BST ampoule pairs followed manufacturer recommendations after steam autoclave exposure, with each run including BST ampoules of each brand unexposed to steam autoclaving as positive controls. Celerity™ BST ampoules were incubated for 20 minutes at 57°C in a special incubator which spectrophotometrically evaluated the BST growth medium for fluorescent α-glucosidase signal changes (no fluorescence change with successful sterilization; increased fluorescence after failed sterilization) (Fig. 1). *G. stearothermophilus* spores surviving after failed sterilization produce α-glucosidase upon germination, which reacts with a 4-methylumbelliferyl-α-D-

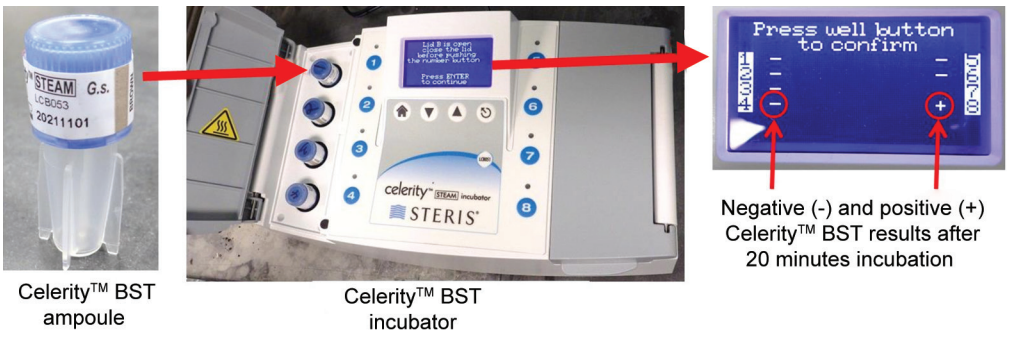

Fig. 1: Celerity™ BTS system

glucopyranoside fluorescent substrate in the BST growth medium to increase fluorescence intensity levels.

Attest™ BST ampoules were incubated for 48 hours at 57°C in a laboratory heating block, after which a potential acid pH-based color change in the BST nutrient culture broth from viable *G. stearothermophilus* spore germination was visually assessed (no color change in purple color with successful sterilization; change to yellow color with failed sterilization) (Fig. 2).

Descriptive data analysis tabulated BST outcomes with successful and failed steam autoclave sterilization. The Fisher's exact test, and a *p*-value of ≤0.05 for statistical significance, were used to evaluate outcome differences between the two brands of BST. The 177 BST pair sample size provided 80% power and a two-sided significance of 5% for detecting a difference of 0.19 between discordant pair outcome proportions.[19] Using 2 × 2 contingency table analysis, sensitivity [true positive (TP) rate], specificity [true negative (TN) rate], and accuracy (the proportion of correctly classified BST outcomes relative to the presence or absence of sterilizing steam autoclave conditions), were calculated to evaluate and compare the performance of Celerity™ and Attest™ BST for detection of steam autoclave sterilization conditions.[20] Sensitivity was calculated as TP outcomes divided by TP outcomes plus false negative (FN) outcomes. Specificity was determined from TN outcomes divided by false positive (FP) outcomes plus TN outcomes. Accuracy was defined as (TP + TN) divided by (TP + TN + FP + FN).[20] The PC-based STATA/SE 16.1 for Windows (StataCorp PL, College Station, Texas, USA) 64-bit statistical software package was used in the data analysis.

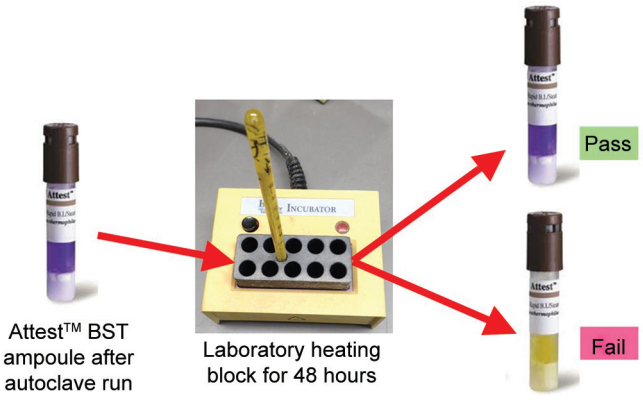

Fig. 2: Attest™ BST system

Table 1: Survival of *G. stearothermophilus* spores in two BST indicators after dental steam autoclave exposure

	Dental steam autoclave conditions	
BST brand	*Sterilizing*	*Non-sterilizing*
Celerity™	0/157[a]	20/20
Attest™	0/157	20/20

[a]Number of BST ampoules with spore survival/number of BST ampoules tested

Results

All Celerity™ and Attest™ BST ampoules not exposed to any steam autoclave conditions and used as positive controls in BST laboratory processing were, as expected, positive for *G. stearothermophilus* spore growth.

All 157 pairs of Celerity™ and Attest™ BST subjected to sterilizing steam autoclave conditions accurately identified successful sterilization, with no *G. stearothermophilus* spore growth from either BST ampoules after steam autoclave exposure to manufacturer-recommended sterilizing temperature and air pressure operating conditions (Table 1). This provided 100% agreement, and no statistically significant difference in the prevalence of successful sterilization outcomes ($p = 1.000$, Fisher's exact test), between the 157 pairs of BST brands after sterilizing steam autoclave exposure.

Celerity™ and Attest™ BST also accurately detected unsuccessful sterilization, with all tested BST ampoules positive for *G. stearothermophilus* spore growth after exposure to non-sterilizing aborted steam autoclave time periods of only 5 minutes, instead of a manufacturer-recommended sterilizing time of 15 minutes (Table 1).

In contingency table analysis, both BST brands exhibited 100% sensitivity, 100% specificity, and 100% accuracy for the detection of sterilizing steam autoclave conditions (Table 2).

Discussion

The present study provides the first reported non-manufacturer evaluation of a new rapid BST for assessing steam autoclave sterilization performance with dental instruments. The most important finding was documentation of the reliability of the Celerity™ BST in rapidly determining the sterilization efficacy of dental steam autoclaves within only a 20-minute incubation and evaluation time period, as compared to 48 hours of incubation required by the widely used Attest™ BST. There was 100% agreement, and no statistically significant difference in the prevalence of successful sterilization outcomes, between 157 pairs of the Celerity™ and Attest™ BST indicators after steam autoclave exposure at manufacturer-recommended sterilizing temperatures, cycle time, and air pressure settings. In addition, when exposed to non-sterilizing steam autoclave conditions for an aborted suboptimal time period, all Celerity™ and Attest™ BST ampoules turned positive for *G. stearothermophilus* spore growth, which properly indicated failed sterilization.

These findings with the Attest™ BST are in agreement with previously published research establishing its reliability in evaluating autoclave sterilization conditions.[16,21] The Celerity™ BST outcomes are in agreement with and independently confirm, available manufacturer data.[18] No other data is presently published on the reliability of the Celerity™ BST for evaluating dental steam autoclave sterilization efficacy.

Table 2: Contingency table analysis of two BST brands for detection of steam autoclave sterilization conditions

BST brand	*True positive*	*False positive*	*False negative*	*True negative*	*Sensitivity*	*Specificity*	*Accuracy*
Celerity™	157[a]	0	0	20[b]	100	100	100
Attest™	157	0	0	20	100	100	100

[a]Number of BST ampoules with *G. stearothermophilus* spore death after exposure to sterilizing steam autoclave conditions; [b]Number of BST ampoules with *G. stearothermophilus* spore survival after exposure to non-sterilizing steam autoclave conditions

Critical to the ability of the Celerity™ BST to provide a test outcome within 20 minutes is its reliance upon spectrophotometric detection of α-glucosidase enzyme production by viable *G. stearothermophilus* after spore germination, and its interaction with a 4-methylumbelliferyl-α-D-glucopyranoside fluorescent substrate in the BST culture medium, resulting in the release of fluorescent 4-methylumbelliferone.[18] Importantly, only viable *G. stearothermophilus* vegetative cells produce α-glucosidase.[22] Thus, the increased levels of α-glucosidase detected by Celerity™ BST after failed sterilization represents enzyme synthesized and released by germinating *G. stearothermophilus* spores surviving steam autoclave exposure.[22]

As a result, the rapid 20-minute Celerity™ BST offers earlier detection of steam autoclave sterilization failure before potentially contaminated dental instruments are used in clinical patient care and provides a reliable alternative to widely employed BST products that require 48 hours of incubation after steam autoclave exposure. However, a limitation is that further commercial product development is needed to improve the application and affordability of the Celerity™ BST system for the dental profession. The specialized incubator plus spectrophotometer is presently designed for running seven tests and a positive control ampoule per 20-minute assay time, and retails for approximately $4,000 USD, with each BST ampoule costing approximately $18 USD. A needed future development is a smaller version of the specialized incubator, with fewer testing wells at a lower retail price, as well as a lower price per BST ampoule, which would be more suitable for dental practice settings.

An important issue not addressed by any current BST is how to detect and inactivate prions, which may persist on dental instruments even after successful sterilization and destruction of all viable microorganisms. Prions are misfolded proteins associated with certain fatal neurological diseases in humans, such as Creutzfeldt-Jakob disease and Kuru,[23] which are highly resistant to moist heat and steam autoclave sterilization conditions.[24,25] Clinical use of dental instruments with protein residues persisting after cleaning and autoclave sterilization,[26] which may include prions from previously treated patients, on oral tissues linked in close proximity to trigeminal nerve nuclei in the brain stem, may render the oral cavity especially vulnerable to prion exposure.[27] Supporting this view, variant Creutzfeldt-Jakob disease was found to be experimentally transmitted to 97% of challenged mice following transient (5-minute), atraumatic, surface exposure of gingiva to a prion-contaminated dental file.[27] In humans, a case-control study in Switzerland, after adjusting for age, gender, and education, reported a 2.6 significantly increased odds of sporadic Creutzfeldt-Jakob disease in adults with a history of invasive dental care,[28] although other studies did not detect such a relationship.[29–31] An urgent need exists to find methods to reliably remove prions from contaminated dental instruments and to accurately detect their presence with testing kits similar to BST indicators of microbial life, in order to prevent their possible iatrogenic transmission to patients during dental care.

Conclusion

Celerity™ BST, after only 20 minutes incubation, performed equally well as a BST requiring 48 hours incubation in determining the sterilization efficacy of dental steam autoclaves. Further product development to improve the application and affordability of Celerity™ BST for dental practice settings is recommended.

Clinical Significance

Rapid BST offer earlier detection of sterilization failure before potentially contaminated dental instruments are used in clinical patient care.

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