EVALUATION OF A RAPID BIOLOGICAL SPORE ASSURANCE TEST FOR DENTAL INSTRUMENT STERILIZATION

A Thesis
Submitted to
the Temple University Graduate Board

In Partial Fulfillment
of the Requirements for the Degree
MASTER OF SCIENCE

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May, 2021

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ABSTRACT

Objectives: Dental instrument sterilization with steam autoclaves is critical to maintaining infection control standards in dental practice, and preventing patient-to-patient transmission of pathogenic bacteria and viruses. The American Dental Association and the United States Centers for Disease Control and Prevention recommend, and many state dental laws require, weekly use of biological spore tests to verify dental instrument sterilization outcomes. However, the most widely used biological spore test needs microbial culture incubation for 2 days after autoclave exposure, which limits swift identification of sterilization failure.

To address this issue, this study evaluated the reliability of a new rapid biological spore test for determining the sterilization efficacy of dental steam autoclaves within 20 minutes.

Methods: Two commercial biological spore tests were evaluated in Temple University dental school steam autoclaves, 1.) the Steris Celerity 20 Steam Biologic Indicator with a 20-minute outcome time requirement, and 2.) the 3M Attest 1262 Biological Indicator with a 48-hour outcome time requirement. Both biological spore tests employed live thermoresistant *Geobacillus stearothermophilus* spores as an indicator of whether sterilization conditions in steam autoclaves were met or not. To compare their efficacy, a total of 157 pairs of the two biological spore tests were placed into dental steam autoclaves with dental instrument cassettes, and subjected to manufacturer-recommended steam autoclave temperature and air pressure operating conditions for an adequate sterilization time of 15 minutes. Two additional groups of 10 pairs each of the two biological indicators were subjected to appropriate steam autoclave

temperature and air pressure settings, but only for aborted non-sterilizing time periods of 10 and 5 minutes, respectively. Subsequent aseptic processing and laboratory incubation of both biological indicators was initiated within 2-24 hours, and followed manufacturer recommendations.

After autoclave exposure, Steris Celerity 20 Steam Biologic Indicator test ampoules were incubated in a specialized instrument for 20 minutes at 57 °C, which also spectrophotometrically evaluated the microbial culture medium for fluorescent α-glucosidase enzyme signal changes. No change in fluorescent intensity represented successful sterilization, whereas increased fluorescence indicated survival of viable *G. stearothermophilus* spores germinating into vegetative bacterial cells after failed sterilization.

3M Attest 1262 Biological Indicator ampoules were incubated for 48 hours in a laboratory heating block at 57 °C, after which a pH-based color change in the microbial culture broth was visually assessed. No change in the color of the culture broth (purple color remains) indicated successful sterilization, whereas development of a yellow color in the culture broth, as a result of viable *G. stearothermophilus* spore germination into vegetative bacterial cells, denoted failed sterilization.

Results: A total of 354 biological indicators were exposed to dental steam autoclaves sterilization cycles, incubated for either 20 minutes or 48 hours, and evaluated for *G. stearothermophilus* spore growth. The Steris Celerity and 3M Attest biological spore tests both uniformly detected successful sterilization, with no *G. stearotherm-ophilus* spore growth, after 15 minutes of steam autoclave exposure at manufacturer-recommended steam autoclave temperature and air pressure operating conditions. This

provided 100% agreement, and no statistically significant difference in the prevalence of successful sterilization outcomes, between 157 pairs of both biological indicator types after 15 minutes of steam autoclave exposure.

Similarly, both biological spore test systems were also in complete agreement after only 5 minutes of steam autoclave exposure, with 100% of both biological indicators positive for *G. stearothermophilus* spore growth, indicating failed sterilization.

In contrast, after 10 minutes of steam autoclave exposure, there was a complete lack of agreement between the two types of biological indicators. All 10 Steris Celerity spore tests were positive, whereas all 10 3M Attest ampoules were negative, for *G. stearothermophilus* spore growth after 10 minutes of steam autoclave exposure. Relative to this disagreement, a non-biological chemical indicator strip that was part of the Steris biological indicator test system failed to have a darkened bar develop and extend into the "Accept (OK)" portion of the strip for all Steris Celerity spore tests exposed to either 5 minutes or 10 minutes of steam autoclave exposure, indicating that adequate autoclave steam, temperature and/or time parameters had not been attained for sterilization.

Conclusions: The Steris Celerity biological spore test was successful in rapidly determining the sterilization efficacy of dental steam autoclaves within only a 20-minute incubation time period, as compared to 48 hours of incubation required by the widely-used 3M Attest biological spore test. As a result, this rapid assay offers earlier detection of steam autoclave sterilization failure before potentially contaminated dental instruments are used in clinical patient care.

The alarming failure of 3M Attest biological spores to grow after a non-sterilizing 10-minute steam autoclave exposure time warrants further product evaluation.

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INTRODUCTION

Sterilization is a process where all life forms are rendered non-viable (Hancock 2013), and the sterility assurance level, known as the probability of finding a single living microorganism in or on a medical device, is at most one in 1,000,000 (van Doornmalen & Kopinga 2008). Sterilization of dental instruments, before and after their use in clinical patient care, is critical to maintaining infection control standards in dental practices, and preventing dental patient-to-dental patient transmission of pathogenic bacteria, viruses, and fungi.

There are a variety of methods for sterilizing dental instruments. They most frequently include use of a steam autoclave, which applies moist heat under increased air pressure, as well as less often employed methods, such as dry heat oven sterilization (dryclave), unsaturated chemical (alcohol and formaldehyde) vapor pressure sterilization (chemiclave), ethylene oxide gas sterilization, peroxide vapor sterilization, ultraviolet light, ozone sterilization, and prolonged immersion into some liquid chemical agents, such as glutaraldehyde (Sebastiani et al. 2017).

Prior to the 1960s, boiling water was regularly used to disinfect dental instruments, but not sterilize them, as bacterial spores are not adequately killed by water heated to only 100 °C (Fulford & Stankiewcz 2020a). Amazingly, it has been pointed out that "even in the mid-1980s, boiling instruments in water remained a popular means of reprocessing instruments in the UK", and that more recently "in some developing

countries, boiling instruments persists as a method of reprocessing [dental instruments]" (Fulford & Stankiewcz 2020b).

By the 1960s, steam autoclaves became more available and employed for sterilization of dental instruments in-between patient use. Such devices use steam because it contains more latent heat to potentially transfer onto dental instrument surfaces than water at the same temperature, and increased air pressure to raise the temperature of steam to levels markedly above 100 °C in order to kill bacterial spores (Fulford & Stankiewcz 2020a).

Inadequate sterilization of dental instruments may result in dental patient-to-dental patient transmission of infectious agents leading to clinical disease, although definitive proof of this phenomenon, and the level of risk encountered, is not available and ethically difficult to experimentally study. The first documented case of dental patient-to-dental patient transmission of a bloodborne pathogen in a dental setting in the United States was reported in 2007 (Redd et al. 2007). The case involved 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 private specialty practice, which occurred despite no identifiable deficiencies in infection control practices at the dental office (Redd et al. 2007). In 2014, two adults treated in an oral surgery practice in Tulsa, Oklahoma developed hepatitis C infections from genetically identical strains, with the investigating epidemiologist suggesting that contaminated surgical instruments were a possible vector of transmission (Weaver 2014). Contaminated dental instruments have also been

implicated in a 2015 outbreak of hepatitis C infections among 5 adult patients attending a general dental practice in England (Volgenant & de Soet 2018). While the incidence of these types of events are reported to be rare (Millership et al. 2007, Cleveland et al. 2016), according to one review, these findings nevertheless "suggest that transmission of pathogens between patients and dental equipment and vice versa does exist," and that "infections related to the dental office are most likely when infection control measures are not followed meticulously" (Volgenant & de Soet 2018). It is likely that additional cases of dental patient-to-dental patient infectious spread of pathogenic microorganisms due to inadequate dental instrument sterilization have occurred in dental practices throughout the world, but have gone unrecognized due to their asymptomatic transmission, or remained unreported by patients and clinicians, and not investigated by regulatory body authorities.

Most frequently, inadequate steam autoclave sterilization occurs due to operator errors, where improper pre-autoclave instrument cleaning fails to sufficiently reduce surface bioburdens that insulate underlying microorganisms, or incorrect positioning or wrapping of instrument loads impairs steam penetration and contact with dental instrument surfaces, or improper autoclave settings are employed relative to autoclave temperature, cycle time, and/or air pressure (Skaug et al. 1999, Miller 2001). Additionally, mechanical dysfunction of the autoclave device itself may further account for some sterilization failure events, particularly among older or poorly serviced units (Skaug et al. 1999).

Monitoring of steam autoclave sterilization effectiveness may be undertaken with chemical indicator strips, vials, or tapes which, by a color change, indicate that certain sterilization conditions were met in an autoclave cycle, and by use of biological spore testing to determine whether or not viable bacterial spores were actually killed by the autoclave exposure (American Dental Association 1988, 1996). Of the two approaches, biological spore testing is considered to be a more stringent assay for determining autoclave sterilization capability (American Dental Association 1988, 1996). The American Dental Association and United States Centers for Disease Control and Prevention recommend, and many state dental laws require (such as California, Florida, Indiana, Ohio, Oregon, and Washington), weekly use of biological spore tests to verify dental instrument autoclave sterilization outcomes (American Dental Association 1988, 1996, Miller 2001).

For dental steam autoclaves, biological spore testing is performed most frequently with spores formed by *Geobacillus stearothermophilus*, a non-pathogenic, Gram-positive, thermophilic bacilli found in various non-human environmental sites, such as in soil, hot springs, and ocean sediment (McMullan et al. 2004). *G. stearothermophilus* spores resist death by high moist heat more than all common pathogenic vegetative bacteria and viruses (Feeherry et al. 1987). Commercial products with *G. stearothermophilus* spores for steam autoclave sterilization testing have been available since the mid-1970s, at which time the first reported use of biological indicators in assessing dental steam autoclaves was in Germany in 1976, and in the United States in 1979 (Skaug et al. 1999).

At present, the most widely used biological spore test in dental practices requires microbial culture incubation for 2 days to see if there is survival and germination of *G. stearothermophilus* spores after autoclave exposure, which limits timely identification of steam autoclave sterilization failures (Palenik et al. 1999).

In the fall of 2019, discussions between the Central Sterilization Facility and the Oral Microbiology Testing Service Laboratory at Temple University School of Dentistry in Philadelphia focused on finding an alternative *G. stearothermophilus* spore-based biological indicator assay for dental steam autoclaves that did not require a 2-day incubation time after autoclave exposure. A relatively new, commercially-available, biological spore test for determining the sterilization efficacy of steam autoclaves within 20 minutes was identified, but no published research was found at that time relative to its reliability.

To address this issue, this study evaluated the reliability of a new rapid biological spore test for determining the sterilization efficacy of dental steam autoclaves within 20 minutes.

CHAPTER 2

MATERIALS AND METHODS

Laboratory Facilities

This study was carried out in the Oral Microbiology Testing Service Laboratory at the Temple University Maurice H. Kornberg School of Dentistry in Philadelphia, Pennsylvania. The Oral Microbiology Testing Service Laboratory is state-licensed for high-complexity bacteriological analysis by the Pennsylvania Department of Health (Clinical Laboratory Permit No. 021872) as an oral microbiology reference laboratory. The Oral Microbiology Testing Service Laboratory is additionally certified by the United States Centers for Medicare and Medicaid Services to be in compliance with Clinical Laboratory Improvement Amendments (CLIA) regulations (CLIA Certificate No. 39D0707385) pertaining to standards required of United States clinical laboratories engaged in diagnostic testing of human specimens (Rauch & Nichols, 2007).

The present study was laboratory-based, and did not involve humans or identifiable private human information or biologic specimens, as defined by United States Department of Health and Human Services regulations at 45 CFR part 46.116(f). As a result, the study did not require review or approval from the Temple University Human Subjects Protections Institutional Review Board.

Biological Spore Tests

Two commercial biological spore tests were evaluated in Temple University dental school steam autoclaves, 1.) the Celerity 20 Steam Biological Indicator (Steris Corporation, Mentor, OH, USA), and 2.) the Attest 1262 Biological Indicator (3M

Corporation, St. Paul, MN, USA). Both of these biological indicators employed thermoresistant *G. stearothermophilus* spores as a marker for determining whether sterilization conditions were met by steam autoclave test cycles.

Steam Autoclave Sterilization Testing - Part 1

The first part of the study involved placement of each of the two biological spore tests into dental steam autoclaves with cassettes of dental instruments, and then subjecting them to manufacturer-recommended steam autoclave temperature and air pressure operating conditions for an adequate sterilization time of 15 minutes. A total of 157 pairs of both biological indicators were evaluated in this way from 14 dental steam autoclaves located in Temple University dental school's Graduate Periodontology Clinic, Graduate Endodontics Clinic, Graduate Orthodontics Clinic, Emergency Oral Surgery Clinic, Sedation Clinic, and Central Sterilization facility (Table 1).

Table 1. Distribution of Dental Steam Autoclaves Tested with Biological Indicators

Autoclave Location	Brand/Model	Model Number
Graduate Periodontology Clinic:		
Autoclaves #1 and 2	Tuttnauer	3870EA/ ELARA11
Graduate Endodontics Clinic:		
Autoclaves #1 and 2	Tuttnauer	ELARA11
Graduate Orthodontics Clinic:		
Autoclave #1	Midmark/ M9 UltraClave	M9-020/ 3861150

Table 1 (continued)

Autoclave Location	Brand/Model	Model Number
Autoclave #2	Midmark/ M11UltraClave	M11-020
Autoclaves #3 and 4	SciCan/ Statim 5000	01-201103/ G4-201103
Sedation Clinic:		
Autoclave #1	SciCan/ Statim 5000	G4-201103
Emergency Oral Surgery Clinic:		
Autoclave #1	SciCan/ Statim 2000	01-121101
Central Sterilization:		
Autoclaves #1 and 2	Getinge	533 HC
Autoclaves #3 and 4	Getinge	733 HC

The autoclaves were operated at a temperature setting of 121 °C (250 °F) and 15 pounds of force per square inches of air pressure for 15 minutes, except for the SciCan Statim steam autoclaves. The Statim 2000 models employed a temperature of 135 °C (275 °F) and 30.8 pounds of force per square inches of air pressure for a holding time of 3.5 minutes, while the Statim 5000 models used a temperature of 135 °C (275 °F) and 30.8 pounds of force per square inches of air pressure for a holding time of 6 minutes.

Dental school staff assigned to each of these areas were responsible, as part of their routine weekly quality control procedures, for placement of the biological indicators into the dental steam autoclaves during sterilization cycles, and then taking the exposed tests to the Oral Microbiology Testing Service Laboratory for independent analysis. Aseptic processing and laboratory incubation of each of these biological indicators was initiated within 24 hours after steam autoclave exposure, and followed manufacturer recommendations.

This part of the study was completed over approximately a 9-month time period, extending from November 25, 2019 to March 16, 2020, and from May 26, 2020 to December 1, 2020.

Steam Autoclave Sterilization Testing - Part 2

In the second part of this study, additional pairs of both biological spore indicators were subjected to manufacturer-recommended steam autoclave temperature and air pressure operating conditions, but for only suboptimal non-sterilizing time periods where dental autoclave sterilization cycles were aborted after only 10 minutes (10 pairs of both biological indicators), as well as after only 5 minutes (10 pairs of both biological indicators). This testing was performed using the Steris Amsco 3021 steam autoclave located in the dental school's CORE research area on the third floor of Building 600 (Old Dental School Building), and set at a temperature of 121 °C (250 °F) and 15 pounds of force per square inches of air pressure. Oral Microbiology Testing Service Laboratory staff (Dr. Thomas E. Rams and Jacqueline D. Sautter) and Dr. Andie H. Lee autoclave-exposed these biological indicators. Aseptic processing and laboratory incubation of

each of these biological indicators was initiated within 2 hours after steam autoclave exposure, and followed manufacturer recommendations.

This part of the study was carried out in January, 2021.

Processing Steris Celerity 20 Steam Biological Indicators

Steris Celerity 20 Steam Biological Indicators were packaged in Steris Celerity 20 Steam Process Challenge Devices with a Type 5 steam integrating indicator (Figure 1).

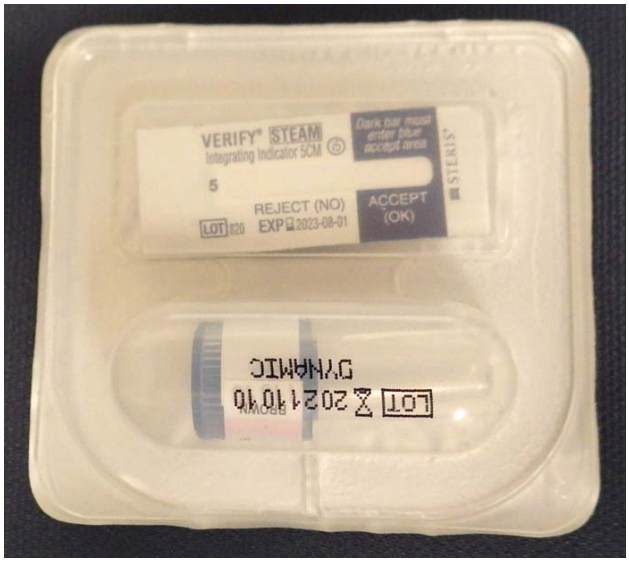

Figure 1. Steris Celerity 20 Steam Process Challenge Device with Type 5 steam integrating indicator strip (top portion) and Steris Celerity 20 Steam Biological Indicator (bottom portion).

Steris Celerity 20 Steam Biological Indicator plastic vials, inoculated with *G. stearothermophilus* spores (mean 1.0 x 10^6 to 4.0 x 10^6 colony forming units/vial), and a proprietary culture media sealed within the cap, were allowed to cool after autoclave exposure. After removal from the Celerity 20 Steam Process Challenge Device, the

biological indicators were activated by twisting the vial cap clockwise to release the culture media, and shaking the vial once to mix it with autoclave-exposed *G. stearothermophilus* spores. The vials were then incubated in a specialized instrument for 20 minutes at 57 °C, which also spectrophotometrically evaluated the microbial culture medium for fluorescent α-glucosidase enzyme signal changes. No change in fluorescent intensity represented successful sterilization, whereas increased fluorescence indicated failed sterilization, as surviving *G. stearothermophilus* spores produced α-glucosidase enzyme activity upon germination, which reacted with a 4-methylumbelliferyl-α-D-glucopyranoside fluorescent substrate in the indicator culture media (Figure 2).

Figure 2. Processing of autoclaved Steris Celerity 20 Steam Biological Indicator (left). After 20-minute incubation in Celerity Steam incubator (center), fluorescent α-glucosidase levels were spectrophotometrically evaluated, with increased fluorescence (+ sign) indicating failed sterilization, and no change in fluorescence (- sign) associated with successful sterilization (right).

A non-autoclave exposed Steris Celerity 20 Steam Biological Indicator plastic vial was processed and incubated with each test run as a positive control, as recommended by the manufacturer.

The Type 5 steam integrating indicator strip packaged in the Steris Celerity 20 Steam Process Challenge Device was a non-biological chemical indicator of autoclave steam, temperature and time parameters. During a steam autoclave sterilization cycle, steam penetrating into the indicator strip melted a steam-sensitive proprietary chemical agent onto one end of a wicking paper strip, turning it dark blue as it soaked towards the other end of the strip. Critical steam sterilization conditions were considered to have been met once the darkened bar extended over time far enough along the indicator strip into a portion marked as "Accept (OK)".

Oral Microbiology Testing Service Laboratory staff (Dr. Thomas E. Rams and Jacqueline D. Sautter) and Dr. Andie H. Lee participated in evaluating these biological indicators.

Processing 3M Attest 1262 Biological Indicators

3M Attest 1262 Biological Indicator plastic vials were allowed to cool for at least 15 minutes after autoclaving, after which an internal glass ampoule was physically crushed to permit mixing of an indicator nutrient culture broth with the autoclave-exposed *G. stearothermophilus* spores (mean 1.0×10^6 to 4.0×10^6 colony forming units/vial). The proprietary nutrient culture broth was comprised of water (99.8%), dextrose (< 0.19%), inosine (< 0.001%), L-alanine (< 0.004%), L-tyrosine (< 0.01%), and the monosodium salt of bromocresol purple (< 0.001%), and had a pH = 7.5 prior to autoclave exposure (3M Company 2020). The activated biological indicator vials were incubated in a laboratory heating block for 48 hours at 57 °C, and then visually assessed. No change in the color of the nutrient culture broth, where a purple color remained,

indicated complete cell death of the autoclave-exposed *G. stearothermophilus* spores and successful sterilization. In contrast, development of an acid-induced pH shift, leading to a yellow color change in the nutrient culture broth, denoted failed sterilization due to germination of *G. stearothermophilus* spores surviving the autoclave exposure (Figure 3).

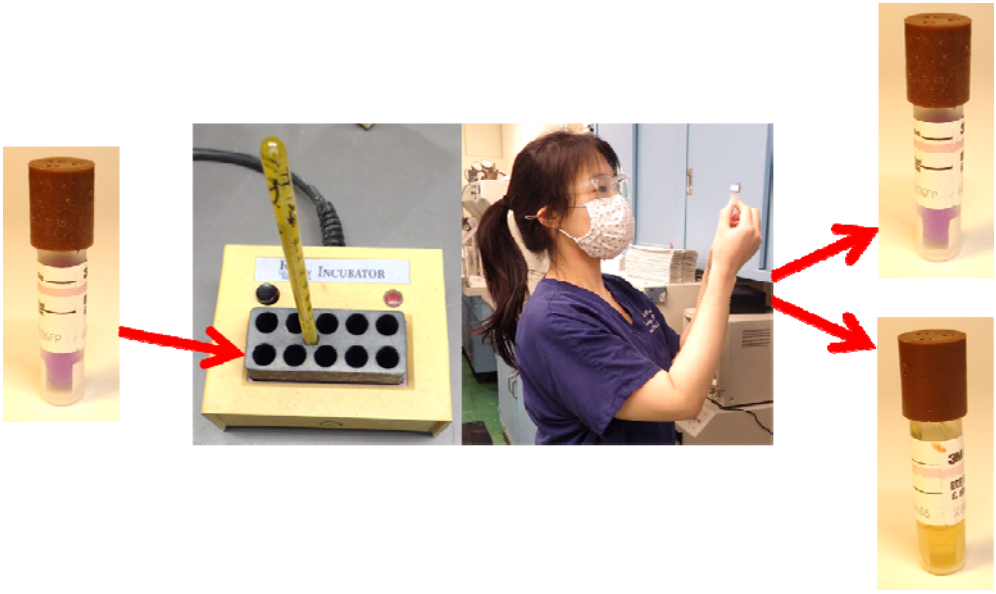

Figure 3. Processing of autoclaved 3M Attest ampoule (left). After 48-hour incubation in a laboratory heating block and visual evaluation (center), successful sterilization was indicated by no change in the purple color of the nutrient culture broth (right top), and failed sterilization by a yellow color change (right bottom).

A non-autoclave exposed 3M Attest 1262 Biological Indicator plastic vial was processed and incubated with each test run as a positive control, as recommended by the manufacturer (3M Health Care 2016), in order to ensure use of correct incubation

conditions, viability of indicators, and capability of the nutrient broth to promote rapid germination of viable *G. stearothermophilus* spores.

Oral Microbiology Testing Service Laboratory staff (Dr. Thomas E. Rams and Jacqueline D. Sautter) and Dr. Andie H. Lee participated in evaluating these biological indicators.

Data Analysis

Descriptive analysis tabulated and compared the number of Steris Celerity 20 Steam Biological Indicator and 3M Attest 1262 Biological Indicator tests associated with successful and failed sterilization after 15, 10 and 5 minutes of steam autoclave exposure. Fisher's exact test evaluated differences between each of the two biological indicators in the prevalence of test outcomes associated with successful sterilization outcomes after 15 minutes of steam autoclave exposure, with a *P*-value of ≤ 0.05 required for statistical significance. The PC-based STATA/SE 16.1 for Windows (StataCorp PL, College Station, TX, USA) 64-bit statistical software package was used in the data analysis.

Oral Microbiology Testing Service Laboratory staff (Dr. Thomas E. Rams and Jacqueline D. Sautter) and Dr. Andie H. Lee participated in the tabulation and/or analysis of the study data.

CHAPTER 3

RESULTS

Biological Spore Test Outcomes

A total of 354 biological indicators were exposed to dental steam autoclaves sterilization cycles, incubated for either 20 minutes or 48 hours, and evaluated for *G. stearothermophilus* spore growth. Table 2 provides the survival prevalence of viable *G. stearothermophilus* spores in Steris Celerity and 3M Attest biological indicators after dental steam autoclave exposure for optimal sterilization times and suboptimal non-sterilizing periods.

Table 2. Survival of *Geobacillus stearothermophilus* Spores in Two Biological Indicators After Varying Times of Dental Steam Autoclave Exposure

	Dental steam autoclave exposure time		
Biological indicator:	5 minutes	10 minutes	15 minutes
Steris Celerity	10/10 *	10/10	0/157
3M Attest	10/10	0/10	0/157

* number of tests with spore survival/number of tests performed

Both biological spore test systems uniformly detected successful sterilization, with no *G. stearothermophilus* spore growth, after 15 minutes of steam autoclave exposure at manufacturer-recommended steam autoclave temperature and air pressure operating conditions. This provided 100% agreement, and no statistically significant

difference in the prevalence of successful sterilization outcomes (P = 1.000, Fisher's exact test), between 157 pairs of both biological indicator types after 15 minutes of steam autoclave exposure.

Similarly, both biological spore test systems were also in complete agreement after only 5 minutes of steam autoclave exposure, with 100% of both biological indicators positive for *G. stearothermophilus* spore growth, indicating failed sterilization.

In contrast, after 10 minutes of steam autoclave exposure, there was a complete lack of agreement between the two types of biological indicators. All 10 Steris Celerity spore tests were positive, whereas all 10 3M Attest ampoules were negative, for *G. stearothermophilus* spore growth after 10 minutes of steam autoclave exposure (Table 2).

The non-biological chemical indicator strip that was part of the Steris biological indicator test system (Figure 1) failed to have a darkened bar develop and extend into the "Accept (OK)" portion of the strip for all Steris Celerity spore tests exposed to either 5 minutes or 10 minutes of steam autoclave exposure, indicating that adequate autoclave steam, temperature and/or time parameters had not been attained for sterilization.

CHAPTER 4

DISCUSSION

Since the development of scientifically-based steam autoclave temperature, time, and air pressure standards by Louis Pasteur and Robert Koch in the 1880s (Hugo 1991), proof of sterility of medical and dental instruments has been problematic, since sterilization is not directly measurable (Volgenant & de Soet 2018). Instead, biological indicator tests provide an indirect measure of sterility attained by autoclaves, based on the supposition that if highly thermoresistant bacterial spores, such as those associated with *G. stearothermophilus*, are destroyed by a sterilization cycle, then all other life forms will also be destroyed.

The present study provides the first known evaluation of a new rapid biological indicator of steam autoclave sterilization performance with dental instruments. The most important finding from this study is documentation of the reliability of the Steris Celerity biological spore test in rapidly determining the sterilization efficacy of dental steam autoclaves within only a 20-minute incubation time period, as compared to 48 hours of incubation required by the widely-used 3M Attest biological spore test. In an analysis of 354 total biological indicator tests exposed to dental steam autoclaves sterilization cycles, incubated for either 20 minutes or 48 hours, and evaluated for *G. stearothermophilus* spore growth, there was 100% agreement, and no statistically significant difference in the prevalence of successful sterilization outcomes, between 157 pairs of the Steris Celerity and 3M Attest biological indicators after 15 minutes of steam autoclave exposure at autoclave manufacturer-recommended sterilizing temperatures and air pressure settings.

In addition, both biological spore test systems had 100% agreement in their outcomes when exposed to only 5 minutes of suboptimal steam autoclave exposure, with all of the tests turning positive for *G. stearothermophilus* spore growth, indicating failed sterilization.

With 10 minutes of suboptimal steam autoclave exposure, all Steris Celerity biological indicator tests were also positive for *G. stearothermophilus* spore growth, indicating failed sterilization, in agreement with a non-biological chemical indicator strip, included with the Steris Celerity 20 Steam Process Challenge Device system, which indicated that adequate autoclave steam, temperature and/or time parameters had not been attained for sterilization during the aborted 10-minute steam autoclave cycle.

Surprisingly, all 3M Attest ampoules failed to yield *G. stearothermophilus* spore growth after only a suboptimal 10-minute steam autoclave exposure. Since the non-biological chemical indicator strips run concurrently with the 3M Attest vials indicated that sterilization conditions had not been met, the biological indicator test should have turned positive with *G. stearothermophilus* spore growth, similar to the accompanying Steris Celerity biological indicator test pair also included in the steam autoclave cycles. The reasons underlying this alarming failure of the 3M Attest biological indicator test to yield *G. stearothermophilus* spore growth after exposure to non-sterilizing conditions are not known and were outside the scope of this study, which was focused on assessing the sterilization assurance performance of the Steris Celerity biological indicator. Further investigation is needed to determine if the inadequate outcomes of the 3M Attest biological indicator were product lot-specific, or reproducible with other product vials.

The results of this study were profound for the Temple University dental school. After the conclusion of the approximately 9-month time period of comparative testing of the two biological indicators in pairs during routine steam autoclave sterilization evaluation, use of the 3M Attest biological indicator test was discontinued, and the Steris Celerity biological indicator was adopted for exclusive use going forward from December, 2020.

Critical to the ability of the Steris Celerity biological indicator to provide a test outcome in 20 minutes is its reliance upon spectrophotometric detection of α-glucosidase enzyme activity by viable *G. stearothermophilus* cells after spore germination, and interaction with a 4-methylumbelliferyl-α-D-glucopyranoside fluorescent substrate in the indicator test culture media. Importantly, only viable *G. stearothermophilus* vegetative cells produce α-glucosidase (Setlow et al. 2016). Thus, α-glucosidase detected in the Steris Celerity biological indicator test appears to represent enzyme levels present and released only from the dormant spore core of germinating *G. stearothermophilus* spores surviving autoclave exposure and new α-glucosidase synthesized during the *G. stearothermophilus* spore outgrowth process (Setlow et al. 2016).

As a result, the rapid 20-minute Steris Celerity spore test offers the possibility of earlier detection of steam autoclave sterilization failure before potentially contaminated dental instruments are used in clinical patient care, and provides for dental practices an alternative to the widely-employed biological spore tests that require 48 hours of incubation after steam autoclave exposure. Further research and commercial product

development facilitating greater availability and affordability of the Steris Celerity biological spore test into dental practices is recommended, since the specialized incubator used to spectrophotometrically detect α-glucosidase enzyme activity by viable *G. stear-othermophilus* is presently designed to evaluate up to 7 biological indicator tests and a control vial during a single 20-minute assay time, and retails for approximately $4,000, with the Celerity 20 Steam Process Challenge Device costing approximately $18 each. A smaller version of the specialized incubator, with fewer testing wells and a lower retail price, would be more appropriate for dental office applications.

A potentially explosive issue not addressed by any current biological indicator test is the problem of how to detect and inactivate prions that may be present on dental instruments, even after successful sterilization and destruction of all viable microbial life. Prions are misfolded proteins, highly resistant to moist heat and steam autoclave sterilization conditions (Casolari 1998), which are associated with certain fatal neurological diseases in humans, such as Creutzfeldt-Jakob disease and kuru (Ritchie & Barria 2021). Future research is urgently needed to find methods to reliably remove prions from contaminated dental instruments, and to accurate detect their presence or absence with testing kits similar to biological indicators of microbial life.

CHAPTER 5

CONCLUSIONS

The Steris Celerity biological spore test was successful in rapidly determining the sterilization efficacy of dental steam autoclaves within only a 20-minute incubation time period, as compared to 48 hours of incubation required by the widely-used 3M Attest biological spore test. Both biological spore tests uniformly detected successful sterilization, with no *G. stearothermophilus* spore growth, after 15 minutes of steam autoclave exposure (100% agreement between 157 pairs of both biological indicators). In addition, both biological spore tests were also 100% positive for *G. stearothermophilus* spore growth after only 5 minutes of steam autoclave exposure, indicating failed sterilization, with similar uniform spore growth found with the Steris Celerity spore test after 10 minutes of steam autoclave exposure.

As a result, the rapid 20-minute Steris Celerity spore test offers the possibility of earlier detection of steam autoclave sterilization failure before potentially contaminated dental instruments are used in clinical patient care, and provides for dental practices an alternative to the widely-employed biological spore tests that require 48 hours of incubation after steam autoclave exposure. Further research and commercial product development facilitating greater availability and affordability of the Steris Celerity biological spore test into dental practices is recommended.

Finally, the alarming failure of 3M Attest biological spores to grow after a non-sterilizing 10-minute steam autoclave exposure time warrants further product evaluation.

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