Sterilization Verification Procedure

Canton City Public Health operates Autoclaves for the purpose of sterilizing reagents, media, equipment used for clinical and drinking water testing as well as dental instruments. These various uses are regulated by multiple entities and provide similar sterilization function check requirements.

The process described here is intended to be comprehensive enough to meet the multiple governing authority requirements and general recommendations. Specific regulatory information can be found on subsequent pages.

INSTRUCTIONS FOR USE

Exposure:

1. Complete the Autoclave Sterilization Record
2. Place a minimum of TWO Biological Indicators (BI) into the sterilization chamber along with the material to be sterilized.
3. Test the most challenging area in the sterilizer (e.g. near the door and where the heaviest load is located, usually in the middle of the sterilizer chamber).
4. Process the load according to the sterilizer manufacturer's instructions.
5. Remove the BI.

Activation and Incubation:

1. Aseptically transfer spore strips into a tube containing soybean casein digest (also referenced as TSB) broth. The tube should be placed in the incubator immediately after the strips are cultured.
2. As a BI control, an unprocessed BI (from the same lot) should be prepared for incubation. The positive control shall become turbid following incubation. Incubation of positive controls should be read at one day and no later than 7 days.
3. As a media control, an unprocessed tube containing the same lot of soybean casein digest broth should be prepared for incubation. The negative media control should not become turbid during the 7-day growth cycle.
4. For Steam Sterilization incubate at 55-60°C and for Dry Heat Sterilization incubate at 35°C for 7 days, checking for spore growth at 1 day and 7 days. (Visual color change in medium and/or turbidity).

Test Results:

1. Record results after 24 hours and full incubation on the EPA Sterility Check record.
2. Sterilization conditions were achieved if BOTH of the following are true:
    a. No visual turbidity in the media control and autoclaved BI **and**
    b. Visual turbidity present in the positive control.
3. Any unexpected result should be reported immediately to a supervisor and the sterilizer taken out of service awaiting repeated test results or situation is resolved.

References:

Dental –

Ohio Administrative Code, 4715 State Dental Board, Chapter 4715-20 Patient and Personal Protection

4715-20-02 Sterilization and disinfection.

(1) Sterilization must be accomplished by an FDA-approved device or method, for example, autoclave, dry heat, or unsaturated chemical vapor.

(4) All heat sterilizing devices must be tested for proper function on a weekly basis by means of a biological monitoring system that indicates microorganism kill. The biological monitoring system used must include a control to verify proper microbial incubation. In the event of a positive biological spore test, the dentist must take immediate remedial action to ensure that heat sterilization is being accomplished.

(5) Biological monitoring documentation:

(a) In-office testing documentation - Documentation must be maintained in the form of a log reflecting dates, person(s) conducting the testing, and the results of the test capsule and control capsule.

(c) Documentation of testing and repairs shall be maintained for a period of at least two years, and shall be maintained in the dental facility and be made immediately available upon request by an authorized agent of the state dental board.

American Dental Association states:

The CDC recommends monitoring sterilizers at least weekly with biological indicators.[2, 3] Check to see whether your state dental board has different requirements.

Biological monitoring can be done in two ways:

1. In-office incubator and spore monitoring strips (contact your dental supplier for a list of products). This method usually gives results in 24-48 hours.
2. Mail-in spore monitoring programs. This process usually takes a week. Although it takes longer to get results using a service, third-party monitoring programs may provide more accuracy than in-house monitoring.

A positive spore test result indicates that sterilization failed. According to CDC recommendations:[1, 3]

1. Take the sterilizer out of service.

2. Review the sterilization process being followed in the office to rule out operator error as the cause of failure.
3. Correct any identified procedural problems, and retest the sterilizer using biological, mechanical, and chemical indicators.

If the repeat biological indicator test is negative and the other test results fall within normal limits, the sterilizer can be returned to service.

Maintain a log of spore test results. Check with your state dental board to determine how long you need to keep spore testing records.

Laboratory Manual for Microbiological Analyses of Public Drinking Water
Ohio Administrative Code Chapters 3745-81 and 3745-82 and rules 3745-83-01, 3745-91-06 and 3745-9-09

b. Autoclave sterility checks are required once every three months, per autoclave.

• If using a biological indicator ampule, follow manufacturer's instructions. Note: After sterilization, remove and allow ampules to cool for 10 minutes prior to incubation. Incubate at 55 - 60°C for 24 hours. Growth is evident by a color change per manufacturer's instructions. If color change occurs, corrective action for the autoclave is required.

• Alternatively, fill an Erlenmeyer flask with 25 to 50 mL of TSB/BHI, inoculate with a known coliform culture, cover flask opening with aluminum foil and incubate at 35 ± 0.5°C for 24 hours. After incubation, when TSB/BHI shows growth, autoclave at 119 - 121°C for 12 to 15 minutes on slow exhaust. Allow to cool to room temperature. Fill a test vessel with approximately 25 mL of TSB/BHI and inoculate the TSB/BHI with the "sterilized" culture from the Erlenmeyer flask. Incubate test vessel at 35 ± 0.5°C for 24 hours. After the 24 hour incubation period, remove the test vessel from the incubator. The inoculated test vessel must not show growth. If growth is present in the inoculated test vessel, corrective action for the autoclave is required.

Clinical Laboratory Improvement Amendments

Title 42 → Chapter IV → Subchapter G → Part 493 Title 42: Public Health

PART 493—LABORATORY REQUIREMENTS

§493.1254 Standard: Maintenance and function checks.

(a) *Unmodified manufacturer's equipment, instruments, or test systems.* The laboratory must perform and document the following:

(1) Maintenance as defined by the manufacturer and with at least the frequency specified by the manufacturer.

(2) Function checks as defined by the manufacturer and with at least the frequency specified by the manufacturer. Function checks must be within the manufacturer's established limits before patient testing is conducted.

(b) *Equipment, instruments, or test systems developed in-house, commercially available and modified by the laboratory, or maintenance and function check protocols are not provided by the manufacturer.* The laboratory must do the following:

(1)(i) Establish a maintenance protocol that ensures equipment, instrument, and test system performance that is necessary for accurate and reliable test results and test result reporting.

(ii) Perform and document the maintenance activities specified in paragraph (b)(1)(i) of this section.

(2)(i) Define a function check protocol that ensures equipment, instrument, and test system performance that is necessary for accurate and reliable test results and test result reporting.

(ii) Perform and document the function checks, including background or baseline checks, specified in paragraph (b)(2)(i) of this section. Function checks must be within the laboratory's established limits before patient testing is conducted.

FDA Definition

A biological sterilization process indicator is a device intended for use by a health care provider to accompany products being sterilized through a sterilization procedure and to monitor adequacy of sterilization. The device consists of a known number of microorganisms, of a known resistance to the mode of sterilization, in or on a carrier and enclosed in a protective package. Subsequent growth or failure of the microorganisms to grow under suitable conditions indicates the adequacy of sterilization.

Centers for Disease Control and Prevention, Guideline for Disinfection and Sterilization in Heathcare Facilities

16.d Use biologic indicators to monitor the effectiveness of sterilizers at least weekly with an FDA-cleared commercial preparation of spores (e.g., Geobacillus stearothermophilus for steam) intended specifically for the type and cycle parameters of the sterilizer.

Manufacturer information:

Mesa’s products are manufactured in accordance with USP and ISO Guidelines and the US Food & Drug Administration’s Quality System Regulations. Spore Strips are 510(k) cleared.

Combined Species Spore Strips – G. stearothermophilus* (105) and B. atrophaeus* (106) on each strip. For use in Steam, EO, Dry Heat or Chemiclave® sterilizers.

A minimum of two test strips in glassine paper should be introduced into the sterilization chamber along with the material to be sterilized. At the end of the sterilization process the strips should be aseptically removed from the glassine paper and transferred to the recovery media (tryptic soy broth). The media with the strip should then be incubated at 56°C (steam) or at 35°C (Dry Heat or EO). A control strip that has not been subjected to the sterilization process also should be incubated at this time in recovery media. The tubes of media should be checked for growth as described in the instructions accompanying each package of strips.

The biological indicator consists of bacterial spores of *Geobacillus stearothermophilus* and *Bacillus. atrophaeus* inoculated onto a paper filter carrier contained within a glassine envelope for use in steam sterilizers and dry heat sterilizers. The spore strip is removed from the load and aseptically transferred into appropriate microbiological culture medium. Biochemical activity of the organisms produce acid by-products that, when transferred to standard broth, will demonstrate noticeable turbidity.BI's are conventional spore growth readout biological indicators specifically designed for reliable monitoring of steam and dry heat sterilization processes without the use of enzyme based technology or specific and specialized incubators or monitoring devices.

STORAGE
Store at controlled room temperature 15-27°C (60-80°F) Protect from light, chemicals and sterilants, excessive heat and moisture. Optimal humidity range for long term storage is 30 to 70%. Do not desiccate.
BI's have a shelf life established by the manufacturer; product will include an expiration date.