GUIDANCE ON THE MICROBIOLOGICAL CHALLENGE TESTING OF HEALTHCARE WASTE TREATMENT AUTOCLAVES

Introduction

Two components of the UNDP GEF Project on Global Healthcare Waste involve the demonstration of non-incineration healthcare waste treatment technologies, the most common of which is the autoclave. This document provides a microbiological challenge test protocol for validation testing of gravity-displacement or vacuum autoclaves used for the treatment of medical waste.

Objective of the Test Protocol

The objective of the test protocol is to demonstrate the ability of an autoclave to effectively treat medical waste according to accepted treatment standards.

Basic Principles and General Approach

Treatment efficacy refers to the capability of an autoclave to alter waste such that its potential for transmitting disease is eliminated or substantially decreased. The terms disinfection and sterilization are often used when discussing treatment efficacy. Disinfection can be defined as the reduction or removal of disease-causing microorganisms (pathogens) in order to minimize the potential for disease transmission. Sterilization is defined as the destruction of all microbial life. Since the complete destruction of all microorganisms is difficult to establish, sterilization of medical and surgical instruments is generally expressed as a 6 $\log_{10}$ reduction of a specified microorganism, corresponding to a one millionth (0.000001) survival probability of the microbial population.

The STAATT classification system, in lieu of the terms disinfection or sterilization, denotes levels of "microbial inactivation" specifically for healthcare waste treatment. It was established to define measures of performance of healthcare waste treatment technologies. The international microbial inactivation standard for healthcare waste treatment based on the STAATT criteria is Level III:

> Level III Inactivation of vegetative bacteria, fungi, lipophilic/hydrophilic viruses, parasites, and mycobacteria at a 6 Log_{10} reduction or greater; and inactivation of *G. stearothermophilus* spores and *B. atrophaeus* spores at a 4 Log_{10} reduction or greater

The representative microbiological indicators generally used to test compliance with this standard are: *Mycobacterium phlei* or *Mycobacterium bovis* (BCG) at a 6 Log_{10} reduction or greater; and heat-

resistant spores *Geobacillus stearothermophilus* or *Bacillus atrophaeus* at a 4 Log_{10} reduction or greater.

Spores of *Geobacillus stearothermophilus*, formerly called *Bacillus stearothermophilus*, are dormant nonpathogenic endospores that are able to withstand the high temperatures of steam treatment as well as dry heat. Spores of *Bacillus atrophaeus*, formerly *Bacillus subtillis var. niger*, are also resistant to moist and dry heat as well as chemical inactivation. Table 1 lists the major types of microorganisms excluding prions according to their resistance to chemical disinfection; the same pattern is found with heat disinfection. Due to the high resistance of the bacterial spores, validation testing with the "gold standard" (spores of *Geobacillus stearothermophilus* or *Bacillus atrophaeus*) is generally all that is required for waste treatment autoclaves.

Table 1. Microorganisms listed in order of *decreasing* resistance to chemical disinfection

MICROORGANISMS	EXAMPLES
BACTERIAL SPORES *[MOST RESISTANT]*	*GEOBACILLUS STEAROTHERMOPHILUS, BACILLUS ATROPHAEUS, CLOSTRIDIUM SPOROGENES*
CYST FORMS OF PARASITES	*CRYPTOSPORIDIUM* CYSTS
MYCOBACTERIA	*MYCOBACTERIUM TUBERCULOSIS VAR. BOVIS*, NON-TUBERCULOUS MYCOBACTERIA
NONLIPID OR SMALL VIRUSES	POLIOVIRUS, COXSACKIE VIRUS, RHINOVIRUS
FUNGI	*TRICHOPHYTON* SPP., *CRYPTOCOCCUS* SPP., *CANDIDA* SPP.
NON-CYST FORMS OF PARASITES	
VEGETATIVE BACTERIA	*PSEUDOMONAS AERUGINOSA, STAPHYLOCOCCUS AUREAUS, SALMONELLA CHLOERAESUIS*, ENTEROCOCCI
LIPID OR MEDIUM-SIZE VIRUSES *[LEAST RESISTANT]*	HERPES SIMPLEX VIRUS, CYTOMEGALOVIRUS, RESPIRATORY SYNCYTIAL VIRUS (RSV), HEPATITIS B VIRUS, HEPATITIS C VIRUS, HUMAN IMMUNODEFICIENCY VIRUS (HIV)

Source: *Sterilization, Part 1: Sterilization in Health Care Facilities*, Arlington: Association for the Advancement of Medical Instrumentation (2006)

The traditional method for testing wet thermal systems such as autoclaves uses endospores placed on dry paper strips protected by glassine envelopes. After processing, the strip is retrieved, carefully removed from the glassine envelope and transferred to a vial of sterile medium. Within 24 hours, the test strip is aseptically inoculated in 5.0ml soybean–casein digest broth medium. This process has to be done carefully to prevent the introduction of environmental bacteria into the medium. The strip with the growth medium is then incubated for at least 48 hours, at 30°C for *Bacillus atrophaeus* and at 55 °C for *Geobacillus stearothermophilus* to allow any surviving spores to grow and replicate. Failure of the autoclave treatment is indicated by a cloudy medium.

An easier method is used today and is recommended in this guidance document. It entails the use of a self-contained biological indicator (SCBI) comprised of a spore strip in a vial with growth medium inside a sealed inner ampoule; a cap with holes coupled with a hydrophobic filter is used as a bacterial barrier. The vial is placed inside a test load and recovered after treatment. The vial is then squeezed to break the ampoule and allow the growth medium to mix with the processed spore

strip. The vial is incubated for 24 or 48 hours at a specified temperature. The self-contained biological indicator for waste treatment autoclaves contains a pre-determined amount of spores (10^4 concentration) such that a negative test result corresponds to a 4 Log_{10} reduction. SCBIs containing a pH indicator show a change of color to indicate surviving spores and a positive result (failure). Another method uses a hermetically sealed glass ampoule with a growth medium and a pH indicator. Each ampoule, containing a known population of endospores, is kept refrigerated until use and requires a 48-hour incubation period. Growth is evident by either turbidity and/or a color change. Another biological indicator system detects the activity of an enzyme found on the bacterial spore. The enzyme's response has been shown to be equivalent to the spore's ability to multiply. This method detects the fluorescence produced by a positive indicator. Due to its high sensitivity, the method provides results in one to three hours, which has been correlated with a seven-day incubation period.

For initial validation tests of a new autoclave, the process cycle should be adjusted until at least three consecutive tests pass the requisite log reduction criteria. The operating parameters corresponding to those successful tests (exposure time, temperature or pressure) should be documented. Operating conditions for all future loads should replicate those parameters and should be recorded.

The approached used in this protocol is a microbiological challenge test using self-contained biological indicators of *Geobacillus stearothermophilus* placed into carriers that are then placed inside the waste bag to demonstrate a $\geq$ 4 log reduction of the bacterial spores.

TEST PROTOCOL

General

The following are general guidelines for the test protocol:

- The autoclave should be run at normal operating conditions.
- Tests should be conducted using waste typical for the particular healthcare facility. Special attention should be given to sharps and lab culture wastes. Test should be conducted using the same containers or bags used by the healthcare facility and sealed in the manner commonly done at the facility.
- The placement of the sample waste bag in the treatment system should reflect typical placement and stacking arrangements used. If multiple bags are treated, the biological indicators should be placed in the waste bags where treatment is most difficult.

Personnel

The test should be conducted by personnel specifically trained for this purpose. The test could be done by an autoclave operator if he or she is properly trained and supervised by a facility manager.

PPE

Occupational safety procedures should be followed when testing infectious waste. The following PPE should be used when opening medical waste bags, placing carriers into the bags, closing bags, and

placing bags into the autoclave: heavy duty gloves, face mask, goggles (or face shield), hard sole shoes (or boots), and apron (or protective gown) over regular clothes. The following PPE should be used when retrieving the carriers: heavy duty gloves, hard sole shoes (or boots), apron (or protective gown) over regular clothes, and face shield to protect from steam as needed. PPE should be cleaned after use.

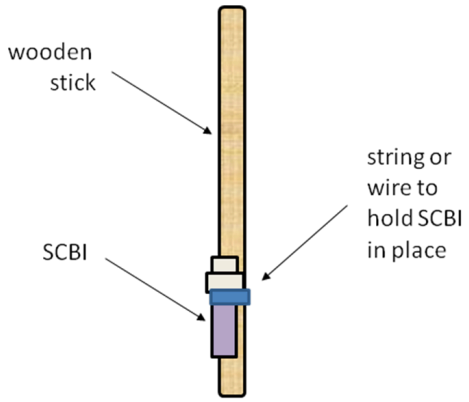
Materials

The self-contained biological indicators (SCBIs) should have the following specifications (see Appendix A for examples):

- Biological indicator: *Geobacillus stearothermophilus* spores
- Concentration: $\geq 1 \times 10^4$ cfu/ml
- D-value: greater than 1.5 minutes at 121°C (D-value > 2 min is suggested if available)

If the autoclave treats multiple bags or containers per load, three SCBIs and one control should be used for a challenge test. Each SCBI should be marked to distinguish the test indicators and the control. The three SCBIs should be placed in three separate bags, one of which should be at the center bottom of the stack. If only one bag is treated per load, one SCBI and one control should be used for a challenge test.

Carriers should be prepared as shown in the drawing below.



SCBI CARRIER
(not drawn to scale)
To be placed inside a sealed medical waste bag

Procedure

The following procedure for placement, retrieval and incubation should be followed:

A) Three individual bags typical of the average size of healthcare waste bags in the load are randomly selected. The bags are opened carefully and a carrier placed inside. The wooden stick is positioned such that the SCBI is in the middle of the bag. If inner bags are found inside the medical waste bag, the carrier is placed inside an inner bag. The bags are then fastened in the manner in which they were originally found.

B) The three bags are placed in the center bottom, middle, and top of the batch of waste in the autoclave. The control is set aside and kept at room temperature.

C) After the autoclave is filled in the usual manner, the treatment process is started.

D) The following data should be recorded: name of person conducting the test, date, operating parameters including start and finish times, general description of the waste, and weight of the bag.

E) At the end of the treatment process as the waste bags are removed, the three bags with the carriers are separated.

F) The SCBIs are retrieved from the carriers and observations made to determine if any damage to the SCBIs has occurred.

G) Once observations have been recorded, the three SCBIs and the control are incubated following the instructions provided by the SCBI vendor (typically 24 hours at 55-60°C). The incubator should be able to maintain the temperature within ±2ºC during the incubation period.

H) After incubation, the SCBI ampoules or vials shall be examined for Growth or No Growth following the instructions provided by the SCBI vendor. The results shall be recorded (see sample form provided in Appendix B).

Frequency Except for the initial validation test of a new autoclave, the normal frequency of microbiological challenge testing is once a week.

If all test indicators show No Growth for four consecutive tests (one month), the frequency of testing can be decreased to once every two weeks.

Failures SCBIs that show any damage that may affect the determination of microbial inactivation (such as cracked vials and exposure of spore strips, leaking glass ampoules containing the growth medium, broken caps) should be noted in the report but not included in the determination. There should be three undamaged SCBIs for a test to be valid, otherwise the test should be repeated.

The process is considered successful if all SCBIs show No Growth. If one or more SCBIs indicate Growth, troubleshooting should be conducted to determine the source of the problem, such as the need for equipment maintenance or adjustment of operating parameters (increasing exposure time, temperature, or the number or depth of the vacuum cycles if a vacuum autoclave is used). The test should be repeated for every run until all SCBIs show No Growth. The test should be continued until three consecutive tests show No Growth for all SCBIs. Subsequently, the tests should be conducted weekly until four consecutive tests show No Growth, after which the frequency of testing can be decreased to once every two weeks.

Records The records of the results of microbiological challenge testing should be made available to regulatory authorities upon request or submitted periodically to regulatory authorities according to existing rules. The records should be kept by the facility for at least three years.

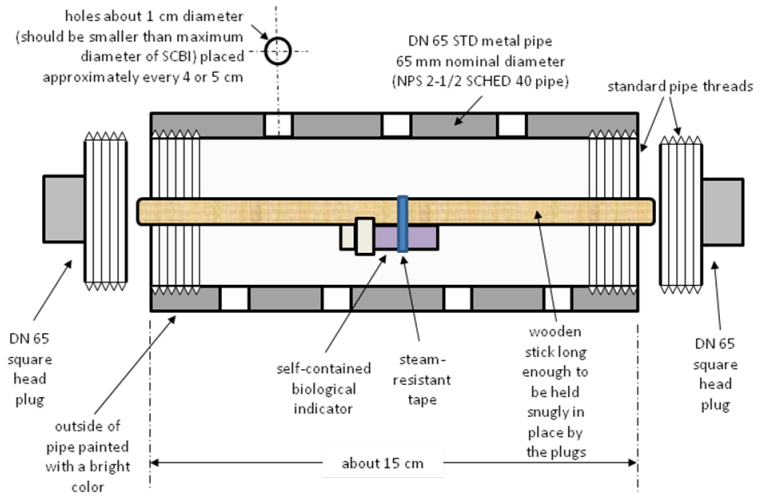
MODIFICATIONS FOR OTHER TREATMENT SYSTEMS

This protocol can be modified to apply to microwave units and steam-based hybrid systems that incorporate internal shredding or mixing such that waste bags are ruptured and the contents released inside the autoclave or microwave chamber.

To prevent the SCBIs from being destroyed by internal shredders, the biological indicators would have to be introduced into the unit in a way that would bypass the shredding section. For units that use a mixing arm or paddle or for autoclaves that rotate, a carrier should be design with the following characteristics:

- Relatively small
- Easy to open and to secure
- Robust enough to protect the SCBI and to be used repeatedly
- Designed to allow steam to penetrate easily
- Easily seen (e.g., painted with bright colors) for retrieval.

Examples of simple carrier designs are tennis balls with holes drilled through them, Teflon® tubes with holes and wooden plugs held in place by heavy gauge wire on both ends, and short metal pipes with holes and threaded caps on both ends as shown in the drawing below. Shredded waste should be placed inside the carrier along with the SCBI.



SCBI CARRIER
(not drawn to scale)
To be placed inside a sealed medical waste bag

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APPENDIX A

Examples of Testing Supplies

Note 1: Mention of the products below does not constitute an endorsement or recommendation by the UNDP GEF Project. The products are presented only as examples. Other products meet the requirements in this guidance.

Note 2: These examples use 1.0 x 10^5 concentrations of the bacterial spores. For waste treatment autoclaves, STAATT only requires concentrations of 1.0 x 10^4 of bacterial spores. A 10^5 concentration is a more stringent requirement but may be easier to obtain commercially.

Raven ProSpore Ampoules or ProSpore 2 Self-Contained Biological Indicators
Geobacillus stearothermophilus ATCC 7953, minimum concentration 1.0 x 10^5 cfu/ml
ProSpore or ProSpore 2 dry bath incubator, 12 wells, 240 Volts, 35 or 55 °C
Raven Labs, P.O. Box 27261, Omaha, NE 68127 USA; TEL: +1-402-593-0781
http://www.mesalabs.com/products-services/raven-labs.html

3M™ Attest™ Self-Contained Biological Indicators
Geobacillus stearothermophilus (derived from ATCC 7953) in a flexible polypropylene vial, minimum population of 1 x10^5 spores per strip, includes a Tryptic Soy broth growth medium with a pH-sensitive indicator dye (bromocresol purple)
Attest incubator, 220/240 Volts, 14 vial capacity, 56 ± 2°C
3M Center, St. Paul, MN 55144 USA
http://solutions.3m.com/wps/portal/3M/en_US/IP/infectionprevention/solutions/sterilization-assurance/load-control/

SporView® Self-Contained Biological Indicators
Geobacillus stearothermophilus, population 2.2 x 10^5, results in 24 hours
Medical Engineering Technologies Ltd., Yew Tree Studios, Stone Street, Stanford North, Ashford, Kent TN25 6DH, UK; TEL: +44 (0)8454 588924
http://www.met.uk.com/6a-medical-sterilisation-indicators.php#self-contained-biologigal

Bionova Terragene Self-Contained Biological Indicators
Geobacillus stearothermophilus bacterial spores on filter-paper packaged within a plastic tubewith a sealed-glass ampoule of culture medium and a color indicator
MedNet GmbH, Borkstraße 10, 48163 Münster, Germany; TEL: +49 (0) 251 32266-0
http://www.medneteurope.com/Sterilisation-Monitori.14.0.html?&L=2

APPENDIX B

Biological Indicator Test Log

Challenge Test

Name: _____ Date of Test:_____

Address:_____

Biological Indicator Brand: _____ Concentration:_____

Product #/Lot -_____ Expiration Date: _____

Autoclave/Model Number:_____

Autoclave Operating Parameters: _____

Cycle Start Time: _____

Cycle End Time: _____

Description of waste bags selected for the SCBIs

Sample #	Weight (kg)	Description of waste	Location in the autoclave
# 1			
# 2			
# 3			

Test results

Neg. Control	
Sample #	Results
# 1	
# 2	
# 3	
Positive Control	

Negative control is an unprocessed BI
Record results as "+" for Growth (failure)
and "NG" for No Growth

Comments / Upset conditions during testing if any:

_____

_____