

Monitoring Bio/Med Waste Bags with Spore Strips as Biological Indicators and 'False' Negatives

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It has been noted that a fairly large number of facilities use spore strips as their Biological Indicator (BI) for the monitoring of biowaste bags to be steam sterilized. These waste bags include regulated medical waste (RMW), biological waste and a variety of lab, clinic or media prep waste bags. The waste contents could be made up of various quantities of discarded agar slants, petri dishes, tubes of tryptic soy broth (TSB) or other such common lab waste that is likely to contain various strains of bacterial cells including spore-forming gram negative bacillus, E. coli, fungi, mold and/or yeast cultures. Isolate and contaminant cultures, possibly pathogen, body fluid wipes and blood bourn swabs and gauze can also be included with RMW are all thrown into the biowaste bags. Waste accumulates from the ICUs (Intensive Care Units), pathology labs and surgical suites. The number and types of possible pathogens is very large. Included in this potpourri may also be antibiotic discard, cleaning tissues or cloths used to sanitize surface areas or clean laminar flow hoods that contain leftover sporicidal or antimicrobial spray and alcohols or disinfectants that may have been used in a cleaning process. As can be easily seen in the following photo's #1 and #2, the waste bags usually contain an abundance of petri dishes and other items that will liquefy under sterilization conditions. Agar slants, tubes of TSB or other growth media and numerous items containing collected or cultured contaminants will soon be free flowing within the bag as temperatures reach the agar melting point and plates and tubes open up under pressure and temperature.



Photo #1



Photo #2: Waste Bag Debris

Other sites contributing waste include: environmental/water testing labs, tattoo facilities, dental offices, veterinary clinics and a large number of public health labs. All of the mentioned facilities are accumulating biowaste and it must be sterilized prior to disposal. Most of these waste bags sit in a collection area until they are sent off to be autoclaved

either on-site or off-site. Many sites use BIs in the form of spore strips to measure the cycle's effectiveness. Could just the presence of this hodgepodge of waste items discarded into the bags have a localized effect upon the spore strips being used to monitor a sterilization cycle? Prior to providing information pertaining to the above question, let's look at why spore strips are even being used as BIs for biowaste cycles.

The most common form of BI used as an indicator for monitoring sterilization cycles involving steam is the spore strip. Spore strips are fairly easy to use, they are the least expensive of BIs to purchase and most individuals using BIs are familiar with the use of spore strips. Of the regulatory standards that require the use of BIs for monitoring medical or biological waste, they specifically mention and allow for the use of spore strips in autoclaves. When one is preparing for environmental laboratory accreditation or certification, autoclaves must be monitored with spore strips or suspensions.¹

The broad category of 'autoclave loads' would also encompass biowaste loads. Numerous agencies, including many State Department of Health agencies follow the four proposed sterility assurance levels for microbial inactivation set by the US State and Territorial Association on Alternative Treatment Technologies (STAATT) for the treatment of medical/clinical waste. RMW/biowaste requires a Level 3 minimum for treatment. With Level 3, the use of a minimum challenge of Log 4 population is used. As an example of citing spore strip use, the recently approved Code of Massachusetts Regulations for the minimum requirements for the management of medical or biological waste (State Sanitary Code Chapter VIII), section 480.150, E 4, states "...testing shall consist of spore strips or a retrievable medium approved by the Department, which contain a 1.0×10^4 minimum challenge population of a bacterial indicator organism that is most resistant to any aspect of the treatment technology as outlined in the most recent medical waste treatment technology guidelines established by the State and Territorial Association on Alternative Treatment Technologies (STAATT) or its successor the International Society of Analytical Analysis of Treatment Technologies (IStAATT)". When first viewing this code, one would quickly see 'spore strips or a retrievable alternative medium approved by the Department'. The easiest choice for compliance would be to use the spore strips with a population of 10^4 and avoid getting approval by the Dept. for an alternative medium. Thus in a majority of cases, spore strips are used and at a population of 10^4 .

The following test was conducted in order to test if the possibility exists that the presence of the disinfectant or bactericidal cloths or wipes put into a waste bag and in close proximity to the spore strips could possibly have an effect upon the spore strips (at a population level of 10^4) in such a way as to provide the user with a false negative.²

Three 'red bag' autoclave waste bags were prepared. Each bag contained approximately the same volume of discarded petri dishes, lab paper towels used for cleaning flow hoods, discarded tubes of growth media and TSB along with usual lab testing waste. Four test spore strips with a population of 10^4 were placed into each of the 3 bags. Included in bags #1 and #2 were moist paper wipes or paper towels that were used to clean flow hoods

¹ US EPA Manual of Methods for Virology, Chapter 4, Quality Assurance, section 6.11

² Born, Zach Raven Labs, Omaha NE, Waste bags and Spore Strips, in-house testing Oct. 2008.

using bactericidal and disinfectant sprays. The spore strips containing spores of *G. stearotherophilus* and the towels mentioned above were intentionally placed near the top area of each bag. In this manner, steam penetration into the lower parts of the bag was not to be considered an issue. In bag #3, no towels containing traces of bactericidal spray were used. All three bags were run in the same cycle, 121°C exposure temperature with a prevac phase used. The exposure time was set for 4 minutes at 121°C. This time was chosen as an ‘all survive’ time for the spore strips under normal 121°C conditions. With this exposure time, all spore strips should survive since this short exposure would be sub-lethal. The 4 minute time was elected from the spore strip certificate of analysis where the population was at 2.5×10^4 and had a resistance of 1.9 minutes. Using USP ‘Survive/Kill’ calculation (log of population minus 2 times the D-Value) we had a ‘survive time’ of 4.5 minutes exposure to 121°C where all the spore strips would survive the exposure.³ In our brief trial, we chose an even shorter time for exposure and used 4 minutes. Following an exposure cycle of 4 minutes at 121°C, all spore strips were removed from the waste bags and the strips were aseptically transferred to tubes of TSB and incubated at 55 to 60°C. Growth and no growth results are listed below in Table #1 following 7 days of incubation.

Table #1

| Waste Bag | # of Spore Strips used | # of Spore Strips showing growth |
|---|------------------------|----------------------------------|
| Bag #1 (contained tissues with bactericidal spray) | 4 | 0 |
| Bag #2 (contained tissues with bactericidal spray) | 4 | 0 |
| Bag #3 (contained no tissues with bactericidal spray) | 4 | 4 |

With an exposure time of 4 minutes, the spore strips should have survived the cycle in all three waste bags. Even though all three waste bags were exposed to the same sub-lethal exposure time of 4 minutes at 121°C, the spore strips in waste bags #1 and #2 did not show signs of growth while waste bag #3 without the tissues did grow and was not killed by the short exposure cycle. It was not the exposure cycle that killed the spore strips in bags #1 and #2 but the waste bag contents that contributed to lethality.

Admittedly, additional tests should be run with various exposure times used and strip placement variation within the bags. However, I feel that this brief investigation allows for the possibility that if one were using spore strips for monitoring waste bag sterilization, a false negative is within reason as a possible risk; a risk that, even if slight, should not be allowed to exist.

With typical waste sterilization cycles dealing with Prevac, exposure time and temperatures, most likely even a waste bag bioburden of 10^{12} or 10^{14} pathogens would likely be killed due to low moist heat resistance. However, if we are monitoring a cycle’s effectiveness with spore strips and a possibility exists where test results could be false,

³ USP 31, *Survival Time and Kill Time*, pg. 1532.

our confidence level for sterility assurance has been greatly lowered. Using an improper biological indicator for a specific cycle type is more deleterious than using no BI at all.

Over the past 15 years I have come into contact with numerous waste bag steam cycles where spore lethality just did not occur. Some were due to inadequate exposure time but the majority were due to inadequate air removal and thus poor steam penetration. Some cycles just did not include a Prevac phase and thus steam penetration was extremely difficult or impossible to obtain and some were mechanical vacuum failures. Time and temperature recordings were fine but the prevac was missing and thermocouples located in the wastebag were measuring dry heat rather than moist heat within the bag's contents. *If* spore strips were used and *if* they were in contact with bactericidal or disinfectants in a very localized area in such a situation where adequate steam penetration did not occur and the strip should have survived, we could possibly have a failed cycle yet the spore strips would indicate adequate cycle parameters were met and a failed cycle not detected.

Prevac cycles should always be used when autoclaving biowaste/RMW bags. The prevac adds additional confidence that heat penetration and air removal within the bag is being accomplished. One could use a wireless temperature data logger located near the waste bag's bottom area to check for temperature penetration. This simple to use data logger will record the temperature obtained by the lower portion of the bag contents during the entire cycle time. At the cycle end, the Logger is downloaded to a PC and the cycle temperature profile is observed.



Photo #3: Temperature Data Loggers

Photo #4 shows what an extreme situation could look like if the strips were allowed to come in contact with waste bag contents. The spore strips could very likely be compromised and not provide valid testing or monitoring results. Even extreme, moisture saturated spore strips at a cycle's end should be suspect of being compromised. Spore strips are not manufactured for or intended to be placed within liquid loads. The spore strip envelope made from glassine paper has uniformly small size porosity when in a dry condition. Soaking the glassine with moisture modifies the porosity. Using them in liquid-type loads is contrary to the spore strip's intended use.



Photo #4: Spore strips covered in waste bag debris

If a biological indicator is to be used in liquid or high moisture accumulation loads, it should be completely sealed from the possible affects that the load contents could have upon the indicator. One should consider using a self-contained or sealed ampoule BI as shown below in photos #5 and #6. It can be pre-placed in the bottom area of an empty waste bag prior to use. This would eliminate the need to place the ampoule into the bags lower portion prior to autoclaving preventing a handling risk. A thin wire can be tied to the ampoule for easy removal after the cycle is finished. At cycles end, simply pull the wire and retrieve the ampoule.

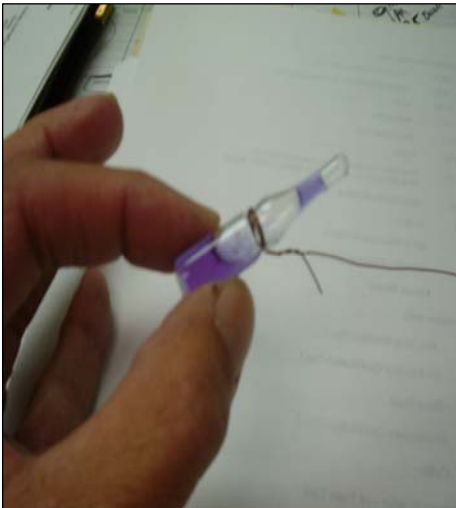


Photo #5



Photo #6

Possible waste from patients with undiagnosed or undetected HIV or Hepatitis may go into the general clinical waste stream with handlers being unaware of the seriousness of the waste bag contents. A sterilization cycle failure can pose a considerable risk for handlers and the environment.

All medical and biowaste should be treated equally with a robust cycle that is easily capable of providing a minimum sterility assurance level of a 10^6 spore reduction. Using a lower standard of 10^4 spore reduction is just not acceptable. Increasing the sterility confidence level from a Log 4 population to a spore reduction level of Log 6 may only increase our needed sterilization exposure time by a few minutes. A few additional minutes are well spent to achieve a bioburden survivor probability level of 1 in a million or a 10^6 spore reduction capability. All autoclave cycles dealing with medical/lab waste should easily be capable of achieving this higher level of sterility assurance. It is this higher level of performance that must be validated and uniformly applied to all RMW/biowaste treatment standards.

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