AMSCO Waste Processing Technical Report

TECHNIQUES FOR STEAM STERILIZING LABORATORY WASTE

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SUMMARY

The traditional method for steam sterilizing petri dishes and other laboratory discards is processing at 250°F in a gravity-displacement sterilizer. Laboratory discards are typically packaged in polypropylene (biohazard) autoclave bags that are either completely sealed after filling, or have a constricted opening for air/steam exchange and, except for this opening, provide complete containment of the enclosed material. Heat-penetration studies by investigators such as Rutula et al.[1], and Lauer et al.[2], have disclosed lengthy load heat-up times for the combination of filled autoclave bag and 250°F processing temperature.

This study examines the efficiency associated with different processing techniques for laboratory waste. Both agar-laden, disposable petri dishes and typical laboratory discards were processed. The effects of processing temperature and waste containment systems were investigated. The results are presented in this report. Processing at 250°F was compared to processing at 270°F. Standard, polypropylene autoclavable packaging was compared to polyethylene meltable bags that expose their contents to steam. In all cases heat penetration into the material was determined and exposure settings were developed to achieve load sterilization. Both load thermal profiles and biological indicators were used in this determination. Because we found that petri dishes required a longer heat-up time, sterilization determinations were based on this worst-case load.

Sterilization efficiency increased when heat transfer into the materials was accelerated. Use of a higher processing temperature was found to be one way to accomplish this. A 270°F cycle produced a 32% increase in cycle efficiency. Enhancing steam penetration into the waste materials also had a positive effect on processing efficiency. Other factors, such as contained moisture within the material, and conduction rate through the packaging, exerted an impact on load heat-up. These factors are addressed in a discussion of the results of this study.

INTRODUCTION

Laboratory waste processing presents some peculiarities from a steam-sterilization perspective. First, because infectious material must be kept contained, conventional waste packaging is impervious to steam penetration. This has a detrimental effect on processing efficiency. Rutula[1] and Lauer[2] have commented on the magnitude of this effect.

Another peculiarity is the type of material processed. Steam sterilizers are designed to process thermostable materials. Disposable petri dishes, a common laboratory discard, melt at sterilization temperatures, encapsulating pockets of media and air. . .further impeding steam penetration. Thus, the packaging and thermal instability of most laboratory waste have a cumulative effect in reducing the efficiency of the steam-sterilization process.

Any method which accelerates load heat-up time increases steam-processing efficiency. Although the majority of laboratory waste is sterilized at 250°F, we investigated processing at 270°F to shorten load heat-up time. Since most modern gravity-displacement sterilizers have a provision for processing at either 250°F or 270°F, this method is both easy to implement and safe to use.

The other area investigated for reducing processing time was the penetrability problem posed by polypropylene biohazard bags. We sought a containment system that would give personnel the protection of a polypropylene bag, but at the same time enhance steam penetration. One approach to this type of system is a bag that remains intact until it is subjected to high heat (i.e., sterilization temperatures). Polyethylene bags, which degrade at sterilization temperatures, were evaluated for their capacity to enhance steam penetration during processing.

METHODOLOGY

Monitoring Equipment

An Eagle® model 2013 sterilizer with a 16x16x26" chamber was used for this testing. This unit provides the option of running gravity-displacement cycles at either 250°F or 270°F. Six to eight thermocouple leads were threaded into the chamber via a Conax® adapter. This adapter gives an airtight seal between the chamber and the outside environment. One thermocouple lead was attached to the temperature-control bulb in the sterilizer's drain line. The remaining thermocouples were used to profile load temperatures.

In addition to the thermal data, biological indicators were also used to evaluate the effectiveness of each sterilization process. For most of the tests Chemspor®, a sealed vial containing *Bacillus stearothermophilus* spores suspended in a growth medium, was used. The advantage of this product is that it can't be contaminated when retrieved from a waste load. As an additional challenge, however, Spordex® spore strips were also used in restricted applications which are explained later in this section.

Table I
TEST LOADS

LABORATORY DISCARDS
• 10 ml glass pipets (quantities from 10 to 30) • 5 ml glass pipets (quantities from 10 to 30) • 1 ml glass pipets (quantities from 10 to 30) • 4x4" squares of Johnson & Johnson gauze pads (quantities of 10) • 2x2" squares of Johnson & Johnson gauze pads (quantities of 100) • 20 cc disposable syringes (quantities of 2) • 1 pair of Flexam gloves • 1 section of rolled, absorbent cotton (quantities of 12)
PETRI DISHES (POLYSTYRENE)
• 1.8 to 4 kilograms • Each dish contained approximately 20 ml of tryptic soy agar

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Comparison of Processing Temperatures

Two types of loads were evaluated:

- Disposable polystyrene petri dishes containing solidified agar (approximately four kilograms for each cycle).
- Typical laboratory discards (itemized in Table I).

Load material was randomly placed in 19x24" polypropylene (biohazard) bags.

For petri dish loads, thermocouples were affixed to plates and Chemspor monitors were placed at the top, middle and bottom of the loads. For typical laboratory discard loads, one thermocouple was attached to a 10 ml pipet at the bottom of the load, another was inserted in a Flexam glove at the load's approximate center, and a third monitored the air space within the barrel of a syringe. Chemspor monitors were placed in the same locations.

Polypropylene bags were sealed with conventional elastomeric closures (supplied with the bags). To minimize agar spillage during processing, the bags were placed in Nalgene® tubs, a common lab procedure. Unlike prior evaluations of this type (i.e., Lauer and Rutula), water was not introduced into the bags for the purpose of internal steam generation.

Both categories of load material were processed in each test cycle. Repeated 250°F and 270°F gravity cycles generated load-temperature profiles which were analyzed in the context of equivalent time at 250°F (e.g., F_0 values). Biological information was used to corroborate the thermal data as well as directly demonstrate the obtainment of sterilizing conditions within each bag. The initial data was used to calculate lethal exposures.

Comparison of Packaging

Material preparation was identical to that described for tests conducted at the two processing temperatures. Because the polyethylene bags we obtained were 12x18", smaller than those used in the "temperature" tests, load volumes were similarly reduced. . .i.e., 1.8 to 2 kilograms of petri dish waste. To establish a basis for comparison, smaller polypropylene bags were also used. . .14x19" (as compared to 19x24" bags used in the "temperature" testing).

In this portion of the study we used both spore strips and Chemspor to monitor the process. For petri dish loads, spore strips were placed in empty dishes and stacked next to the thermocouples. For laboratory discard loads, the spore strips were inserted into the lumens of the pipets.

Since the focus of the tests was to compare packaging effects, test cycles were run using one type of load material packaged in both polyethylene and polypropylene (biohazard) bags. Thermal and biological data were compiled and analyzed as they were in the "temperature" testing.

Petri dishes were processed at 250°F with a 70-minute exposure time, and at 270°F with exposure times of 25, 35 and 45 minutes. Laboratory discards were processed at 270°F for 45, 60 and 65 minutes.

RESULTS

Comparison of Processing Temperatures

Tables IIa and IIb indicate the come-up times for each type of load. For petri dishes, more than 90 minutes exposure was needed at both temperatures for all thermocoupled areas to reach the processing temperature. For laboratory discards, the 270°F cycles produced average come-up times of 58 minutes, while an average of 77 minutes was needed at 250°F. This data indicates that petri dishes are more difficult to sterilize than laboratory discards. Figure 1 shows the come-up characteristics for petri dishes (the worst-case load) processed at 250°F and 270°F. Note that, while load come-up is essentially the same, the 270°F cycle accelerates the attainment of 250°F within the load (as indicated by the intersection of the segmented lines) after 48 minutes of exposure.

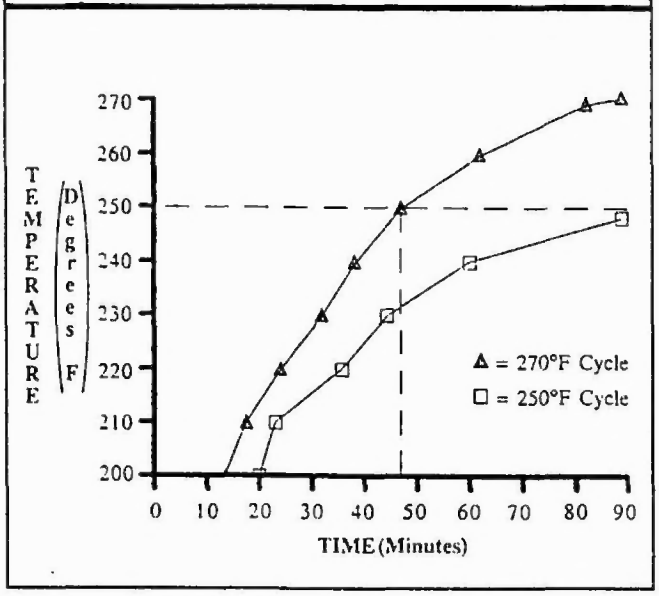
Table IIa
COMPARISON OF PROCESSING TEMPERATURES - COME-UP TIMES FOR 4 KG OF PETRI DISHES

PROCESSING TEMPERATURE	EXPOSURE SETTING	COME-UPTIME DURING EXPOSURE
250°F	90 Minutes	>90 minutes
270°F	90 Minutes	>90 minutes

Table IIb
COMPARISON OF PROCESSING TEMPERATURES - COME-UP TIMES FOR LABORATORY DISCARDS

PROCESSING TEMPERATURE	COME-UPTIME DURING EXPOSURE
250°F	77 Minutes (Syringe)
270°F	58 Minutes (Bottom)

Figure 1
COMPARISON OF COME-UP TIMES - 250°F VERSUS 270°F - PETRI DISHES



Based on this thermal data, it was calculated that lethality would be obtained with exposures of 91 minutes at 250°F and at approximately 60 minutes at 270°F. Table III shows that incomplete biological kill occurred as a function of these parameters. By increasing the exposure time to 65 minutes at 270°F, all retrieved biological indicators were negative for growth. It was projected from the 250°F data that, at 250°F, a 95-100 minute exposure would be necessary for complete kill. The 270°F cycle, therefore, provides approximately a 33% increase in processing efficiency.

Table III
COMPARISON OF PROCESSING TEMPERATURES - BIOCIDAL DATA

PROCESS CYCLE	PACKAGING TYPE	TEST LOAD	BIOLOGICAL KILL	
			Chemspor	Spordex
250°F 91 minutes	Polypropylene	Petri Dishes	1/8+	—
		Lab Discards	0/8+	—
270°F 60 minutes	Polypropylene	Petri Dishes	1/8+	—
		Lab Discards	0/8+	—
270°F 65 minutes	Polypropylene	Petri Dishes	0/9+	—
		Lab Discards	0/8+	—

Comparison of Packaging

Tables IVa and IVb list the come-up times for loads processed in polyethylene and polypropylene (biohazard) bags. Petri dishes in polypropylene bags, exposed to 250°F for 70 minutes, required more than 65 minutes to reach temperature. The corresponding time for petri dishes in polyethylene bags was 48 minutes. A 45-minute exposure at 270°F produced load come-up times in excess of 45 minutes for polypropylene bags and 39 minutes for polyethylene bags. Laboratory discards in polypropylene bags, exposed for 65 minutes at 270°F, failed to reach temperature. By contrast, polyethylene-bagged discards attained 270°F in 43 minutes.

Table IVa
COMPARISON OF PACKAGING - COME-UP TIMES FOR PETRI DISHES

TEMPERATURE	EXPOSURE TIME	PACKAGING	COME-UP TIME DURING EXPOSURE
250°F	70 minutes	Polypropylene (Biohazard)	>65 minutes
		Polyethylene	48 minutes
270°F	45 minutes	Polypropylene (Biohazard)	>45 minutes
		Polyethylene	>39 minutes

Table IVb
COMPARISON OF PACKAGING - COME-UP TIMES FOR LABORATORY DISCARDS

TEMPERATURE	EXPOSURE TIME	PACKAGING	COME-UP TIME DURING EXPOSURE
270°F	65 minutes	Polypropylene (Biohazard)	>65 minutes
		Polyethylene	43 minutes

Figure 2 shows the come-up characteristics of the petri dish load as a function of packaging for 270°F cycles. Intercepts drawn through the curves at 250°F indicate come-up times of 27 minutes for polyethylene bags and 33 minutes for polypropylene bags.

Figure 2
COMPARISON OF COME-UP TIMES - POLYPROPYLENE VERSUS POLYETHYLENE PACKAGING - PETRI DISHES

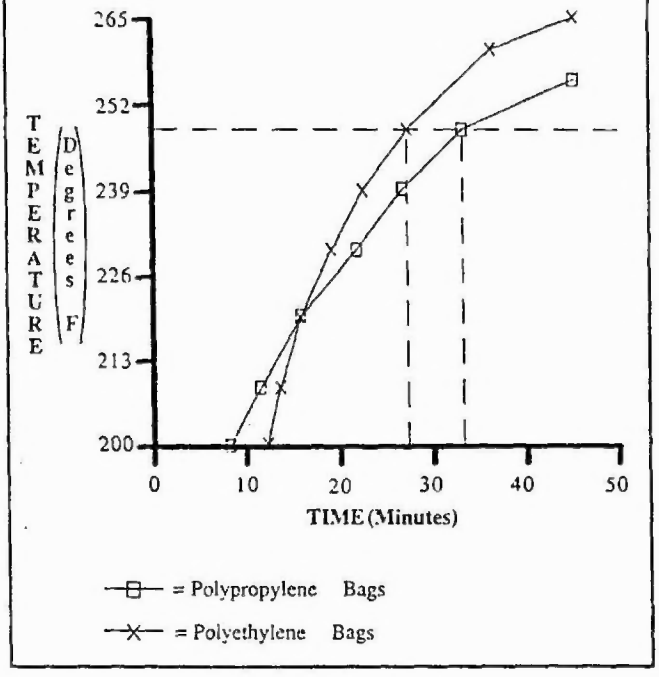


Table V shows the biological results. For 270°F exposures, polyethylene packaging of petri dishes produced greater cidal activity than polypropylene (biohazard) packaging. The most dramatic effect was noticeable with a 35-minute exposure time. For polyethylene-packaged petri dishes, 75% of the Chemspor retrieved were negative for growth, while for those packaged in polypropylene bags, only 28% were negative. For both types of packaging, exposure for 45 minutes at 270°F produced complete kill in Chemspor and in most of the spore strips (one positive occurred in a polypropylene bag). At 250°F, a 70-minute exposure resulted in complete kill in polyethylene bags, but left fractional positives in polypropylene bags.

Table V
COMPARISON OF PACKAGING - BIOCIDAL DATA

PROCESS CYCLE	TEST LOAD	PACKAGING TYPE	BIOLOGICAL KILL	
			Chemspor	Spordex
270°F 25 minutes	Petri Dishes	Polyethylene	4/6+	1/2+
		Polypropylene	5/6+	2/2+
270°F 35 minutes	Petri Dishes	Polyethylene	4/16+	2/6+
		Polypropylene	11/18+	3/6+
270°F 45 minutes	Petri Dishes	Polyethylene	0/34+	0/6+
		Polypropylene	0/31+	1/6+
250°F 70 minutes	Petri Dishes	Polyethylene	0/15+	0/6+
		Polypropylene	1/16+	2/6+
270°F 45 minutes	Lab Discards	Polyethylene	0/7+	2/4+
		Polypropylene	0/7+	4/4+
270°F 60 minutes	Lab Discards	Polyethylene	0/7+	1/3+
		Polypropylene	0/7+	0/3+
270°F 65 minutes	Lab Discards	Polyethylene	0/16+	1/6+
		Polypropylene	0/16+	0/6+

Exposure times of 45 minutes or more at 270°F killed all Chemspor contained within laboratory discard loads in both types of packaging. Partial positives were obtained from spore strips retrieved from pipets in polyethylene bags, even after 65 minutes of exposure. Complete lethality was obtained in polypropylene bags after a 60-minute exposure.

DISCUSSION

Certain implications regarding laboratory waste processing in a steam sterilizer are apparent from the data gathered. First, prior studies which demonstrated protracted come-up times for petri dishes processed at 250°F are substantiated. In this study, more than 90 minutes were needed for a four-kilogram mass to reach the processing temperature. This may appear surprising at first glance since many items can be sterilized within 30 minutes. Consider, however, that AMSCO recommends a 55-minute exposure for two liters of water which weigh approximately two kilograms. It may be reasoned that four kilograms of water would require a much longer exposure time. One variable, then, which impacts sterilization of laboratory waste is the mass of material.

Another consideration is the thermal conductance of the material being sterilized. In the case of petri dishes, heat energy is expended when the polystyrene and the agar melt. This melting produces a distortion of the petri dishes as well as a migration of the agar toward the bottom of the bag. Continued heating of the agar appears to fractionate its components and produce water vapor (steam). This may have a desirable effect in terms of sterilization, but the effect may be somewhat offset by potential air entrapment due to encapsulation of the plastic. These factors (melting and air encapsulation) impede thermal conductance.

As suggested in the introduction, polypropylene packaging also slows heat penetration. Its barrier properties do not permit direct steam admittance to the material. Instead, steam entry is limited to the constricted orifice, formed by the elastomeric closure, at the top of the bag.

To combat some of these effects, a higher processing temperature of 270°F may be used. It was demonstrated that the obtainment of sterilization temperatures is accelerated by this method. This translates into a 32-33% reduction in the exposure time needed to effect sterilization. A change from 250°F to 270°F processing is easy to accomplish with today's sterilizers and does not require modifications to existing equipment (note, however, that older sterilizers may not be designed to process items at the 28-30 psig steam pressure present during a 270°F cycle; check the equipment before trying this procedure).

Use of polyethylene packaging also accelerates load heat-up. The data indicates that about twice as many positive biological indicators occurred in polypropylene bags than in polyethylene bags. This was observed for a sub-lethal exposure of 35 minutes at 270°F using two kilograms of petri dishes. To eliminate agar spillage, a double-bagging technique can be used. The polyethylene bag is placed inside a larger polypropylene bag. Before processing, the sides of the outer bag are rolled down to expose the polyethylene bag. After processing, the sides of the polypropylene bag are gathered around the contained material and the top is sealed. This procedure maximizes steam penetration into the load and can be safely implemented with infectious material.

A disparity is seen, however, when laboratory discards are processed in polyethylene. Table IVb suggests that these bags offer a decided advantage for steam penetration (43-minute come-up time versus 65-minute come-up time for polypropylene bags). A 45-minute exposure at 270°F produced about twice as many positives in polypropylene bags than in polyethylene bags. Longer exposures, however, resulted in a low level of positives for items in polyethylene bags but complete kill for those in polypropylene bags. The partial positives, found in pipets, may have been caused by the polyethylene melting over the pipets and forming an air pocket by sealing off the opening. Or the contaminants may have been introduced after retrieval. We believe, however, that these positives may be a product of the testing procedure since the pipets were actually too long for the dimensions of our bags. Further testing of laboratory discards with larger polyethylene bags may show results similar to those for petri dish loads. In any case, this data indicates the necessity for biological-indicator use in conjunction with thermal data for efficacy determinations.

At the present time, we see the use of polyethylene bags, combined with 270°F processing as the safest, easiest and most effective way to enhance heat penetration into infectious waste material.

REFERENCES

1. Rutula, William A., Marsha M. Stiegel, and Felix A. Sarubbi, Jr. 1982. *Decontamination of Laboratory Microbiological Waste by Steam Sterilization.* **Applied Environmental Microbiology**, Vol. 43, No. 6: 1311-1416.

2. Lauer, James L., Donald R. Battles, and Donald Vesley. 1982. *Decontaminating Infectious Waste by Autoclaving.* **Applied Environmental Microbiology**. Vol. 44, No. 3: 690-694.