Influence of Packaging and Processing Conditions on the Decontamination of Laboratory Biomedical Waste by Steam Sterilization

Gérard Ozanne, Roger Huot and Claude Montpetit
***Appl. Environ. Microbiol.* 1993, 59(12):4335.**

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1993, p. 4335–4337
0099-2240/93/124335-03$02.00/0

Vol. 59, No. 12

Influence of Packaging and Processing Conditions on the Decontamination of Laboratory Biomedical Waste by Steam Sterilization

GÉRARD OZANNE,* ROGER HUOT, AND CLAUDE MONTPETIT

Laboratoire de santé publique du Québec, 20045 chemin Ste-Marie, Ste-Anne-de-Bellevue, Québec, Canada H9X 3R5

Received 14 July 1993/Accepted 21 September 1993

The conditions for optimal steam decontamination of polypropylene bags half loaded with laboratory biomedical waste were studied (276 bags were processed). Controls were single-closed bags without water added or incisions made in the top, standing freely in an autoclave set at 121°C. The average time required to reach 121°C at the load center was 46 min for controls. A significant increase in this time occurred following addition of water to bags without incisions (60 min), with double bagging (60 min), or when using vertical containers (82 min). A significant decrease occurred when bags were slashed (37 min) or processed at 123°C (32 min) or 132°C (19 min). Horizontal containers or addition of water to slashed bags had no significant effect.

Pressure steam sterilization is generally used to decontaminate biomedical waste before discarding it because it is practical, effective, and one of the less expensive ways of doing it (1, 4). However, previous studies on the processing conditions required to obtain optimal waste decontamination have produced paradoxical results (3, 4, 6), making an optimal steam waste decontamination protocol difficult to establish.

In our institution, biomedical waste is processed on site by steam sterilization. A study was undertaken to determine the optimal conditions required for processing of polypropylene autoclave bags half loaded with contaminated biomedical waste. To duplicate the real-life situation, no attempt was made to standardize the contents of the bags. However, 276 bags were processed, allowing us to compare statistically the impact of different processing conditions.

To our knowledge, this is the first large-scale study of the impact of processing conditions on decontamination of biomedical waste carried out at one site with one autoclave. This design allowed us to exclude confounding factors such as site, personnel, or equipment. The results obtained were used to develop a waste decontamination protocol for our institution.

A gravity displacement autoclave (Medallion General Purpose; American Sterilizer Co., Erie, Pa.) was used for all experiments. On average, the autoclave chamber reached a temperature of 121°C within 2 min of sterilization cycle initiation. The autoclave timer was checked against a manual timer. The accuracy of the temperature gauge was checked regularly against a thermocouple located at the lower front end of the autoclave chamber (coldest location).

Temperature was measured with type K thermocouples (Thermo-Kinetics, Toronto, Ontario, Canada) connected to a digital temperature monitor (model 3900; Digitron Instrumentation, Hertford, Hertfordshire, United Kingdom) through a multiple-thermocouple adaptor (American Sterilizer Co.). Thermocouple accuracy was checked against a mercury-in-glass thermometer. The temperature was recorded at 2-min intervals throughout the sterilization cycle.

Loads were heated to 121°C, as detected with a thermocouple centrally located in the load, and then processed for 20 min as indicated in the sterilization protocol used in our laboratory at the time. Because of the high plastic content of the load, which made postautoclaving sampling difficult, cultures of samples from autoclaved bags were not carried out to verify the sterilization process. Instead, sterilization effectiveness was checked with biological indicators (*Bacillus stearothermophilus*, Chemspor vials [American Sterilizer Co.]) centrally located in the load. Following sterilization, Chemspor vials were incubated at 60°C for up to 7 days. Also, known bacterial cultures (*Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Listeria monocytogenes*) in the exponential growth phase in broths and on agar slants were used to check sterilization effectiveness. Following sterilization, broths (Trypticase soy broth; BBL Microbiology Systems, Cockeysville, Md.) were inoculated with the autoclaved bacterial cultures and then incubated at 35°C for 18 h. For both sterilization controls, absence of growth was interpreted as an indication of a successful sterilization process.

To be considered sterilized, a load must reach an internal temperature of 121°C (1) and stay at that temperature for at least 15 min. Thus, the indicator used in our study for comparison of processing conditions was the average time required to reach 121°C as measured by a thermocouple centrally located in a bag half loaded with biomedical waste (average heat-up time). Emplacement of the bags in the autoclave chamber was permuted between runs to allow for each type of bag to be located in turn at the lower front end of the autoclave chamber. The bags were laid out in the autoclave chamber in a way that allowed free circulation of steam around them.

Throughout all of the experiments, the same size and brand of autoclave bags were used (63 by 91 cm; Bio-Check Biohazard Bags; Baxter Healthcare Corp., Scientific Products Div., McGaw Park, Ill.). The bags were made of polypropylene 0.0464 ± 0.005 mm thick, as measured with a micrometer. They were designed to withstand a temperature of 133°C.

Bags were loaded with contaminated biomedical waste from our bacterial identification laboratories. They were

* Corresponding author.

TABLE 1. Time required to reach 121°C at the center of a load under different autoclaving conditions

Data set no.	Incisions in top of bag[a]	Bagging method[a]	Water added[b]	Stainless steel containers[c]	Temp set on autoclave (°C)	No. of bags tested	Time (min) to reach 121°C at center of load			
							Mean	Minimum	Maximum	SD
1	No	Single	No	No	121	50	46	24	70	12
2	No	Single	Yes	No	121	15	60	38	78	11
3	Yes	Single	No	No	121	63	37	12	72	12
4	Yes	Single	Yes	No	121	42	36	16	62	10
5	No	Single	No	No	123	42	32	14	54	10
6	No	Single	No	No	132	14	19	10	28	5
7	No	Single	No	Yes (V)	121	20	82	56	124	16
8	No	Single	No	Yes (H)	121	16	50	26	70	14
9	No	Double	No	No	121	14	60	32	78	12

[a] Bags were closed with elastomeric bands.
[b] Water (750 ml) was added before processing.
[c] Unless otherwise stated, bags stood freely on a stainless tray (48 by 103 cm). H, container (diameter, 330 mm; height, 330 mm) horizontal; V, container vertical.

processed when half full (mean weight, 4.2 ± 1.62 kg). No attempt was made to standardize the contents of the bags. Most of the time, bacterial cultures on agar media in plastic disposable petri dishes accounted for at least 50% of the load, which was completed with borosilicate test tubes containing either broth or agar, disposable 96-wells microplates, and other small objects. The usual autoclave load was six bags (26.9 ± 4.15 kg).

Processing conditions and the number of bags processed to test each set of experimental conditions are given in Table 1. The reference bags to which the others were compared are described in data set 1. Each one of the eight sets of experimental conditions (data sets 2 to 9) was compared to the same unique set of reference conditions (data set 1). Some sets of experimental conditions were compared in groups of two (data sets 3 and 4, 5 and 6, and 7 and 8). For all of the experiments, except those comparing the influence of the processing temperature set on the autoclave, experimental and reference bags were autoclaved simultaneously, giving several data sets for the reference conditions. There was no difference between the results from different reference data sets (analysis of variance; $P < 0.05$). Thus, all of the results obtained with the reference conditions were pooled in data set 1 for final analysis.

Data compilation was done with Quattro Pro, version 4.0 (Borland International, Scott Valley, Calif.). Data were analyzed with TRUE EPISTAT, version 4.0 (Epistat Σ Services, Richardson, Tex.). For each data set, descriptive statistical parameters were computed. Since there was no multiple comparison between group means in the experimental design, an unpaired two-tailed t test was used to analyze the data.

No growth was observed after proper incubation of the 69 Chemspor vials or the 27 broths inoculated with autoclaved bacterial cultures that were used to check sterilization effectiveness.

In the following sections, closed bags with three or four 20-cm-long incisions made in the top are referred to as open bags. Bags without incisions are referred to as closed bags.

The mean heat-up time for open bags (data set 3) was significantly shorter ($P = 0.00016$) than that for closed bags (data set 1). The 20% reduction of the average heat-up time when open bags were used was probably due to greater direct steam contact with the load in open bags (6). Even though these results and those obtained by previous workers (6) suggest that opening bags before autoclaving would decrease processing time, we do not recommend this practice because of the risk of biological contamination of the staff and the surroundings.

The mean heat-up time for open bags with added water (data set 4) was not significantly different ($P = 0.3012$) from that for similar bags without added water (data set 3). Researchers have previously reported that addition of water to bags before autoclaving did not decrease heat-up time (3, 6). Others have reported paradoxical results (4). Our results support that the addition of water to open bags does not significantly decrease the heat-up time. In open bags, the waste material comes into direct contact with the steam; consequently, the effect of additional steam from vaporization of the added water has little impact on the heating process.

The mean heat-up time for closed bags to which 750 ml of water was added (data set 2) was significantly longer ($P = 0.00048$) than that for similar bags without added water (data set 1). The temperature inside closed bags with added water increased more rapidly than inside the other type of bags during the first 35 min of the process, and then the temperature increases became similar. Since water has a higher thermal conductivity than air (4), addition of water to a closed bag should increase thermal transfer. The apparent slowing of thermal transfer after 35 min may be due to accumulation of poor-quality steam (wet steam) within the bag, which would decrease thermal transfer (5) after a certain time. Also, since heat-up time is directly related to the mass of the load (6), addition of 750 g of water, which represented, on average, an 18% increase in mass, may have contributed to the increased heat-up time. Our results, from experiments with open or closed bags, indicated that addition of water is not recommended for processing of biomedical waste material such as agar plates, which already have a high water content.

The mean heat-up time for double bags (data set 9) was significantly longer ($P < 10^{-6}$) than for single bags (data set 1). These results confirmed those of previous studies (6). Double bagging seems to interfere with thermal transfer, probably because of the thermal isolation provided by the two polypropylene bags and the air trapped between them.

The mean heat-up time was longer when bags were inside vertical containers (data set 7) instead of horizontal ones (data set 8) ($P = 0.00079$) or free standing (data set 1) ($P < 10^{-6}$). There was no significant difference in the heat-up time for free-standing bags compared with bags in horizontal containers ($P = 0.3886$). Temperature increased very slowly inside bags in vertical containers compared with the two

other types of bags. Even though it was shown that solid vertical containers increased the sterilization time compared with wire baskets (3), some researchers have suggested that autoclave bags be placed in stainless steel containers before processing to contain spillage (3, 4, 6). In our study, the heat-up time was longer when bags were inside vertical containers, probably because the steam forced the air to the bottom of the container (6), creating pockets of air; air is less efficient than steam for transmission of heat (4). This phenomenon did not occur when the container was on its side or when a bag was free standing. Free-standing bags on a tray with low sides allowed for optimal heating of the bags while permitting containment of spillage within the tray.

The mean heat-up time for bags processed at 121°C (data set 1) was significantly longer than that for bags processed at 123°C (data set 5) ($P < 10^{-6}$) or 132°C (data set 6) ($P < 10^{-6}$). The mean heat-up time for bags processed at 123°C was significantly longer than that for bags processed at 132°C ($P = 0.0000306$). These findings support those of Cooney (2), who reported that increasing the processing temperature from 121 to 132°C significantly decreased the processing time. However, since bags processed at 132°C were in poor condition after autoclaving compared with those processed at 121 or 123°C, rebagging may be necessary for these bags before disposal. When a processing temperature of 123°C was used, the mean heat-up time decreased by 32% and the maximum heat-up time decreased by 22% for closed bags. Compared with open bags processed at 121°C (data set 3), closed bags processed at 123°C (data set 5) had a mean heat-up time that was 13% shorter. Since some laboratories may not have sterilizers designed to process items at 132°C and because of the poor condition of the bags after autoclaving at this temperature, increasing the processing temperature of closed bags from 121 to 123°C seems to be a good alternative to processing them at 132°C or opening them to decrease the heat-up time.

This study was undertaken to determine the optimal conditions for processing of laboratory biomedical waste with the goal of developing a waste decontamination protocol for our institution. By using bags loaded with biomedical waste instead of standardized loaded bags as in previous studies (2, 4, 6), we were able to evaluate not only the average processing time but also the maximum processing time which might be necessary to decontaminate biomedical waste in a real-life situation. This information is essential for proper decontamination of biomedical waste before it is disposed of in dump sites.

The optimal conditions selected for processing of our laboratory biomedical waste were as follows: half-loaded single polypropylene bags closed with elastomeric bands without added water or incisions made in the top, free standing on a tray during processing at 123°C. These conditions allowed for fast processing and safe handling of the waste since the bags remained closed throughout the procedure and were intact after processing. Since the maximum heat-up time observed with the conditions described above was 54 min (data set 5) and a load must stay at 121°C for at least 15 min to be considered sterilized (1), we now use a processing time of 70 min for most of our laboratory biomedical waste. This time excludes the cooling period. Once a week, a Chemspor vial (American Sterilizer Co.) is placed at the center of a load located at the lower front end of the autoclave to verify sterilization effectiveness. With the settings described above, all Chemspor vials are negative after proper incubation.

We thank Michel Samir Zariffa and Manon Lorange for technical assistance during this work. We thank Marie-Alix d'Halewyn, Manon Lorange, Louise Thibert, Michel Brazeau, and Luc Massicotte for critical reviews of the manuscript.

REFERENCES

1. **Block, S. S.** 1991. Infectious medical wastes: treatment and sanitary disposal, p. 730–748. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation, 4th ed. Lea & Febiger, Philadelphia.
2. **Cooney, T. E.** 1988. Techniques for steam sterilizing laboratory waste. Technical note DB-3014. American Sterilizer Co., Erie, Pa.
3. **Gillepsie, E. H., and S. A. Gibbons.** 1975. Autoclaves and their dangers and safety in laboratories. J. Hyg. **75**:475–487.
4. **Lauer, J. L., D. R. Battles, and D. Vesley.** 1982. Decontaminating infectious laboratory waste by autoclaving. Appl. Environ. Microbiol. **44**:690–694.
5. **Perkins, J. J.** 1983. Principles and methods of sterilization in health sciences, 2nd ed., p. 95–121. Charles C Thomas, Publisher, Springfield, Ill.
6. **Rutala, W. A., M. M. Stiegel, and F. A. Sarubbi, Jr.** 1982. Decontamination of laboratory microbiological waste by steam sterilization. Appl. Environ. Microbiol. **43**:1311–1316.