APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1982, p. 1311–1316
0099-2240/82/061311-06$02.00/0

Vol. 43, No. 6

Decontamination of Laboratory Microbiological Waste by Steam Sterilization

WILLIAM A. RUTALA,* MARSHA M. STIEGEL, AND FELIX A. SARUBBI, JR.

Division of Infectious Diseases, Department of Medicine, University of North Carolina School of Medicine, and Department of Hospital Epidemiology, North Carolina Memorial Hospital, Chapel Hill, North Carolina 27514

Received September 9, 1981/Accepted 1 February 1982

A steam sterilizer (autoclave) was tested to determine the operating parameters that affected sterilization of microbiological waste. Tests involved standardized loads (5, 10, and 15 lb [ca. 2.27, 4.54, and 6.80 kg, respectively]) of contaminated petri plates in autoclave bags placed in polypropylene or stainless steel containers. Thermal and biological data were obtained by using a digital potentiometer and a biological indicator containing spores of *Bacillus stearothermophilus*, respectively. The transfer of heat was more efficient when smaller loads of microbiological waste were tested and stainless steel rather than polypropylene containers were used. A single bag with the sides rolled down to expose the top layer of petri plates allowed heat to pass better than did a single bag with the top constricted by a twist-tie. The presence of water in the autoclave bag did not significantly improve heat-up time in stainless steel or polypropylene containers. The results of biological tests substantiated the temperature data. When 10 or 15 lb of microbiological waste was exposed to various test conditions, the only condition that ensured the destruction of *B. stearothermophilus* involved the use of a stainless steel container (with or without water) for 90 min. Autoclaving for 45 min resulted in the destruction of bacteria included in 10 lb (136 ± 3 plates) or 15 lb (205 ± 6 plates) of microbiological waste when stainless steel containers with or without water or polypropylene containers with water were used, whereas 60 min was required to kill all bacteria if polypropylene containers without water were used.

The increasing concern expressed by federal agencies and the general public about hazardous solid waste dictates that we identify methods for rendering our hazardous microbiological waste nonhazardous before disposal. One recognized method for waste treatment involves the steam sterilization (autoclaving) of microbiological waste. Although it is generally assumed that steam sterilization kills bacteria associated with this waste, evidence to support this claim is not available. Since the factors affecting sterilization efficiency (type of container, presence of water, volume of waste, and type of bag) of microbiological waste have not been thoroughly studied, this investigation was initiated to ascertain the conditions and processing times that must be employed to achieve sterilization of microbiological waste. The study was accomplished by using standardized loads (5, 10, and 15 lb [ca. 2.27, 4.54, and 6.80 kg, respectively]) of contaminated petri plates in polypropylene and stainless steel containers with and without water. A digital potentiometer and a biological monitor (*Bacillus stearothermophilus* spores) were centrally located in the loads to identify the time-temperature profile and the efficacy of the sterilizing cycle, respectively.

Our experiments demonstrate that steam sterilization constitutes a reliable method for the sterilization of microbiological waste only if extended exposure periods are used.

MATERIALS AND METHODS

Commercially available plastic autoclave bags measuring 24 by 35.8 in. (ca. 61 by 90.9 cm; Fisher Scientific Co., Pittsburgh, Pa.) were used for tests. These bags are constructed of 1.5-mil polyethylene and are designed to withstand a temperature of 125°C. Bags were tested in two modes: (i) in the open position, with the sides of the bag folded down to expose the top layer of petri plates, and (ii) with the opening in the bag loosely constricted with a twist-tie. Four holes were punched in the tops of all twist-tied plastic bags. Water (500 ml) was added to the plastic bags containing the microbiological waste only when specified.

Standardized loads of 5, 10, and 15 lb (±1 ounce [28 g]) of contaminated 100-mm petri plates from our hospital's clinical microbiology laboratory were tested. They consisted of 67 (±3), 136 (±3), and 205 (±6) petri plates, respectively. An average of 85% of these plates was contaminated with viable bacteria.

The bags were placed in a container and then put into a gravity displacement autoclave (model M65CD5-4, American Sterilizer Co., Erie, Pa.). The interior dimensions of this sterilizer were 53.25 by 24 by 36 in. (ca. 135.26 by 61 by 91.44 cm). The sterilizer reached a temperature of 121°C in an average of 1 min 54 s (±14 s) after the initiation of the cycle. Microbiological waste in autoclave bags was placed in a stainless steel tray (21 by 14 by 5 in. [ca. 53 by 36 by 13 cm]) or a polypropylene container (24 by 17 by 5 in. [ca. 61 by 43.2 by 12.7 cm]) to prevent spillage. The accuracy of of the autoclave's time and temperature gauges and the sterilization efficiency of the autoclave were checked at regular intervals.

The bags were monitored for time-temperature profiles by a digital potentiometer and for sterilization efficacy by a biological indicator. A copper-constantan thermocouple (American Sterilizer) to measure temperatures was passed through the sterilizer chamber via a conax connector (American Sterilizer). The thermocouple was connected to a Doric 400A digital potentiometer (Doric Scientific, San Diego, Calif.). The instrument was checked against mercury-in-glass thermometers. The temperatures were recorded throughout the processing cycle at 5-min intervals, and unless otherwise stated, the thermocouple was placed in the front bag of the three bags in the sterilizer.

The biological monitoring system used was Kilit (BBL Microbiology Systems, Cockeysville, Md.). Each Kilit ampoule contained a suspension of *B. stearothermophilus* spores in culture media. These biological indicators are a sealed system that are standardized so that spores survive when autoclaved for 5 min at 121°C and spores are killed when autoclaved for 15 min at 121°C. After the test cycles, the biological indicators were removed and incubated at 56°C for 7 days. Positive cultures were determined by a color change, from purple to yellow, of the pH indicator contained within the ampoule. The thermocouple and Kilit biological indicators were placed centrally, 5.5 cm from the bottom of the bag.

All autoclave bags and containers were sterilized before the contaminated petri plates were placed in the bags to allow for an assessment of the level of kill of the bacteria on the plates after a sterilizer cycle. At the completion of the cycle, a sterile cotton-tipped applicator was dipped into the molten agar left after a cycle and swabbed onto a blood agar plate. Plates were incubated aerobically at 35°C for 48 h before being evaluated for growth. In 10% of the experiments, plates were also incubated anaerobically at 35°C for 96 h. All microorganisms present after a processing cycle were identified by standard microbiological techniques (3).

A statistical analysis was performed on the transformed data with $y = \log_e[(250 - \text{temperature})^{-1}]$. After the strength of the linear relationship $y = (a \times \text{time} + b)$ was measured by the Pearson product-moment correlation ($\bar{\rho} = 0.985$), the resulting straight lines were compared by the general linear test procedure.

RESULTS

In the first series of experiments, we attempted to identify the location most difficult to heat in a load of microbiological waste. This was done by placing the thermocouple centrally in 10 lb of microbiological waste at various locations in the load (0, 5.5, 11, 16.5, and 22 cm from the bottom). The greatest delay in heating was observed when the thermocouple was placed 5.5 cm from the bottom of the bag. The bag nearest the door (front) heated more slowly than the middle and back bags, and there was no difference in heat transfer when one versus three bags were used. The thermocouple and the biological indicator were therefore placed 5.5 cm from the bottom of the front of three bags in subsequent experiments.

Table 1 presents data from experiments in which various preparations of microbiological waste were tested to assess the conditions affecting the sterilization process. These data were derived from temperature tracings with the digital potentiometer under the described conditions. The results showed that heat transfer was significantly facilitated by smaller volumes of microbiological waste and a stainless steel container. A single autoclave bag with the sides rolled down also hastened heat transfer when compared with a single bag tied with a twist-tie. The presence of water did not significantly improve the heating of microbiological waste in stainless steel or polypropylene containers. Representative temperature traces of each of the four conditions that affect the sterilization of microbiological waste are shown in Fig. 1. The results show the importance of varying load size in a polypropylene container (Fig. 1A). The 15-lb load of microbiological waste allowed slower heat transfer than did the 5- or 10-lb load of waste. An equally important parameter in the transfer of heat is the type of container (Fig. 1B). In a typical temperature tracing comparing stainless steel and polypropylene containers with 10 lb of waste, the stainless steel container was at the sterilizing temperature (121°C) for 30 min, whereas the polypropylene container under the same conditions did not reach 121°C. In contrast, heat-up time was not significantly improved when water was added to a bag of microbiological waste that was placed in either type of container (Fig. 1C). The bagging technique was an important factor in heat transfer. Placing the microbiological waste in two bags rather than one generally hindered heat transfer under identical test conditions. Folding down the sides of the bag to expose the top layer of plates allowed good heat transfer but might not be practical (Fig. 1D).

The biological data substantiated the temperature tracing data. When 5 lb of microbiological waste (67 ± 3 plates) in stainless steel containers with or without water or polypropylene containers with water was exposed to a steam sterilizing cycle of 30 min, no growth of vegetative or

TABLE 1. Comparison of conditions that affect sterilization of microbiological waste[a]

Condition	Bag	Vol (lb)	Container	Water (500 ml)	*P* value
Vol	S	5 vs 10	SS	Yes	<0.01
	S	5 vs 15	SS	Yes	<0.001
	S	10 vs 15	SS	Yes	NS
	S	5 vs 10	P	Yes	<0.001
	S	5 vs 15	P	Yes	<0.001
	S	10 vs 15	P	Yes	NS
	SSD	5 vs 10	P	No	<0.001
Container	S	10	P vs SS	Yes	<0.001
	S	10	P vs SS	No	<0.001
Water	S	10	SS	Yes vs no	NS
	S	10	P	Yes vs no	NS
Bag	S vs SSD	5	P	No	<0.001
	S vs SSD	10	P	No	<0.001

[a] Data represent duplicate runs under the same conditions, except the two SSD (5- and 10-lb) experiments, which are single runs. Abbreviations: P, polypropylene; SS, stainless steel; S, single autoclave bag; SSD, single autoclave bag with sides rolled down; NS, not significant ($P > 0.05$).

sporeforming bacteria or both was observed. Growth of sporeforming bacteria was observed when 5 lb of microbiological waste was placed in a polypropylene container without water, but all organisms were killed after 45 min.

Since microbiology laboratory waste containers are often not emptied until they are full (containing about 125 petri plates), larger volumes of microbiological waste were examined in more detail. When 10 lb of microbiological waste (136 ± 3 plates) was tested in polypropylene containers with and without water, growth of *B. stearothermophilus* occurred even after a sterilizing cycle of 90 min (Table 2). The highest temperature achieved under these conditions was 118°C. Growth of vegetative or sporeforming bacteria or both was observed after 15, 30, and 45 min in polypropylene containers without water and 15 and 30 min with water. The vegetative bacteria identified included *Escherichia coli, Acinetobacter* sp., *Staphylococcus aureus, Staphylococcus epidermidis, Enterobacter* spp., *Pseudomonas* spp., *Proteus* spp., *Klebsiella pneumoniae*, nonhemolytic *Streptococcus*, and *Streptococcus viridans; Bacillus* was the only sporeforming bacterial genus identified. In stainless steel containers holding 10 lb of waste, growth of vegetative or sporeforming bacteria or both was observed after 15 and 30 min with and without water. When a 90-min processing cycle and stainless steel containers were tested, *B. stearothermophilus* was killed.

The efficiency of killing the biological indicator (*B. stearothermophilus*) in 15-lb loads of microbiological waste (205 ± 6 plates) in stainless steel or polypropylene containers was essentially identical to that of 10-lb loads (Table 3). A minor exception was noted in the experiment with a polypropylene container with water at 90 min. Minor differences were also noted in the recovery of vegetative and sporeforming bacteria when comparing the 10- and 15-lb loads after 30 min of autoclaving. Whereas occasional growth of vegetative or sporeforming bacteria or both was noted in the 10-lb load after 30 min, growth of these organisms occurred only when 15 lb of waste was placed in the polypropylene or stainless steel container without water.

DISCUSSION

Steam sterilizers are commonly used by hospitals for rendering potentially hazardous microbiological waste nonhazardous before final disposal. A recent survey of North Carolina hospitals indicates that 41% of the hospitals use steam sterilizers for rendering microbiological waste sterile before discarding it in a sanitary landfill. About two-thirds of these hospitals use a time-temperature profile of ≤30 min at 121°C. Many hospitals use the standard time and temperature recommendations (e.g., 121°C for 15 min) when processing hazardous microbiological waste. These recommended times are valid for items in direct contact with steam but do not include the penetration time, which will vary with the physical conditions of the load and which must be determined by sensing the load temperature. This investigation examined some of the load conditions that may affect sterilization.

The results of our study show that the type of container holding the waste is an important factor in successful heat transfer. A comparison of the slopes of the lines from the transformed data demonstrates that the stainless steel con-

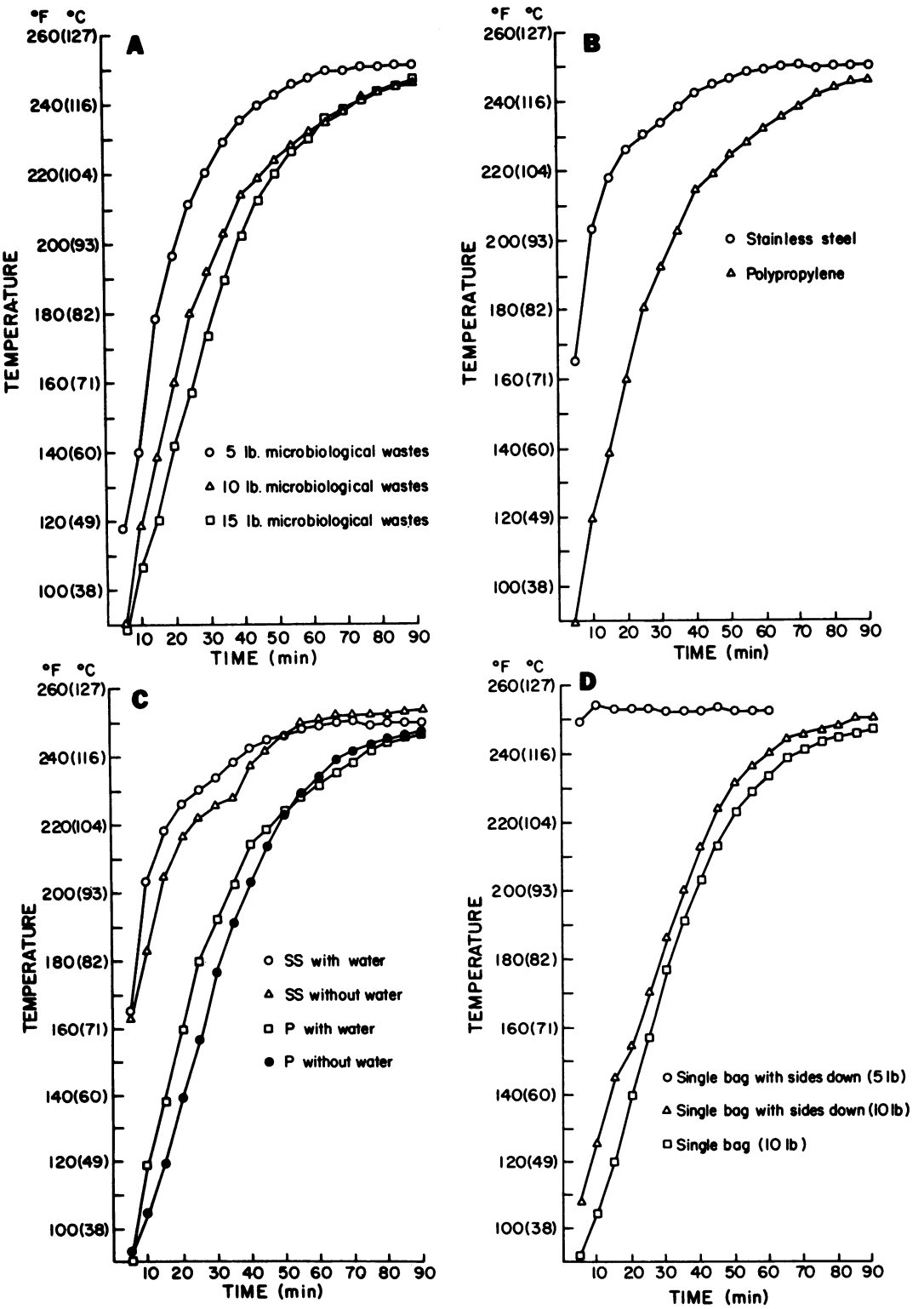

FIG. 1. Time-temperature curves for conditions that affect steam sterilization of microbiological waste. (A) Polypropylene container with water; (B) 10 lb of waste with water; (C) polypropylene (P) or stainless steel (SS) container and 10 lb of waste; (D) polypropylene container without water.

TABLE 2. Exposure conditions required for sterilization of 10 lb of microbiological waste[a]

Container	Water (500 ml)	Time (min)	Growth of BI	Highest temp achieved (°F [°C])	Bacteria after cycle
Polypropylene	Yes	15	+	147 (64)	+ (SFB and VB)
		30	+	198 (92)	+ (33% SFB)
		45	+	219 (104)	−
		90	+ (66%)	244 (118)	−
Polypropylene	No	15	+	147 (64)	+ (SFB and VB)
		30	+	195 (91)	+ (33% SFB)
		45	+	213 (101)	+ (33% SFB)
		60	+	241 (116)	−
		90	+	245 (118)	−
Stainless steel	Yes	15	+	205 (96)	+ (66% VB)
		30	+	232 (111)	+ (SFB and VB)
		45	+	249 (121)	−
		90	−	250 (121)	−
Stainless steel	No	15	+	220 (104)	+ (33% VB)
		30	+	230 (110)	+ (33% VB)
		45	+	234 (112)	−
		90	−	255 (124)	−

[a] Polyethylene bags containing waste were loosely constricted with a twist-tie, and holes were punched in the top of the bag before placement into the container. All results are from three to six replicates per condition. A + or − result in columns 4 and 6 means that all replicates gave identical results; the percentage figure indicates the percentage of positive replicates per condition. Abbreviations: BI, biological indicator; SFB, sporeforming bacteria; VB, vegetative bacteria.

tainer allowed for optimal heat transfer. This apparently occurs because of the improved conduction of heat through the walls of a stainless steel container. We chose to use shallow containers because deeper containers require more time for air removal. Although perforated or wire mesh containers allow better air removal, they are not suitable for discarded cultures since

TABLE 3. Exposure conditions required for sterilization of 15 lb of microbiological waste[a]

Container	Water (500 ml)	Time (min)	Growth of BI	Highest temp achieved (°F [°C])	Bacteria after cycle
Polypropylene	Yes	30	+	202 (94)	−
		60	+	240 (116)	−
		90	+	245 (118)	−
Polypropylene	No	30	+	199 (93)	+ (33% SFB)
		60	+	242 (117)	−
		90	+	245 (118)	−
Stainless steel	Yes	15	+	199 (93)	+ (SFB and VB)
		30	+	236 (113)	−
		60	+	248 (120)	−
		90	−	250 (121)	−
Stainless steel	No	15	+	171 (77)	+ (SFB and VB)
		30	+	209 (98)	+ (66% SFB and VB)
		45	+	229 (109)	−
		60	+	242 (117)	−
		90	−	251 (122)	−

[a] Polyethylene bags containing waste were loosely constricted with a twist-tie, and holes were punched in the top of the bag before placement into the container. All results are from three to six replicates per condition. A + or − result in columns 4 and 6 means that all replicates gave identical results; the percentage figure indicates the percentage of positive replicates per condition. Abbreviations: BI, biological indicator; SFB, sporeforming bacteria; VB, vegetative bacteria.

the molten agar that results after a sterilizing cycle may escape within the autoclave and can block the chamber drain upon cooling.

The physical characteristics of the load affected heat penetration, but this condition is not unique to microbiological waste. When the sides of the autoclave bag were rolled down to expose the top layer of a 5-lb load of petri plates, heat-up time was almost instantaneous. However, when the weight of the load was increased to 10 lb and the sides of the bag were rolled down, heat-up time was significantly delayed. This finding is most likely related to the difference in the depth of the loads. In the 10-lb load, the thermocouple is covered by about 16 cm of petri plates, whereas in the 5-lb load it is essentially on top of the 6-cm load. Whenever a container is placed in a gravity sterilizer in an upright position, the incoming steam (being lighter than air) forces air to the bottom of the sterilizer and the container (4). The removal of entrapped air and resultant steam permeation is increasingly difficult as the height of the material (container) is increased. Thus, some investigators have recommended that water be added to the container (1). The premise is that water placed among the waste will generate its own steam and replace residual air that might interfere with sterilization. This variable was evaluated in our study, and although we found an improvement in heat-up time with microbiological waste in shallow stainless steel or polypropylene containers and autoclave bags, it was not statistically significant. These findings are consistent with the results of Gillespie and Gibbons (2), but they are in contrast to the data of Lauer and Battles (Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, Q128, p. 221).

The characteristics of the autoclave bag also affected heat penetration. Two bags reduced heat penetration under the tested conditions when compared with heat penetration in one bag. In contrast, improved heat-up time was observed when the sides of the bag were folded down. The findings are presumably due to the reduced transmission of steam and air through a polyethylene film. Perkins found that whereas 1-mil polyethylene exhibits good vapor transmission rates, 4-mil polyethylene is practically impermeable to steam and air (4). Thus, the manufacturers of autoclave bags generally recommend loosely constricting the top so that there is a free passage of air or steam. Laboratories that use 3- to 4-mil polypropylene bags without a loosely constricted top and without holes punched in the top of the bag should expect a slower heat-up time than that determined in this investigation.

It seems apparent that our continued use of the autoclave for decontamination of microbiological waste should be linked to some assurance that sterilization is taking place. The most conservative approach, which provides an exceptional margin of safety, would involve the use of biological indicators in loads of microbiological waste. If the elimination of *B. stearothermophilus* is used as evidence for sterilization, then the autoclaving of 10- or 15-lb bags of waste in a stainless steel container would require a minimum sterilizing cycle of 90 min. This practice is likely to be viewed as quite unrealistic and the argument raised that it is not necessary to ensure the elimination of all bacterial spores in such a process. It thus seems reasonable to support the selection of an autoclave processing time that provides consistent destruction of pathogenic vegetative and sporeforming bacteria but that does not necessarily eliminate the spores of *B. stearothermophilus*. Our data show that autoclaving for 45 min resulted in the destruction of bacteria included in 10 or 15 lb of microbiological waste when stainless steel containers with or without water or polypropylene containers with water were used, whereas 60 min was required to kill vegetative and sporeforming bacteria when polypropylene containers without water were used.

In conclusion, factors that facilitated heat transfer and sterilization of microbiological waste included the type of container in which the waste was placed, the physical characteristics of the load, and the autoclave bag. The results indicated that the presence of water did not appreciably improve heat-up time when 10 lb of waste was placed in a stainless steel or polypropylene container. Steam sterilization of microbiological waste is reliable if extended exposure periods are used and the conditions that affect heat transfer (container, bag, volume of waste, and possibly water) are optimized. The decontamination of hazardous microbiological waste by steam sterilization is complex, for by varying test conditions, one can markedly alter the thermal and biological result. Unless specific minimal conditions are attained, bacteria will remain viable.

ACKNOWLEDGMENT

We thank Greg Samsa for his statistical analysis of the data.

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