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Validation of Autoclave Protocols for Successful Decontamination of Category A Medical Waste Generated from Care of Patients with Serious Communicable Diseases

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ABSTRACT In response to the Ebola outbreak in 2014, many hospitals designated specific areas to care for patients with Ebola and other highly infectious diseases. The safe handling of category A infectious substances is a unique challenge in this environment. One solution is on-site waste treatment with a steam sterilizer or autoclave. The Johns Hopkins Hospital (JHH) installed two pass-through autoclaves in its biocontainment unit (BCU). The JHH BCU and The Johns Hopkins biosafety level 3 (BSL-3) clinical microbiology laboratory designed and validated waste-handling protocols with simulated patient trash to ensure adequate sterilization. The results of the validation process revealed that autoclave factory default settings are potentially ineffective for certain types of medical waste and highlighted the critical role of waste packaging in successful sterilization. The lessons learned from the JHH validation process can inform the design of waste management protocols to ensure effective treatment of highly infectious medical waste.

KEYWORDS Ebola, sterilization, medical waste, serious communicable diseases, autoclave

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The Ebola outbreak in West Africa in 2014 revealed potential gaps in the abilities of U.S. hospitals to safely provide care for patients with highly infectious diseases. Prior to the outbreak, the capacity to care for patients in the United States infected with high-consequence pathogens was limited to a few specialized facilities, or biocontainment units (BCUs) (1–3). In response to the crisis, the Centers for Disease Control and Prevention (CDC) recommended a tiered approach wherein U.S. hospitals serve as frontline health care facilities, Ebola assessment hospitals, or Ebola treatment centers (ETCs) (4). The Office of the Assistant Secretary for Preparedness and Response (ASPR), a federal office in the Department of Health and Human Services (HHS), created a regional response plan, which called for the creation of Regional Ebola and Other Special Pathogen Treatment Centers (RETCs) (5). These RETCs were modeled in part on the U.S. facilities that provided care for Ebola patients, namely the University of Nebraska Medical Center, the National Institutes of Health, Emory University, and Bellevue Hospital Center, and also include design elements based on local capabilities and lessons learned from the outbreak (6).

One of the unique challenges of caring for patients with highly infectious diseases is the handling of patient medical waste containing category A infectious substances (7–11). A category A infectious substance is "capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs" (12). Only a small number of civilian facilities process category A substances, and the cost and logistical barriers to transporting waste to those facilities are substantial. The amount of waste generated during the care of an Ebola patient is also significantly greater than that for routine medical care. This is partly due to the high volume of gastrointestinal losses, but also reflects the higher staff-to-patient ratio and the large amount of disposable personal protective equipment (PPE) required (9–11). Medical waste that is treated onsite through the use of steam sterilizers, or autoclaves, can be handled as regulated medical waste (9). The CDC and ASPR recommend that facilities preparing to care for patients infected with Ebola consider installing on-site autoclaves to handle category A infectious substances (7).

The Johns Hopkins Hospital (JHH) BCU is the ASPR region 3 RETC serving Maryland, Delaware, Pennsylvania, Virginia, West Virginia, and Washington, DC (5). The JHH BCU includes two pass-through autoclaves for treating infectious waste prior to transporting it off the unit (6). A preliminary risk assessment (PRA) was conducted to identify potential high-consequence events during autoclave use and to offer opportunities for risk reduction. The PRA identified the following two main risks associated with waste disposal and autoclave use: (i) the exposure of a health care worker to infectious material, and (ii) the failure to effectively sterilize waste. To address the risk of sterilization failure, the JHH BCU conducted a series of validation experiments using mock patient care trash loads. These experiments demonstrated that autoclave factory default settings are potentially inadequate for sterilizing highly infectious waste and that careful attention to waste packaging prior to autoclave processing is a critical factor for successful sterilization. The lessons learned from this validation process can inform waste management protocols to ensure effective treatment of highly infectious medical waste at facilities that utilize on-site autoclaves.

RESULTS

We found that 16 of 19 (84%) autoclave cycles performed using factory default settings failed to sterilize the biological indicators in the center of the load. This included all runs performed using a liquid or gravity cycle for 30 min or a vacuum cycle for 15 min at 123°C or 134°C, respectively. These failed runs contained simulated loads composed of liquids (0.5 to 1 liter) in suction canisters or sharps containers, as well as PPE and other paper products. Water-saturated and unsaturated bed linens (blankets, sheets, and pillow cases) treated with a vacuum cycle for 15 min or with either of the other two default cycles (liquid or gravity) for 30 min also failed to be sterilized. Failure to sterilize the biological indicators occurred regardless of the type of bag closure used, including those that were goose-necked and secured lightly with autoclave tape or were just lightly folded and placed in the autoclave tray. The autoclave service contractor (Modular Component Systems, LLC, Stevensville, MD) was notified of these failures and confirmed that each autoclave was operating within manufacturer specifications.

These runs on simulated loads were repeated multiple times with various sterilization cycle parameters. Initially, attempts were made to identify a single cycle type (liquid, gravity, or vacuum) that would work well for all waste packaged together, as the sorting of trash by type may be too great a safety risk. Repeated runs using a liquid cycle for 60 or 120 min with goose-necked, double, or triple autoclave bags failed, regardless of cycle type. In fact, all runs conducted in which any of the bags were goose-necked or tightly sealed failed. All runs in which a solidifier was used failed. Dissolvable autoclave bags began to break apart within 1 min after coming into contact with moist or wet materials, such as bed linens, so they were not tested in the autoclave.

For liquid cycles, purge times were adjusted and maximized at 14 min with a

TABLE 1 Optimized cycles and parameters used in this study for adequate sterilization of simulated waste

Parameter	PPE and dry trash	Saturated linen	Liquid
Cycle type	Vacuum	Vacuum	Liquid
Sterilization time (min)	30	60	120
Sterilization temperature (°C)	134	134	123
Dry/cool time (min)	1	10	35
Purge time (min)	4	5	14
Precharge (psig)	20	20	20
No. of prevacs[a]	3	3	3
Prevac vacuum point (in. Hg)	10	10	10
Autoclave bag type and configuration	Standard clear bags, double bagged		
Bag closure	Clamped outer bag, loosely twisted inner bag[b]		

[a]Prevac, prevacuum pulse.
[b]Remove clamp and place in tray prior to autoclaving.

sterilizing temperature of 123°C for 60 to 120 min. Large volumes of liquids (>3 liters) required the longest time for sterilization (120 min). Dry waste, such as PPE and other paper and plastic items, required the least amount of time (30 min) when using a vacuum cycle at 134°C with a precharge set to 20 psig (pounds per square inch gauge), a purge time of 4 min, a prevacuum set point of 10 in. Hg (inches of mercury), and 3 prevacuum pulses. The most difficult loads to sterilize were those containing saturated linens (soaked with >1 liter of water) comprising a cotton blanket, sheets, and pillow cases, which required a vacuum cycle of a minimum of 60 min to achieve adequate sterilization using the settings as described for other dry waste. Nine of nine runs (100%) containing multiple saturated linens and using a shorter sterilizing time (3 runs each of 15, 30, and 45 min) failed.

We found that the double bagging of waste was optimal with the inner bag lightly secured by a 7 in. by 1/8 in. rubber band (Pale crepe gold, item no. 909713; Alliance Rubber Company, Hot Spring, AR), which allowed for a small opening at the point where the bag was gathered. For safety, the optimal closure for the outer bag was a 2-in. binder clamp (item no. 308957; Office Depot, Boca Raton, FL), which was removed and placed in the autoclave tray with the bag prior to sterilization. The optimized parameters are summarized in Table 1.

Since initiating the use of the optimized parameters, we have completed two consecutive quarterly validations of the autoclave system. We found that 18 of 18 (100%) mock patient loads (6 PPE, 6 linen, and 6 liquid loads) passed with the optimized parameters compared to only 3 of 19 (16%) mock loads that passed with use of the factory default settings.

DISCUSSION

Current protocols for sterilizing waste from patients with serious communicable diseases, such as Ebola, are based on guidelines for biosafety levels (BSLs) 3 and 4 laboratories (13). While these protocols have been developed to enhance laboratory safety in the handling of infectious materials, they may not be adequate for the type and volume of waste generated from patient care activities. The validation process of the JHH waste-handling system identified several critical issues that need to be considered in the design of protocols for sterilizing waste generated from the care of patients with highly infectious diseases, such as Ebola.

First and foremost, the JHH experience highlighted the need to validate waste management protocols using simulated patient care loads. The simulated loads need to reflect the expected volume and type of waste that will be generated from a patient with a particular disease, and they need to feature the materials that will be used in patient care, including the same autoclave bags, personal protective equipment (PPE), linens, and liquid waste containers. The validation cycles with simulated waste must be processed with biological indicators buried within the trash load, since indicators outside the autoclave bag may not accurately reflect the conditions inside the bag

during the autoclave cycle. Based on the validation results, individual facilities may need to reassess the use of particular patient care items. For example, the JHH BCU no longer uses heavy cotton blankets in patient care rooms, since heavy linens saturated with water were the most likely to fail the validation protocol, even at the highest settings of pressure and temperature.

This validation process led to several important changes in the JHH BCU protocols for the packaging of in-room waste. Since we found that each type of load requires different autoclave cycle parameters to ensure proper sterilization, waste is now separated according to the overall composition (paper and PPE, linens, and liquids of $>$0.1 liters) in separate bags within the patient's room before transporting it to the waste management area. Providers will never reach into trash containers to re-sort waste once it has been discarded. If waste types are inadvertently packaged together, the entire load will be run on the liquid cycle to ensure adequate sterilization. The reason that all loads are not run on the liquid cycle is that this cycle takes 2 to 4 times as long as the other available cycles. It would be challenging to sterilize the anticipated large amounts of waste from an Ebola patient if each cycle took 2 h to complete. The tradeoff for this is the extra step of separating waste in the patient's room.

One of the most important findings of this study is that autoclave bags should not be sealed prior to treatment, so as to allow steam to penetrate into the center of the bag. Despite testing many different types of closures, we found that biological indicators in the center of a load are not sterilized unless there is an opening in both the inner and the outer autoclave bags. This point is critical, since within-bag biological indicators were not adequately sterilized even in loosely taped autoclave bags, especially those with tightly packed loads or those containing saturated linens. Individual facilities will need to develop their own protocols to safely transport waste from the site of patient care to the autoclave, being careful to not load sealed bags into the autoclave. For example, at JHH, a metal clamp is used to close the outer autoclave bag for transport, and the clamp is then removed and placed in the autoclave tray just prior to placing the load into the autoclave. After autoclaving, the sterilized clamp can be reused.

Indicators placed in solidified liquids did not pass the validation process regardless of the autoclave parameters used or the type of cycle selected. This raises potential safety concerns, since currently, there is a paucity of data regarding the use of solidifying agents in the care of patients with highly infectious diseases. Further investigation is warranted, as large volumes of highly infectious liquid waste are likely to be encountered with Ebola patients as well as those with other diseases. Current JHH BCU patient care protocols do not utilize solidifying agents.

Finally, autoclaves need to be operated and tested on a regular basis to ensure that they achieve the proper temperature and pressure parameters before being used for patient care. At JHH, each autoclave is operated four times per week in accordance with manufacturer's recommendations. Quarterly validation of each autoclave cycle is conducted using the three types of simulated trash loads and biological indicators. If changes are made to waste-handling protocols or new equipment is used in patient care, the validation process is repeated to ensure adequate treatment of patient care waste. Detailed logs of all weekly and quarterly validation runs are maintained, and preventive maintenance is conducted on an annual basis. It is also important that autoclave settings for all load types be reviewed following preventive maintenance or other repairs, as settings may inadvertently be reset to factory defaults, which would result in run failure. For this reason, all optimized autoclave settings should be recorded should the need to reinstall them arise.

Conclusions. The sterilization of waste containing category A infectious substances using steam sterilizers, or autoclaves, has been adopted by a number of hospitals preparing to care for patients with Ebola and other serious communicable diseases.

TABLE 2 Initial factory default cycles and parameters tested

Cycle type	Sterilize temp (°C)	Sterilize time (min)	Dry/cool time (min)	No. of prevacs[a]
Vacuum	134	15	30	3
Gravity	123	30	30	0
Liquid	123	30	15	0

[a]Prevac, prevacuum pulse.

While autoclave sterilization may be an effective and safe way to process infectious waste for transport and disposal, this study shows that factory default settings and laboratory waste guidelines are likely insufficient to adequately sterilize pathogens in the center of medical waste autoclave loads. Autoclave parameters may need to be adjusted, with particular attention paid to the way that waste loads are packaged prior to treatment. Each facility utilizing autoclaves for the treatment of infectious medical waste should validate their waste management protocols with simulated patient trash loads and within-bag biological indicators to ensure that waste is properly decontaminated.

MATERIALS AND METHODS

Design of the JHH waste-handling system. To facilitate the unidirectional flow of waste through the unit, the JHH BCU installed two pass-through steam sterilizers (PSS-500, software version 7923; Primus Sterilizer Co., Omaha, NE). Waste is transported in sealed containers from patient care areas to a special waste-handling room at the far end of the unit. Contaminated waste is loaded on the unit side, and once treated, is unloaded on the clean side and packaged for transport and disposal. The autoclaves have a special "Bioseal" function, which allows biological separation of the clean and dirty sides. When the autoclave cycle is completed, the door gasket on the clean side retracts, while the gasket on the dirty side remains sealed. The doors cannot be opened simultaneously, which prevents cross-contamination of the autoclave's clean and dirty sides. Each autoclave is a stand-alone unit, which allows for continued operation of one autoclave if the other unit requires maintenance. Steam intake and electrical and mechanical infrastructures are located on the clean side of the waste-handling area to facilitate autoclave maintenance even while the BCU is caring for a patient. The entire system is connected to the hospital's backup power system, which has two substations and enough backup fuel to maintain power for up to 90 h in the event of a citywide loss of electricity (6).

Autoclave validation process. The BCU team partnered with experts in The Johns Hopkins Hospital biosafety level 3 (BSL-3) laboratory in the division of medical microbiology to design and test autoclave sterilization protocols. The BSL-3 lab uses an autoclave to sterilize laboratory waste in accordance with current federal guidelines from the CDC, NIH, and HHS (13). The BCU autoclaves were validated using simulated loads consistent in quantity and composition with items expected from patient rooms in the BCU. These items included unsaturated (dry) and saturated (soaked with at least 1 liter of water) linens (cotton blankets, sheets, and pillow cases), personal protective equipment (PPE), such as gowns, gloves, booties, and hoods, dry trash (paper and plastic products, etc.), and liquids (0.1 to 1 liter), including suction canisters and laboratory sharps containers. A solidifying agent (MediChoice fluid solidifier; Owens and Minor, Mechanicsville, VA) was tested to determine if liquids were effectively treated after the conversion to a solid state.

Three types of bags were tested, namely, standard red biohazard bags (MediChoice can liners, 30 in. by 43 in.; Owens and Minor, Mechanicsville, VA), clear autoclave bags (autoclavable biohazard waste bags, 25 in. by 30 in. and 31 in. by 28 in.; Medline Industries, Inc., Mundelein, IL), and dissolvable bags (water-soluble bags, 36 in. by 39 in.; Elkay Plastics, Commerce, CA). The test loads for the validation were packaged based on published protocols utilized in existing biocontainment units (8–10). The loads were double or triple bagged, and each bag was filled to 50% to 75% of capacity. The bags were secured using the following variety of closures: a goose-necked closure, with and without autoclave tape; a lightly folded closure, with and without autoclave tape; a rubber-banded closure; and a clamped closure. Different combinations of closures for inner and outer bags were tested. For example, a lightly folded inner bag was tested with a rubber-banded outer bag.

Three separate biological indicators were used to test each load. A rapid biological indicator (3M Attest 1292; 3M, St. Paul, MN) and a standard biological indicator (3M Attest 1262) were placed into the center of each load (or directly into liquids) and affixed by a string to the outside of the bags for easy retrieval after autoclaving. In addition, an individual test pack containing a rapid biological indicator (3M Attest 1296/1296F rapid readout biological indicator steam pack) was placed in the autoclave tray next to the autoclave bags. This test pack served as a control to ensure that the autoclave cycle was sufficient to sterilize a biological indicator that was not buried inside a waste load. When the cycle was completed, the rapid biological indicators were incubated for 3 h according to the manufacturer's instructions using a 3M Attest auto reader 390 before being read; standard biological indicators were incubated at 56 ± 2°C (3M Attest steam incubator) for 48 h before being read. Nonautoclaved rapid and standard biological indicators were used as positive controls for all of the runs. The lot numbers for all biological indicators were recorded to ensure that test indicators and controls were obtained from the same lot.

TABLE 3 Autoclave cycles and parameters modified from factory default settings and tested in this study

Parameter	Settings/range tested	Purpose or comment
Type of cycle	Vacuum, gravity, liquid	Various types were selected based on load type
Sterilization time (min)	15–180	Various times were tried based on load type
Sterilization temp (°C)	123–134	Various temps were tried based on load type and duration of sterilization phase
Exhaust	Slow	For liquid cycles
	Rapid	For vacuum and gravity cycles
Dry time/liquid cool time (min)	1–45	Time for removing moisture/ cooling the chamber
Purge time (min)	2–14	Time for removing air from the chamber
Precharge (psig)	1–20	Pressure to be achieved during the charge portion of all prevacs[a]
No. of prevacs	1–6	No. of pulses autoclave pulls before starting a cycle
Prevac vacuum point (in. Hg)	5–10	Sets vacuum endpoint for pulses

[a]Prevac, prevacuum pulse.

Autoclave cycle types and sterilizations parameters. Table 2 shows the initial factory default settings tested for all of the load types. Liquid, gravity, and vacuum cycles were used depending on the type of load to be sterilized. A liquid cycle was used for all loads containing >0.1 liters of liquid, and gravity or vacuum cycles were used for loads containing dry waste or linens. The individual cycle parameters modified from factory default settings that were tested are shown in Table 3. The modifications included presterilization steps, such as the time spent removing air from the chamber (purge time), the pressure achieved during the charge portion of all prevacuum phases relative to atmospheric pressure (precharge), the vacuum endpoint in in. Hg for all pulses (prevacuum endpoint), and the number of prevacuum pulses. The sterilization parameters included temperature, cycle length, type of exhaust, and dry time. Forty-two different load-run configurations were tested, including unique cycle programs developed as a result of this study.

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