Journal of the American Association for Laboratory Animal Science
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Vol 62, No 1
January 2022
Pages 48–54

Comparison of Low- and High-temperature Cagewash Cycles for Sanitation of Rodent Housing Equipment in Research Facilities

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Sanitation guidelines for animal research facilities state that disinfection is achieved by application of high-temperature water (143 to 180 °F [62 to 82 °C]) or detergents and disinfectants. However, these guidelines are based on requirements for pasteurization, which may be unnecessarily stringent for the sanitation of nonfood items and do not address the theoretical sanitation potential of water at temperatures below 143 °F (62 °C). Recent literature indicates that water temperatures below 143 °F (62 °C) can also provide effective sanitation. In this study, we compared cagewash cycles at low (100 °F [38 °C] and 120 °F [49 °C]) and high (standard) (180 °F [82 °C]) temperatures and evaluated sanitation efficacy by using ATP swabs and RODAC plates. Low-temperature loads were washed either with or without prior treatment of a chemical disinfectant (10% bleach). The 100 °F (38 °C) cycle was not sufficient for sanitization without bleach pretreatment. However, the 120 °F (49 °C) cycle effectively sanitized cages without bleach pretreatment. Validation of effective sanitation at a lower water temperature (120 °F [49 °C]) can improve cagewash logistics and reduce costs as compared with standard (180 °F [82 °C]) high-temperature cycles.

Abbreviations: B, pretreatment with bleach; NB, no bleach pretreatment

DOI: 10.30802/AALAS-JAALAS-22-000088

Introduction

The *Guide for the Care and Use of Laboratory Animals* states that sanitation, which "involves bedding change (as appropriate), cleaning and disinfection," helps to maintain environmental conditions "conductive to health and well-being" in research animal facilities.[7] Disinfection, a key component of sanitation, is defined as an activity that "reduces or eliminates unacceptable concentrations of microorganisms," and "can be achieved with wash and rinse temperature at 143–180 °F or more."[7] In addition, "sanitation of cages and equipment by hand with hot water and detergents or disinfectants can also be effective."[7]

Cage-disinfection guidelines using high-temperature water (143 to 180 °F [62 to 82 °C]) are derived from pasteurization standards, where a cumulative heat factor (a combination of temperature and time) is used to assure effective pasteurization. Both longer exposure at lower temperatures and shorter exposure at higher temperatures can achieve equivalent pasteurization.[14] Formal recommendations for cycle times at temperatures below 145 °F (63 °C) are not available, and temperatures below 140 °F (60 °C) are a food-safety concern in the dairy industry.[5]

However, providing the recommended high-temperature water to cagewash systems comes with challenges, including 1) regulations limiting domestic building hot water to 120 to 140 °F (49 to 50 °C) at the source, 2) regulations of maximum water temperature at the outlet, and 3) heat loss during transport from the heat source to the cagewash system.[15,16] Therefore, cagewash machines often rely on the steam at the facility to achieve the standard high temperatures. The additional equipment and energy costs required to provide high temperature water are substantial.

Current alternatives to high-temperature water are not practical or expedient. If high-temperature water is not available, such as during mechanical outages, sanitation can be achieved by using low-temperature water (less than 143 °F [62 °C]) with the addition of chemical detergents or disinfectants. In our facility, 10% bleach is used as a disinfectant to sanitize cages when mechanical outages prevent the use of high-temperature water for sanitation. However, application of chemical detergents or disinfectants is labor intensive because surfaces must be "rinsed free of residual chemicals" and require a specified surface-contact time. In addition, these detergents and disinfectants pose an occupational health risk, and chemicals such as chlorine products and phenolics are corrosive to equipment.[6,7] Due to the challenges of chemical sanitation, high-temperature water sanitation is preferable.

Pasteurization standards, which apply to food for human consumption, may not be necessary for sanitization of equipment in research animal facilities because surfaces are inert and ingestion is not a concern. Sous vide cooking (cooking vacuum-sealed food in a water bath for a longer time under lower temperature as compared with traditional cooking) demonstrates that exposure to temperatures as low as 122 °F (50 °C) for several hours significantly reduces microorganism counts.[2,11] Furthermore, performance studies in research animal management have demonstrated that exposure to temperatures as low as 140 °F (60 °C) for 25 s[12] or 110 °F (43 °C) for 4 min[1] can effectively sanitize equipment without the need for additional disinfectants.

In this study, we tested cagewash sanitation at 3 temperature conditions: 100 °F (38 °C, the lower limit of domestic hot water),

Submitted: 09 Sep 2022. Revision requested: 10 Nov 2022. Accepted: 16 Nov 2022.
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120 °F (49 °C, the upper limit of domestic hot water), and 180 °F (82 °C, the high-temperature standard). We hypothesized that all 3 temperature conditions would effectively sanitize equipment in research animal facilities.

Materials and Methods

Preliminary processing after cage change. Dirty rat cages were emptied of bedding and scraped clear of organic debris prior to experimental testing. Consistent with our institutional guidelines, cages were changed once a week for singly housed rats and twice a week for group-housed rats. All preliminary cage processing was performed by the same person.

Experimental overview. Dirty equipment from rat housing (cages [polysulfone plastic], wire tops [stainless steel], water bottles [glass], and feeders [stainless steel]; model RS10147U40MVSP-CD3-R Rodent Cage System, Allentown Caging, Allentown, NJ) was collected after cage change and preliminary processing. Prior to cagewash, equipment either received no further treatment or was sprayed with 10% bleach (Pure Bright Germicidal Ultra Bleach, KIK International, Vaughan, Ontario, Canada) for 10 min and then rinsed. Equipment was processed through a rack cagewash system (Basil 4600, Steris, Mentor, OH) at 100 °F (38 °C; lower limit of domestic hot water) or 120 °F (49 °C; upper limit of domestic hot water). Dirty (unwashed) and clean (standard [unbleached, processed through cagewash at 180 °F]) equipment controls were used for comparison (Figure 1). The 100 °F cycle ran for approximately 60 min, the 120 °F for about 55 min, and the 180 °F (82 °C) cycle for approximately 25 min (Figure 2).

In summary, sampled groups were as follows: 1) pretreatment with bleach and then washed at 100 °F (100 B); 2) no pretreatment and washed at 100 °F (100 NB); 3) pretreatment with bleach and washed at 120 °F (120 B); 4) no pretreatment and washed at 120 °F (120 NB); 5) no pretreatment and washed at 180 °F (180 NB, control consistent with institutional standards); and 6) dirty control (sampled after cage change, no pretreatment or wash). Each treatment group was replicated at least 3 times. Each day, wash cycles were run in order of low to high temperature to avoid inadvertent heating.

Equipment tested. On the first day of the study (180 °F and 120 °F cycles), 3 cage bottoms, 1 wire top, 1 bottle, and 1 feeder were processed and tested. Cage bottoms were emphasized as consistent with previous cagewash studies, and other dirty items were added depending on availability.[1,12] The items tested were changed on the second day (100 °F cycle) to 1 cage bottom, 1 wire top, 2 bottles, 2 sipper tubes, and 1 feeder, after a review of historic data (data not shown) revealed that bottles and sipper tubes appeared to be particularly difficult to sanitize (Table 1).

Temperature monitoring and cagewash cycles. Temperature was measured by using the built-in cagewash display and was documented manually every 5 min during the cycles. The built-in cagewash temperature probe was located in the pump outlet piping that led into the washer spray header. The cagewash display temperature had previously been validated to be reasonably accurate; when the steam was off, the sump temperature measured 99 °F (37 °C) via infrared gun, consistent with the cagewash display reading of 97 to 100 °F (36 to 38 °C). In addition, a nonreversible temperature indicator (105 to 160 °F range [41 to 71 °C; 8 Dot Water, Omega Engineering, Norwalk, CT, USA] for the 100 and 120 °F cycles; 160 to 190 °F range [71 to 88 °C; catalog no. NB225, TEMP-A-SURE, Steris] for 180 °F cycles) was attached to the side of the cage washing rack prior to cycling and read afterward. For the 180 and 120 °F cycles, steam was used to keep the water at the target temperature. For the 100 °F cycle, steam was turned off, and the cycle was run on the building water alone. A detergent (Cage-Klenz 180 Alkaline Cage Wash Detergent, Steris) was dispensed during cagewash cycles.

For the 100 and 120 °F cycles, the cycle consisted of a 90-s prewash, a 999-s wash, 2 rinses of 999 s each, and a 360-s exhaust for a total of about 57 min. For the 180 °F cycle, the cycle consisted of a 90-s prewash, 180-s wash, 2 120-s rinses, and a 360-s exhaust for a total of approximately 15 min. Discrepancies between the calculated and measured times were attributed to the time needed to heat the water to the appropriate starting temperature.

Sanitation monitoring. ATP swabs were used to test for the presence of organic matter. ATP activity was measured by using a swab and sampler collector system (AccuPoint Advanced Healthcare ATP Cleaning Validation System and AccuPoint Advanced ATP Surface Sampler Collection System, Neogen, Lansing, MI). A 4 × 4 in. area was swabbed back and forth and then up and down when the geometry was flat. For sipper tubes, the black stopper was swabbed in its entirety, and then the surface of the sipper tube was swabbed in an up-and-down motion. Equipment was sampled as illustrated (Figure 1). ATP activity was quantified in relative light units (RLU) and defined as pass (1 to 149 RLU),

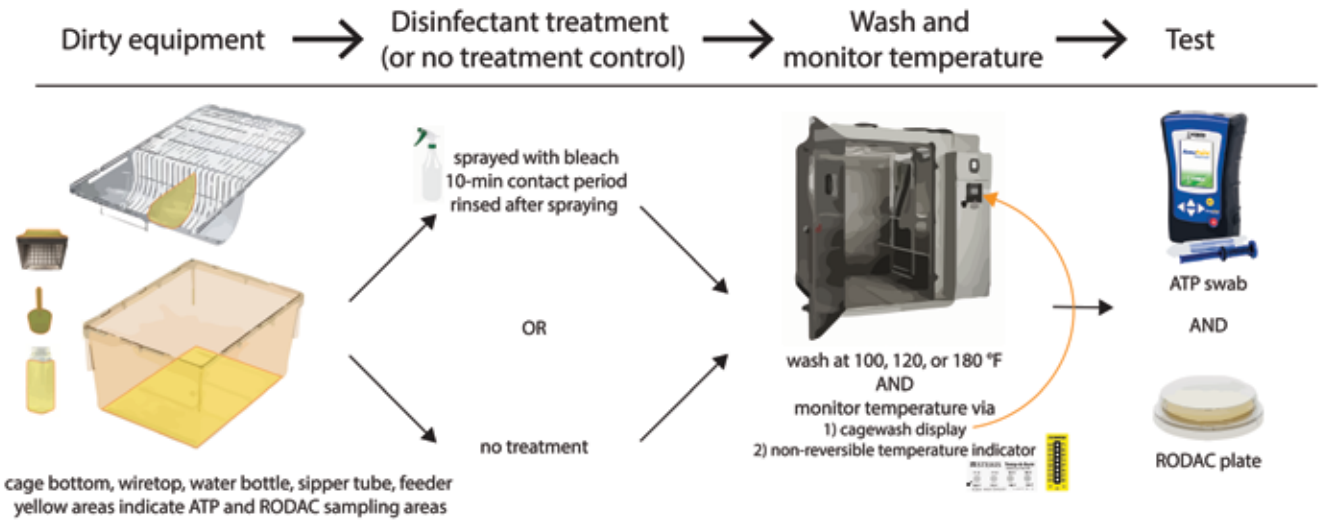

Figure 1. Experimental overview. Dirty equipment was disinfected with 10% bleach (or no treatment control), washed at various temperatures, and then tested for sanitation by using a RODAC plate or ATP swab.

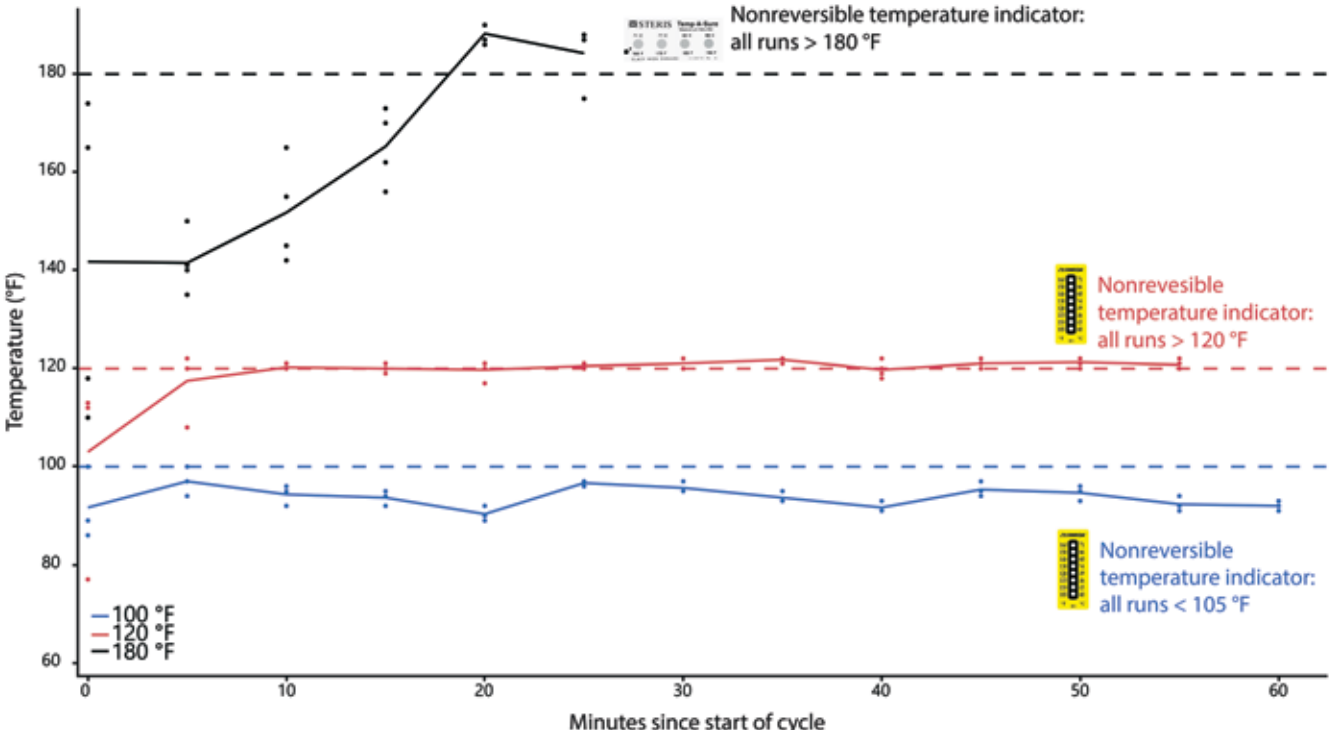

Figure 2. Temperature measured during various cycles, as indicated both by nonreversible temperature indicators and temperature readings displayed on the cagewash equipment. Data points are readings recorded manually from the cagewash display. Lines connect means for each temperature setting. Temperature indicator readings were consistent between temperature groups and are displayed next to the icon for the temperature indicator.

marginal (150 to 299 RLU), or fail (300 RLU and greater), according to the default threshold settings in the Accupoint reader. ATP and RODAC sampling areas did not overlap.

RODAC plates (Remel Contact I Tryptic Soy Agar w/Lecithin, Polysorbate 80, ThermoFisher Scientific, Waltham, MA) were used to measure living and culturable microorganisms. Equipment was sampled as illustrated except for the sipper tube, which was not sampled due to geometry (Figure 1). RODAC plates were pressed once briefly onto the surfaces. Plates were incubated (catalog no. 151030513, Isotemp 60L Incubator Gravity, ThermoFisher Scientific) at 37 °C in ambient air for 48 h. Plates were checked at 48 h and CFU were counted. Plates with more than 100 CFU were quantified as too numerous to count (TNTC). Using previous standards of research animal management and the American Public Health Association,[4] CFU counts were interpreted as good (fewer than 25 CFU), fair (25 to 50 CFU), and poor (over 50 CFU). ATP and RODAC sampling areas did not overlap.

All sanitation monitoring was performed by the same person.

Additional cycles. To conserve cycles, bleach-treated and -untreated samples were run in the same cycle for a given temperature. Because the bleached equipment was rinsed before cagewash, we assumed that bleach contamination of the unbleached equipment would be negligible. At the end of the experiment, we ran a no-bleach cycle at the lowest effective temperature (120 °F, no. 4) to verify that the target temperature would effectively sanitize the equipment without any bleach in the system (Table 1). In addition, a no-bleach cycle comprising 2 cage bottoms at 180 °F was run after an earlier run under the same conditions (180 °F, no. 4) suggested potential contamination on RODAC plates.

Statistical analysis. Sanitation monitoring results were compared between groups by using JMP Pro 16 (JMP Statistical Discovery, Cary, NC). Pairwise comparisons between the various temperature conditions were made by using the Steel–Dwass test (nonparametric version of the Tukey HSD). For RODAC results, values of TNTC were calculated as 100 CFU. Confirmatory cycles were included in the statistical analysis. Significance was defined as a P value of less than 0.05.

Results

Temperature monitoring. The 120 °F and 180 °F cycles both reached their target temperatures during the cycle, as indicated by the cagewash machine display (Figure 2) and nonreversible temperature indicator. The 100 °F cycle did not reach target temperature according to either measure (Figure 2).

The highest temperatures documented during the 180 °F cycles were 187, 190, 190, and 187 °F. The highest temperature documented during the 120 °F cycles was 122 °F for all 4 runs. The highest temperatures documented during the 100 °F cycles were 100, 97, and 97 °F. For the nonreversible indicators, the highest recorded temperature for the 180 °F condition was 180 °F (all 4 runs), for the 120 °F condition was 120 °F (all 4 runs), and for the 100 °F condition was 'not registered' (less than 105 °F [41 °C]; all 3 runs).

Sanitation monitoring. ***100 °F cycles with and without bleach treatment.*** Of the 3 pretreated 100 °F runs, ATP testing results showed that 1 of 2 bottles failed (387 RLU) but all other samples passed, and all RODAC results were good (fewer than 25 CFU). Of the 3 nontreated 100 °F runs, ATP testing results showed that 1 bottom failed (832 RLU), 1 bottle failed (523 RLU), and another bottle was marginal (292 RLU), with all other items returning passing results. RODAC results were poor (56 CFU) for 1 bottom and fair (25 CFU) for another, and 1 bottle yielded poor (TNTC CFU) results, with all other test results good (Tables 1 and 2).

Table 1. Efficacy of sanitation according to ATP testing

		RLU											
Condition	Run no.	Bottom			Wire top	Bottle		Sipper tube		Feeder
100 °F, pretreated										
	1	56			0	0	387	0	76	0
	2	0			0	0	0	0	0	0
	3	0			0	0	15	0	0	0
100 °F, untreated										
	1	20			0	0	0	0	0	0
	2	832			0	0	523	0	0	0
	3	0			0	91	292	0	0	0
120 °F, pretreated										
	1	0	0	0	0	0		N/A		0
	2	0	0	38	0	0		N/A		0
	3	0	0	0	0	0		N/A		0
120 °F, untreated										
	1	0	0	44	0	0		N/A		0
	2	0	0	0	0	0		N/A		0
	3	0	0	0	0	0		N/A		0
	4 (validation of standard)		0		0	0	287	0	24	0
180 °F, untreated										
	1	0	0	0	0	0		N/A		0
	2	0	0	0	0	0		N/A		0
	3	0	98	130	0	0		N/A		0
	4 (additional clean controls)	0		12	N/A	N/A		N/A		N/A
Dirty control										
	1	452	705	3808	0	232	9920	21	7195	363
	2	4718	6204	5640	1141	1406	1788	1263	306	635
	3	3270	4003	4331	1061	479	996	996	306	443

N/A, not applicable

Split columns are used when multiple pieces of the same type of equipment were run in the same cycle, with the number of sub-columns correlating to the number of items tested in each run. Results are color-coded as green (pass, 1 to 149 RLU), orange (marginal, 150 to 299 RLU), and red (fail, 300 RLU and greater).

120 °F cycles with and without bleach treatment. Of the 3 pretreated 120 °F runs, ATP and RODAC results for all samples were pass or good. Of the 3 nontreated 120 °F runs, all ATP results passed; RODAC results showed that 1 of 3 bottoms sampled was fair (44 CFU). All other samples returned good results. An additional untreated 120 °F cycle was run (no. 4, no-bleach only to avoid bleach contamination). This confirmatory cycle showed that 1 of 2 bottles was marginal (287 RLU) on ATP testing and that that the 2 bottles tested as fair (28 CFU) or poor (56 CFU) on RODAC testing. In addition, 1 bottom had a fair (27 CFU) result on RODAC testing. All other tests were interpreted as passing or good (Tables 1 and 2).

180 °F cycles (clean control, untreated only). For the initial 3 untreated 180 °F runs, all ATP results were interpreted as pass. On RODAC testing, 3 bottoms and 1 bottle resulted in poor (TNTC CFU) results. During the RODAC incubation process, we suspected contamination after sampling, as this cycle is the standard for sanitation, and ATP results were very low. A confirmatory untreated 180 °F cycle was run a 4th time with 2 bottoms; both bottoms rated good with ATP testing, and one bottom tested on the lower threshold of 'fair' (25 CFU) with RODAC testing (Tables 1 and 2).

Dirty controls. According to ATP testing, all 9 bottoms, 2 of 3 wire tops, 5 of 6 bottles, 5 of 6 sipper tubes, and all 3 feeders failed. Of the remaining items, 1 wire top passed (0 RLU), 1 bottle was marginal (232 RLU), and 1 sipper tube passed. For RODAC testing, 8 of 9 bottoms, 2 of 3 wire tops, and 1 or 3 bottles tested as poor; 1 of 3 bottles and 2 of 3 feeders tested as fair; 1 of 9 bottoms, 1 of 3 wire tops, 1 of 3 bottles, and 1 of 3 feeders tested as good (Tables 1 and 2).

Statistical analysis. Pairwise comparisons of different temperature and bleaching conditions showed that ATP readings from dirty control cage bottoms were significantly higher than those of 120 NB (P = 0.0017), 120 B (P = 0.0030), and 180 NB (P = 0.0017). ATP sipper tubes were significantly higher in dirty control than in 100 B (P = 0.0308) and 100 NB (P = 0.0145). Sipper tubes were not tested for the 120 and 180 °F conditions, so lack of statistical significance is due to lack of testing, not failure to sanitize at the 120 or 180 °F cycles. RODAC cage bottom readings were significantly higher in the dirty control condition as compared with 120 B (P = 0.0062) and 120 NB (P = 0.0255). No significant differences were detected for any other comparisons (including the 100 or 120 conditions to the 180 NB gold standard).

Discussion

Consistent with previous low-temperature sanitation studies,[1] we demonstrated that a 120 °F × 55-min cagewash cycle without bleach pretreatment sanitizes equipment as well as the 180 °F high-temperature standard. As with the 180 °F cycle, the

Table 2. Efficacy of sanitation according to RODAC testing

Condition	Run no.	no. of CFU at 48 h									
		Bottom			Wire top	Bottle		Sipper tube	Feeder		
100 °F, pretreated											
	1	0			0	0	6	N/A	8		
	2	9			0	0	0	N/A	0		
	3	0			0	0	0	N/A	1		
100 °F, untreated											
	1	56			9	1	11	N/A	0		
	2	23			5	14	TNTC	N/A	0		
	3	25			1	0	17	N/A	5		
120 °F, pretreated											
	1	0	0	2	1	0		N/A	3		
	2	3	9	11	0	4		N/A	7		
	3	0	0	0	8	5		N/A	0		
120 °F, untreated											
	1	3	13	17	1	1		N/A	4		
	2	0	1	1	1	1		N/A	3		
	3	20	24	44	4	0		N/A	1		
	4 (validation of standard)	27			2	28	56	N/A	N/A		
180 °F, untreated											
	1	TNTC	TNTC	TNTC	2	1		N/A	2		
	2	0	1	1	2	1		N/A	2		
	3	6	6	9	0	TNTC		N/A	0		
	4 (additional clean controls)	4		25	N/A	N/A		N/A	N/A		
Dirty control											
	1	10	28	57	19	TNTC		N/A	0		
	2	82	89	TNTC	TNTC	26		N/A	27		
	3	TNTC	TNTC	TNTC	62	11		N/A	48		

N/A, not applicable; TNTC, too numerous to count

Split columns are used when multiple pieces of the same type of equipment were run in the same cycle, with the number of sub-columns correlating to the number of items tested in each run. Results are color-coded as green (good, <25 CFU), orange (marginal, 25–50 CFU), and red (poor, >50 CFU).

ATP swabs for the 120 °F cycle all yielded passing results, with the exception of one 'marginal' bottle reading, although several RODAC results were fair or poor. Because ATP testing consistently returned passing results and because the 120 °F cycles did not yield more fair or poor results than did the 180 °F cycles, we considered this outcome acceptable. In addition, statistical analysis of ATP and RODAC readings for cage bottoms demonstrated significant differences between dirty controls and the 120 °F temperature cycles for both testing tools, regardless of bleach application, at significance values similar to or exceeding those between dirty controls and the untreated 180 °F standard.

Although the lack of bleach in the 120 °F cycles was associated with higher numbers of marginal, fair, or poor results than were the no-bleach 120 °F samples, results for both the bleach and no-bleach groups were within acceptable limits, with no significant difference between the pretreated and untreated 120 °F groups or between the pretreated 120°F cycles and the dirty control.

We believe that the combination of low temperature and extended cycle time (55 min) resulted in a cumulative heat factor that achieved effective sanitization. Our results contrast with a previous study,[12] in which a 125 °F cycle did not achieve effective sanitation. The difference in results is likely due to cycle time; in the previous study, the cycle time was 120 s, whereas in our study, the cycle time was 55 min (Figure 2).

We found mixed results regarding the ability of the 100 °F × 60-min cagewash cycle to effectively sanitize equipment. Because we ran this cycle by using domestic building hot water alone (no steam), only 1 of the 3 cycles consistently reached 100 °F, and the other 2 cycles achieved a maximum temperature of only 97 °F, according to the reading from the cagewash machine display. We anticipate that at facilities that have the cagewash machines closer to the heat source, cycles are more likely to consistently reach 100 °F on building water alone. However, we do not anticipate that having all 3 cycles reach 100 °F would significantly alter our results. Although we hoped that the longer contact time would help the 100 °F cycle reach an acceptable cumulative heat factor for sanitation, we acknowledge that this temperature is similar to that of the human body (98 °F), at which many pathogenic organisms have optimal growth. In addition, 100 °F is below the minimum recommended temperature (113 °F) for sous vide cooking.[8] Because both ATP and RODAC testing showed that multiple items processed at 100 °F as had fail or poor results, we do not recommend using the untreated 100 °F condition as the sole method for equipment sanitization. However, adding bleach pretreatment to a 100 °F wash temperature sanitized most pieces of equipment (only 1 of the 6 water bottles failed ATP testing, all other items passed). Although chemical disinfection may be logistically viable in

small facilities or provide a short-term solution in the case of mechanical malfunction (for example, steam outage), the additional time and labor required to apply bleach, provide 10 min of contact time, and rinse thoroughly make this option problematic for large-scale operations.

We confirm that the 180 °F standard cagewash effectively sanitized equipment in research animal facilities. Although only 1 to 15 s of water exposure at 180 °F is required to effectively eliminate microorganisms according to milk pasteurization standards,[14] we found that 5 to 10 min of the 25-min cycle was at or above 180 °F. Although several items had fair or poor outcomes for RODAC testing, all items had passing ATP results. The failures in the RODAC tests may have been due to contamination after cagewash and ATP sampling, as ATP results are considered more reliable than those from RODAC plates.[3] Excluding potential contamination after the wash cycle, the failure to effectively kill bacteria is likely due to factors that prevent effective exposure of the bacterial load to high-temperature water rather than inadequate water temperature.[13] In our experience, bottles and sipper tubes tend to fail 180 °F sanitation most frequently, likely due to water being unable to contact hard-to-reach surfaces. In the case of cage bottoms, debris is sometimes left on the surface of the cage, creating a physical barrier that prevents high-temperature water from sanitizing the surface.

Together, these results suggest that effective sanitation of equipment in research animal facilities can be achieved using temperatures lower than the 143 to 180 °F range suggested by the *Guide for the Care and Use of Laboratory Animals* and pasteurization literature.[7,14] Instead, domestic hot water, if it achieves outlet water temperatures of 120 °F, will sanitize equipment even without pretreatment with 10% bleach or an additional steam heat source, thus providing both accessible sanitation in small facilities and alternatives to chemical disinfection during mechanical outages. We caution that because many factors differ between cagewash setups at different facilities, such performance standards should be validated on a facility-by-facility basis.

Even at our facility, where the domestic hot water does not reach 120 °F at the cagewash level, validation of low-temperature cycles may support the purchase of more cost-effective equipment and lower utility rates. For example, we were able to replace an aging high-pressure steam boiler (180 °F heat capacity) with a smaller electric water heater (120 °F heat capacity). We continue to use routine sanitation monitoring to verify that the 120 °F cycle is adequate for sanitation at this facility.

The cost for an electric water heater (80-gallon Hubbell) was estimated to be $7,662 (all estimates in USD). The total price after additional costs ($8,288 labor regular time, $1,390 miscellaneous valves and fittings, $144 expansion tank) was approximately $17,484. In comparison, a new high-pressure steam boiler would have cost $29,500. Assuming similar parts and installation costs for the larger commercial boiler, being able to run cycles at 120 °F saved over $21,000 ($7,662 instead of $29,500) in equiment costs. The total cost savings likely would be even greater, given that labor costs and parts probably would be more expensive with the larger boiler system.

In terms of operating cost, we calculated small savings from running a 120 °F cycle compared with a 180 °F cycle ($0.16 per cycle, $41.48 savings per year; assuming a small facility [5 cycles per week, 52 wk per year], select cagewash parameters [60 gallons per minute, 100 °F temperature in], and our facility's 2022 fiscal year utility rates [$11.41/Klb steam, $4.66/kGal water]). The longer cycle time of the low-temperature condition balanced out the cost savings of heating the water to a higher temperature. However, savings may be more substantial if the low temperature cagewash cycle is shorter and the system is used more frequently. Similar studies have demonstrated that lowering the cagewash temperature from 180 °F to either 110 °F or 140 °F can save $8,000 to $67,000 per year.[1,13] Because each situation is unique, calculations should be made on a case-by-case basis.

We recommend future studies with additional replications to confirm our findings and to further optimize the costs and logistics of sanitation. This study was limited by small and variable replicates of equipment type. In addition, we did not control for standardization of animal housing (density, size, age) when selecting soiled equipment, but we reasoned that our institutional cage-change frequency of weekly for singly housed rats and twice weekly for group housed rats would maintain a consistent level of soil at cagewash. These factors may contribute to the large variability in the ATP and RODAC results. Although we followed previously published standards when interpreting our ATP and RODAC results, additional studies are necessary to determine whether these criteria are appropriate for determining sanitation in animal facilities or whether our thresholds should be adjusted. In addition, both ATP and RODAC testing have limitations with regard to evaluating sanitation. ATP-based tests can underestimate levels of gram-negative bacteria due to lower lysis, whereas RODAC plates can fail to detect anaerobes and fastidious organisms.[3,10]

Further study of temperatures between 100 to 120 °F, in addition to the assessing the effect of cycle length on sanitation, also would provide useful information. In vivo and in vitro studies both have demonstrated that fevers of 106 °F (41 °C) inhibited the growth of pathogenic bacteria such as *Streptococcus pneumoniae*, compared with the growth rate at body temperature (99 °F [37 °C]).[9] Another study reported that temperatures of 110 °F prevented the spread of common murine pathogens (mouse parvovirus, *Helicobacter* spp., *Mycoplasma pulmonis*, *Syphacia obvelata*, and *Myocoptes musculinus*)[1].

Because cagewash logistics are an integral part of animal facility sanitation and maintenance, additional studies on this topic could significantly influence the resources devoted to the care of research animals. In conclusion, we have demonstrated that with adequate cycle durations, a 120 °F temperature cycle sanitizes rodent housing equipment in research facilities as well as the 180 °F high-temperature standard. Validation of low-temperature cagewash cycles can improve cagewash logistics and costs.

Acknowledgments

We thank Paul Foote for his assistance with energy calculations and Holly Fairfield, Elizabeth Parker, Dack Shearer, Holly Clem, and Christopher Learned for their help in facilitating the project.

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