
Optimal conditions for the removal of house dust mite, dog dander, and pollen allergens using mechanical laundry

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Background: Mechanical laundry is an effective tool for the environmental control of allergens, but the optimal conditions for removing allergens are not yet clear.

Objective: To evaluate the optimal conditions of mechanical laundry for the removal of house dust mite (HDM), dog dander, and pollen allergens.

Methods: The 4 washing modes of 30°C (86°F), 40°C (104°F), 60°C (140°F), and steam water (SW) with detergent were evaluated. Allergen removal performance was assayed using a 2-site enzyme-linked immunosorbent assay (ELISA) or an ELISA inhibition test.

Results: Using the 30°C and 40°C washing modes, only 6.5% and 9.6% of *Dermatophagoides farinae*, respectively, were killed. However, using the 60°C and SW washing modes, all HDMs were killed. The amounts of Der f 1 remaining after the 30°C, 40°C, 60°C, and SW washing modes were 26.8%, 2.4%, 1.3%, and 0.6%, respectively, with unmanipulated contaminated sheets. The effects of rinse on Der f 1 levels after the 30°C washing were greater compared with those after the 40°C, 60°C, and SW modes. The amounts of Can f 1 in the extractions after washing were 0.3% to 1.3% for all modes, and all extracts, even without a rinse, did not inhibit specific IgE binding to dog allergens according to ELISA. The remaining pollen allergen levels after washing were lower in the 60°C and SW modes than in the lower temperature modes. However, the levels did not differ among the various washing modes after rinsing once.

Conclusion: Water temperature and number of rinses are critical factors for the removal of HDM, dog dander, and pollen allergens.

Ann Allergy Asthma Immunol. 2008;100:583–588.

INTRODUCTION

Environmental control has been recognized as an essential component of treatment for allergic patients.^{1–4} Indoor allergens, such as those from house dust mites (HDMs) and pet dander, are found in high amounts in bedding and clothes.^{5,6} Some children who are allergic to pets may develop symptoms at school on exposure to allergens carried from classmates who have pets at home.^{7,8} In addition, pollens may be spread to indoor environments by clothes contaminated during outdoor activity.⁹ Although HDMs, dog dander, and pollen are recognized as inhalant allergens that aggravate respiratory allergic diseases, direct contact of skin with contaminated clothes and bedding may also aggravate atopic

dermatitis. Hence, adequate environmental control of HDMs is also helpful for atopic dermatitis.³

Mechanical laundry is an effective component of environmental control for patients with respiratory allergies^{2,5} or atopic dermatitis.¹⁰ The process of mechanical laundry consists of washing, rinsing, and drying. Water temperature in the washing process is especially important for the eradication of living HDMs. Temperatures greater than 55°C (131°F) are critical for killing HDMs,¹¹ and the removal of HDM or cat allergens from bedding dust may be affected by water temperature during the wash cycle.¹² However, until now, few data have been available about the effects of rinsing in relation to water temperature for the removal of HDM allergens, and few studies have been conducted to determine the optimal conditions of mechanical laundry for the removal of dog dander and pollen allergens. In this study, we evaluated various water temperatures and numbers of rinses during mechanical laundry to determine optimal conditions for the removal of HDM, dog dander, and pollen allergens.

MATERIALS AND METHODS

Laundry Machine

A commercially available laundry machine (model WD-FR335D; LG Electronics, Seoul, Korea) was used for this

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Disclosures: Authors have nothing to disclose.

Funding Sources: This work was supported by a grant from LG Electronics and by Clinical Research Center for Chronic Obstructive Airway Disease grant 0412-CR03–0704-0001 from the Korean Ministry of Health and Welfare.

Received for publication October 2, 2007; Received in revised form December 10, 2007; Accepted for publication December 11, 2007.

study. Four laundry modes—30°C (86°F), 40°C (104°F), 60°C (140°F), and steam water (SW)—were evaluated for the removal of HDM, dog dander, and pollen allergens. The laundry machine was filled with 3 pillows (50 × 70 × 22 cm) for the washing process. The wash cycle lasted 40 minutes, and a commercially available powdered detergent containing enzymes (Tech; LG Household and Health Care Ltd, Seoul) was used at the recommended concentration (35 g per 70 L of water). After washing, the laundry machine rinsed the laundry with cold water for 10 minutes.

Viability of HDMs

The type of HDM evaluated in this study was *Dermatophagoides farinae*. They were cultured at a mean (SD) of 25°C (3°C) and 75% (2%) of relative humidity. The viability of HDMs was determined using a microscope with magnification ×20. The HDMs were considered to be alive if they were translucent and moving when exposed to heat, whereas dead HDMs generally were nonmoving and shrunken or denatured. Live HDMs were wrapped in filter paper that was not permeable to HDMs, and the folded sections of paper were placed in a spherical capsule constructed of stainless steel mesh. Three wrapped filter papers were packed in each mesh ball, and the balls were placed in the core of an unused pillow (50 × 70 × 22 cm). Each wrapped filter paper contained 100 HDMs, so 1 mesh ball contained 300 living HDMs. These pillows were exposed to the different washing modes of 30°C, 40°C, 60°C, and SW. All test results were obtained in triplicate.

Making Contaminated Cotton Sheets and Extracts From the Sheets After Laundry

Individual sections of cotton sheets (10 × 10 cm) were contaminated with extracts from *D farinae*, dog dander, and oak pollens. For HDM-, dog dander-, and pollen-contaminated cotton sheets, allergens were extracted from 50 g of *D farinae* culture media, 5 g of dog dander, and 20 g of oak pollen, respectively, with 500 mL of phosphate-buffered saline for 24 hours at 4°C. The cotton sheets were immersed in the allergen extraction buffers for 1 hour at 4°C and were dried at room temperature. The contaminated cotton sheets were stapled to pillows and were washed using the different washing modes of 30°C, 40°C, 60°C, and SW. After laundry, the remaining allergens were extracted from the contaminated sheets. The sheets were incubated with 10 mL of borate-buffered saline (170-mmol/L boric acid, 125-mmol/L sodium chloride, pH 7.4) buffer for 18 hours at room temperature using an orbital shaker.

Measurement of Remaining Allergens in Extracts From the Contaminated Cotton Sheets After Laundry

The concentrations of the remaining group 1 major allergen *D farinae* (Der f 1)¹³ and dog dander (Can f 1)¹⁴ in each extracted solution were measured by means of 2-site enzyme-linked immunosorbent assay (ELISA) (Indoor Biotechnologies, Manchester, England) following the manufacturer's instructions. Detection limits of Der f 1 and Can f 1

measurement were 2.0 ng/mL and 1.0 IU/mL, respectively. All results were obtained in triplicate. The remaining allergens were also measured by means of an ELISA inhibition test using atopic pooled serum samples to *D farinae* (n = 5), dog dander (n = 5), and oak pollen (n = 5). The ELISA microplates (Costar, Cambridge, Massachusetts) were coated with 10 µg/mL of extracted *D farinae*, dog dander, or oak pollen allergens in a 0.05M carbonate buffer (pH 9.6) overnight at 4°C. The 1:2 diluted pooled serum samples were preincubated with equal volumes of extraction solutions from the contaminated cotton sheets overnight at 4°C. After being washed with phosphate-buffered saline with Tween (PBS-T), each microplate well was blocked by means of incubation with 200 µL of 1% bovine serum albumin in PBS-T for 1 hour. Specific IgE (sIgE) detection was performed by incubating 50 µL of preincubated pooled serum for 1 hour at room temperature. After the solution was washed 3 times with PBS-T, 50 µL of 1:1,000 biotin-labeled goat anti-human IgE (Vector, Burlingame, California) was added, and it was incubated for 1 hour. After washing, the wells were incubated with 1:1,000 streptavidin-peroxidase (Sigma-Aldrich Corp, St Louis, Missouri) for 30 minutes. Colorimetric reactions were developed using 50 µL of 1mM 2,2 azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) in citrate buffer, pH 4.2, and 1:1,000 dilution of hydrogen peroxide for 5 minutes. The reaction was stopped by 50 µL of 2-mmol/L sodium azide, and optic densities were determined using a UV spectrophotometer (Dynatec, Alexandria, California) at 410 nM.

Statistical Analysis

Statistical analysis was performed using a software program (SPSS 9.0; SPSS Inc, Chicago, Illinois). One-way analysis of variance was used to evaluate changes in allergen concentrations during the laundry process. Only *P* < .05 was considered statistically significant.

RESULTS

Viability of D farinae After Mechanical Laundry Using Different Washing Modes

The viability of *D farinae* after mechanical laundry differed in the different water temperatures and washing modes. After the 30°C and 40°C washing modes, approximately 6.5% and 9.6% of *D farinae*, respectively, were dead. However, after the 60°C and SW washing modes, all 900 *D farinae* were dead, without exception.

Removal of D farinae Allergens From the Contaminated Cotton Sheet Using Different Washing Modes

Incubation of the extracted allergens at different water temperatures for 40 minutes did not affect Der f 1 and Can f 1 measurements (Fig 1). The mean (SEM) Der f 1 concentration in extracted solutions from the unmanipulated contaminated cotton sheet was 6.6 (1.0) µg/mL. The mean (SEM) concentration of Der f 1 in the extraction buffers obtained from 30°C washing without a rinse (1.7 [0.7] µg/mL; 26.8%

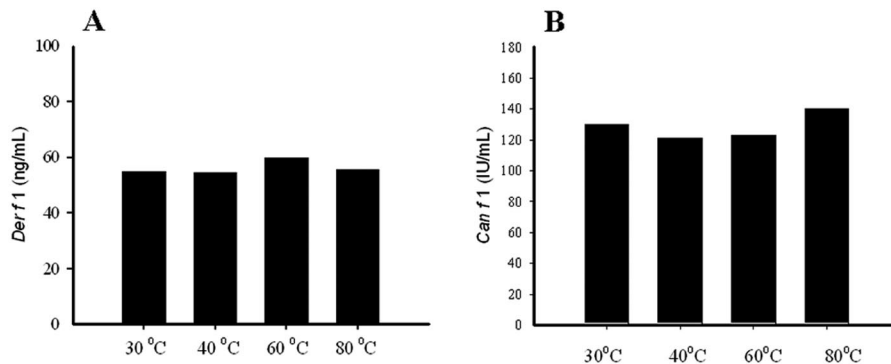


Figure 1. Effects of water temperature on the measurement of Der f 1 (A) and Can f 1 (B) levels by means of 2-site enzyme-linked immunosorbent assay.

of control sheets) was significantly higher than that from the 40°C (161 [19] ng/mL; 2.4%), 60°C (83 [14] ng/mL; 1.3%), and SW (42 [4] ng/mL; 0.6%) washing modes. The concentration of Der f 1 decreased as the number of rinses increased in the 30°C washing mode, but for the 40°C, 60°C, and SW modes, the effect of the number of rinses on the removal of Der f 1 was less apparent. The remaining Der f 1 concentrations in the extracts after washing with the 60°C or SW modes were significantly lower than those remaining after the 30°C and 40°C washing modes, even after rinsing 2 or 3 times (Fig 2). The ELISA inhibition test shows that the level of remaining *D. farinae* allergen immediately after washing was significantly lower in the extract after SW washing than after the other 3 modes. However, there was no difference in the percentage of ELISA inhibition among the 4 different washing modes after rinsing 2 times (Fig 3).

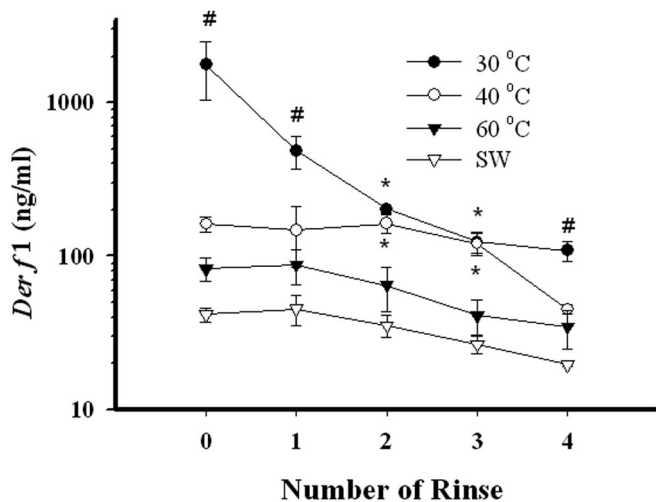


Figure 2. Concentrations of Der f 1 in extracts from contaminated cotton sheets after using different washing and rinsing conditions. The experiments were run in triplicate. * $P < .05$ compared with the other 3 washing modes. * $P < .05$ compared with the 60°C and steam water (SW) washing modes. Error bars represent SEM.

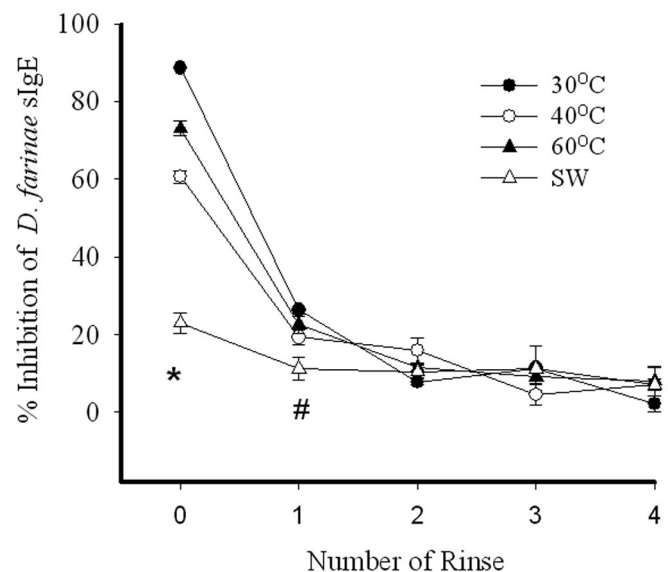


Figure 3. Enzyme-linked immunosorbent assay test for inhibition of *Dermatophagoides farinae* specific IgE (sIgE) measurement with extracts from contaminated cotton sheets after using different washing and rinsing conditions. * $P < .05$ compared with the other 3 washing modes. * $P < .01$ compared with the 60°C and steam water (SW) washing modes. Error bars represent SEM.

Removal of Dog Allergens From the Contaminated Cotton Sheet Using Different Washing Modes

The mean (SEM) concentration of Can f 1 in the extraction of unmanipulated contaminated cotton sheets was 1,950 (202) IU/mL. The amount of Can f 1 was markedly diminished by all the washing modes: 30°C (25.7 [1.9] IU/mL; 1.3%), 40°C (26.5 [1.8] IU/mL; 1.4%), 60°C (7.8 [2.2] IU/mL; 0.4%), and SW (6.1 [0.1] IU/mL; 0.3%). The Can f 1 levels immediately after the 60°C and SW washing modes were significantly lower than those after the 30°C and 40°C modes. However, Can f 1 numbers were below the detection limit after rinsing 2 times in all washing modes (Fig 4). No extracts from the 4 washing modes, even without a rinse, showed inhibited sIgE binding to dog dander allergens according to the ELISA

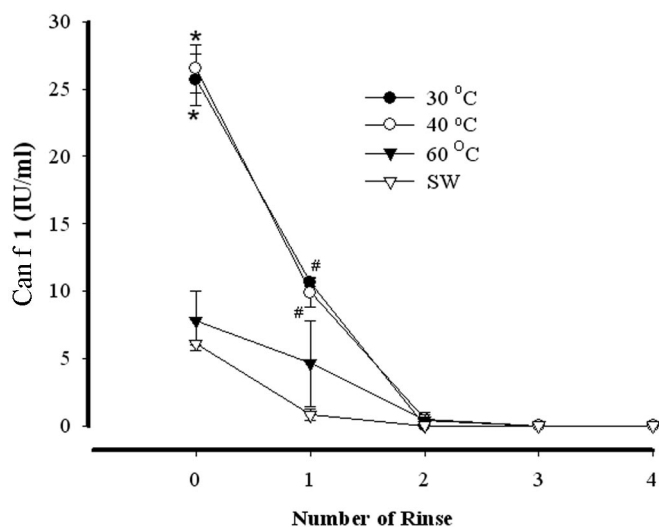


Figure 4. Concentrations of Can f 1 in extracts from contaminated cotton sheets using different washing and rinsing conditions. * $P < .01$ compared with the 60°C and steam water (SW) washing modes. # $P < .05$ compared with the 60°C and SW washing modes. Error bars represent SEM.

analysis, but the extract from the unmanipulated control sheets inhibited dog dander sIgE by a mean (SEM) of 72.9% (0.9%).

Removal of Pollen Allergens From the Contaminated Cotton Sheet Using Different Washing Modes

The remaining oak allergen levels in the extracts of washing without a rinse were different from those of the other washing modes. For the 30°C and 40°C washing modes, oak sIgE binding was inhibited by a mean (SEM) of 63.0% (0.9%) and 59.5% (2.2%), respectively, in ELISA, which was significantly higher than for the 60°C (12.6% [3.5%]; $P < .001$) and SW (1.5% [1.5%]; $P < .001$) modes. However, after rinsing once, there were no differences in the remaining oak allergen levels in all washing modes (Fig 5).

DISCUSSION

Pet and HDM allergens are often found in bedding and clothes,⁶ and laundry is an essential component of environmental control. In this study, we showed that allergens can be removed more effectively using the 60°C and SW washing modes than the 30°C and 40°C modes. The temperature of the water has been recognized as the most important factor in mechanical laundry for controlling HDMs. Water hotter than 55°C is sufficient for killing HDMs during washing.¹¹ In addition, at higher water temperatures, more HDM allergens can be removed from the laundry.^{11,12} This study agrees with previous studies concerning water temperature for killing HDMs. All HDMs were dead after the 60°C and SW modes were used, whereas only 6.5% and 9.6% of HDMs were dead after using the 30°C and 40°C washing modes, respectively. Although a previous study¹¹ showed that the sodium ion concentrations of detergents in the washing water may reduce

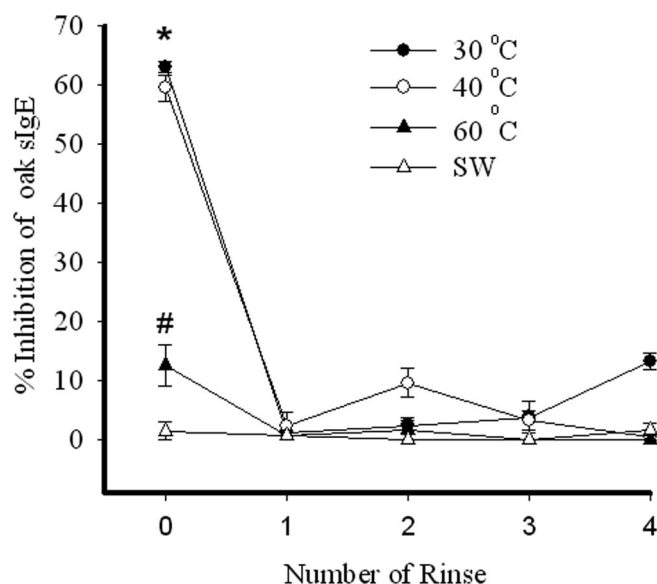


Figure 5. Enzyme-linked immunosorbent assay test for inhibition of oak specific IgE (sIgE) with extracts from contaminated cotton sheets using different washing and rinsing conditions. * $P < .01$ compared with the 60°C and steam water (SW) washing modes. # $P < .05$ compared with SW washing. Error bars represent SEM.

the killing of HDMs in hot water, we thought that a water temperature greater than 60°C is sufficient for killing HDMs. Water temperature was also important for the removal of HDM allergens in this study. The level of remaining HDM allergens after the 30°C washing was significantly higher than that after the other modes, which is consistent with a previous study.¹² Effects of rinsing were apparent only with the 30°C washing because the remaining level of HDM allergens was higher in the 30°C washing mode. The effect of rinsing for the removal of HDM allergens plateaued after 2 rinses, and there was little additional effect after that point. Most laundry machine programs adopt 2 or more rinses after washing, and this is sufficient for removal of the allergens. However, there are small differences, likely insignificant, in the efficiency of removing HDM allergens among laundry machines from different manufacturers (data not shown). For the removal of HDM allergens, the SW washing mode was better than the 30°C and 60°C modes. During the SW mode, the water temperature increases to 60°C owing to steam, and the temperature distribution in the laundry drum is more even.¹⁵ The SW washing mode has other advantages, including energy efficiency, damage to textiles, and water requirements for laundry,¹⁵ and laundry machines with the SW mode are now available in Korea, Japan, China, Europe, and the United States.

Dog dander allergens can be spread through contact with pet-contaminated clothes,⁷ and the laundry of contaminated clothes may be necessary for patients with dog dander allergies.¹⁶ Until now, there have been no data on

the removal of dog dander allergens by laundry machines. In this study, dog dander allergens were easily removed by the laundry machine. Although the remaining Can f 1 levels can be affected by water temperature and mode, no extracts of contaminated cotton sheets, even immediately after washing, inhibited sIgE binding to dog dander allergens in ELISA. The remaining percentage of Can f 1 in the extracts without rinsing was also much lower than that of Der f 1. Furthermore, the remaining Can f 1 levels were below the level of detection after rinsing 2 times in all washing modes. Few data are available regarding clothes contaminated with pollen,⁹ and the effects of different laundry conditions on the removal of pollen allergens have not been studied. The present study shows that pollen allergens can be easily removed by mechanical laundry, and after rinsing just once, no differences were found in the remaining pollen allergen levels among the 4 different washing modes.

Methods of evaluating the removal of allergens or killing of HDMs by laundry machines are different among researchers. Some investigators sprayed sampled dust to textiles for evaluation of the effects of removing allergens¹⁷ or extracted allergens from sampled dust in tubes.¹² In this study, we immersed cotton sheets in the extracts of the allergens and dried the sheets at room temperature. This method may contaminate the sheets more aggressively than the others. However, in real situations, the mechanisms of contamination of the clothes or bedding with allergens are very complex. Sometimes, allergens can be dissolved in sweat or saliva and contaminate clothing during everyday activities, and the present methods may reflect real situations. However, the fecal materials of HDMs may deposit simply onto clothing and can be easily removed by washing; for these situations, differences in the modalities or conditions of washing may be insignificant. Until now, there has been no proven method that reflects the real situation of contamination with allergens. In this study, we evaluated the remaining allergens by means of a 2-site ELISA method or an ELISA inhibition test. These 2 methods are widely used to measure allergens, but there are some differences. The ELISA inhibition test measured the whole allergenicity of the extracts, and the 2-site ELISA used in this study measured the binding activity of monoclonal antibodies to the major allergens. Some allergens are easily denatured by heat, and ELISA inhibition tests may reflect the effects of water temperatures on allergenicity. Human IgE epitopes of the group 1 major allergen of HDM, such as Der f 1, have been reported to be easily denatured by heat.¹⁸ However, the present data show that measurements of 2-site ELISA adapting murine IgG as capture or detective antibodies are not seriously affected by differences in water temperature in the range of 30°C to 80°C.

This study has some limitations. We did not evaluate the clinical effects of mechanical laundry on allergic patients, and the suggested beneficial effects of mechanical laundry

are speculative. Although there were differences in the remaining allergen levels among the different washing modes, we do not know whether the differences are clinically important, especially after rinsing 2 times.¹² Recent studies showed that to obtain clinical effects, the environmental control modalities should be multifaceted and tailored to the individual allergic patients¹ and that intervention in 1 aspect only did not produce clinical benefit.¹⁹ However, mechanical laundry has been recognized as an essential component of environmental control for allergic patients.⁵

In conclusion, this study shows that water temperature and number of rinses are important factors for the removal of HDM, dog dander, and pollen allergens. Rinsing is especially important for washing modes with low water temperatures.

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