Ultrafiltered dog allergen skin test compared with acetone precipitated and conventional dog: A retrospective study

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ABSTRACT

Background: *Various formulations of dog allergen extracts, including conventional dog (also known as dog epithelium) and acetone precipitated (AP) dog, have been used for skin-prick testing (SPT), with AP dog showing improved antigen content but experiencing stability issues due to precipitant formation. Ultrafiltered (UF) dog extract has been developed to address these concerns by offering comparable allergen content to AP dog. This study retrospectively compared UF dog with conventional dog and AP dog in SPT.*

Objective: *To compare the efficacy of UF dog extract with conventional dog and AP dog extracts in detecting dog sensitization through SPT.*

Methods: *Retrospective analysis of SPT results from a single U.S. allergy clinic was conducted. Tests performed between October 2022 and March 2024 were included. Primary and secondary outcomes were analyzed by using descriptive statistics and statistical tests.*

Results: *UF dog, AP dog, and conventional dog showed positivity rates of 24.2%, 23.5%, and 16.3%, respectively. UF dog demonstrated significantly higher average wheal and erythema sizes compared with conventional dog and AP dog, but UF dog was not statistically different from AP dog in terms of test positivity.*

Conclusion: *UF dog extract showed comparable number of positive tests to AP dog and a greater number of positive tests to conventional dog. Results of the study suggest UF dog as a viable alternative to AP dog, which offered improved stability and similar test responses. Further research with larger sample sizes is recommended to confirm these findings.*

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BACKGROUND

Skin-prick testing (SPT) has long been used in allergy clinics to establish dog allergen sensitization.[1,3] Dog allergen extract formulations in the United States have historically included nonstandardized dog epithelium (referred to here as conventional dog) and acetone-precipitated (AP) dog (Hollister-Stier, Jubilant HollisterStier LLC, Chicago, IL). AP dog is superior to conventional dog in detecting dog sensitization.[4–6] Can f 1 and Can f 3 are recognized as clinically relevant allergens, with Can f 1 being the major dog allergen. According to published technical reports and individual sample details, 1:100 wt/vol AP dog extract contains ~171 μg of Can f 1 and 40 μg of Can f 3 per mL, whereas 1:10 or 1:20 wt/vol conventional dog extract typically contains a maximum of 5–10 μg of Can f 1 and anywhere from 2 to 300 μg of Can f 3 per mL.[7,8] Of note, AP dog has been shown to form precipitants during storage, which results in wasted product.[7] A novel formulation, ultrafiltered (UF) dog allergen extract (1:650 wt/vol; Hollister-Stier) has been developed to reduce precipitant formation.[7] UF dog extract has higher levels of Can f 1 and Can f 3 (183 μg/mL and 70 μg/mL, respectively) when compared with AP dog.[7,8] Our study evaluated SPT results in a single center and compared UF dog, AP dog, and conventional dog extracts.

METHODS

SPT was performed to UF dog, AP dog, and conventional dog as part of standard diagnostic evaluations for allergic rhinoconjunctivitis, allergic asthma, or atopic dermatitis per the institution's standard aeroallergen testing panels. We included data from SPT performed between October 10, 2022, and March 1, 2024. A positive SPT result was defined as a wheal diameter ≥3 mm larger than the negative control.[2,9] All SPTs were performed by using Greer single-site picks (item no. GP-1; Greer Laboratories, Stallergenes Greer, Lenoir, NC) with valid positive and negative controls (6 mg/mL of histamine and 50% glycerin solution, respectively). Results were collected for UF dog (1:650 w/vol), AP dog (1:100 w/vol), and conventional dog (1:20 w/vol) for each test (conventional dog manufactured by Stallergenes Greer). The primary outcome was a SPT positivity rate. Secondary outcomes included wheal and erythema diameters in millimeters. We received institutional

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Table 1 **Characteristics by test**

	UF Dog (n = 153)	AP Dog (n = 153)	Conventional Dog (n = 153)	p, UF vs C. Dog*	p, UF vs AP Dog*	p, AP vs C. Dog*
Positivity (all tests), no. (%)#						
Positive	37 (24.2)	36 (23.5)	25 (16.3)	0.05	0.99	0.07
Negative	116 (75.8)	117 (76.5)	128 (83.7)			
Wheal (sensitized)§						
Mean ± SD	6.02 ± 5.47	5.96 ± 5.05)	3.71 ± 4.10	0.01	0.94	<0.01
Median (min, max)	4.50 (0, 27.0)	5.00 (0, 19.0)	3.00 (0, 20.0)			
Erythema (sensitized)§						
Mean ± SD	16.7 ± 14.0	16.0 ± 12.6	10.1 ±10.4	<0.01	0.67	0.01
Median (min, max)	12.0 (0, 50.0)	10.5 (0, 45.0)	6.00 (0, 40.0)			

UF = Ultrafiltered; AP = acetone precipitated; SD = standard deviation; min = minimum; max = maximum; C. Dog = conventional dog.
**The p values are from the paired t-test for continuous measures or the McNemar test for matched percent positive.*
#Overall positive and negative tests were included from all skin-prick tests.
§Wheal and erythema sizes are included from overall dog sensitized positive tests only.

review board exemption for this retrospective study by using existing testing data and secondary research classification with regard to individual patient consent. All data were deidentified before inclusion in the final analysis and were limited to the values discussed above.

Analyses included 153 tests that had available primary and secondary outcome measures for each extract. Descriptive statistics were reported for test positivity (binary) and for wheal and erythema diameters (mm) by test type. Measures from paired samples were compared by test type by using the McNemar test for test positivity and paired *t*-tests for continuous outcomes (wheal and erythema). Proportions positive were reported with their 95% exact binomial confidence intervals (CI). The primary analysis compared proportions positive in UF dog versus conventional dog by using generalized estimating equations with binomial variance distribution and logit link, allowing for dependence of paired binary measures within patients. Differences in proportions with their 95% CIs were reported by using estimated marginal means. Superiority of AP or UF versus conventional dog was established if the lower bound of the 95% CI for the difference in proportions positive (AP or UF dog − conventional dog) was >0. The noninferiority criterion was defined *a priori* as established if the lower limit of the 95% CI for the difference in proportions positive (UF dog − AP dog) was greater than the noninferiority margin of −0.07. Analyses were performed in R (version 3.6.3) by using the "gee". (gee: Generalized Estimation Equation Solver. R package version 4.13–20) and "emmeans". (emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.4.5).

RESULTS

Primary and secondary outcome measures are described by test type in Table 1. Proportions positive with their 95% CIs were 0.24 (95% CI, 0.18–0.32) for UF dog, 0.24 (95% CI,

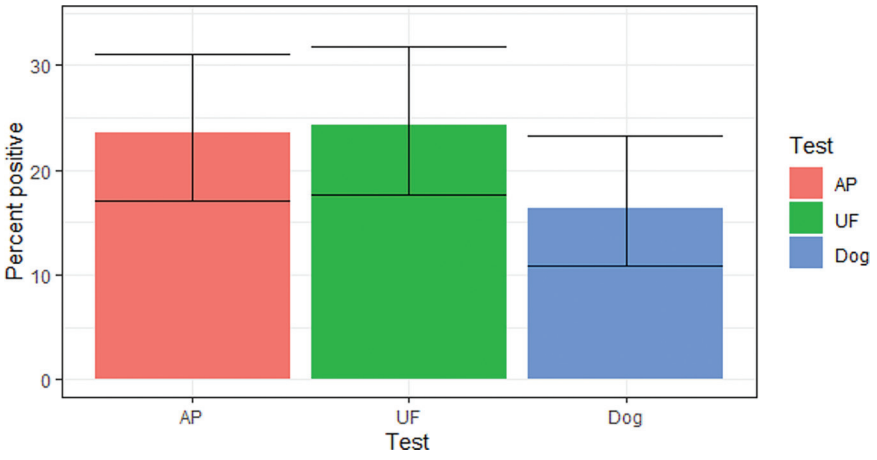

Figure 1. *Positivity by test. Overall positive test results for all three extracts. AP dog (red), UF dog (green), and conventional dog (blue, labeled as "Dog"). Error bars represent 95% exact binomial confidence intervals for the percent positive. AP = acetone precipitated; UF = ultrafiltered.*

0.17–0.31) for AP dog, and 0.16 (95% CI, 0.11–0.23) for conventional dog. Proportions of positive test results are shown in Fig. 1 and include all test results regardless of overall detected dog sensitization. The estimated difference in proportions positive in UF dog versus conventional dog (UF dog − conventional dog) was 0.08 (95% CI, −0.01 to 0.17), in AP dog versus conventional dog (AP − conventional dog) was 0.07 (95% CI, −0.02 to 0.16), and in UF dog versus AP dog (UF dog − AP dog) was 0.01 (95% CI, −0.09 to 0.10). With regard to the primary outcome, both UF dog and AP dog trended toward but did not achieve statistical superiority when compared with conventional dog. The p value for the difference in proportions positive was 0.05 and 0.07 for UF versus conventional dog and for AP dog versus conventional dog, respectively. The lower bounds of the CI for the difference in proportion for both UF versus conventional dog and AP versus conventional dog were <0, and so only approach statistical significance. UF dog approached but did not achieve the primary outcome of statistical noninferiority when compared with AP dog. Mean wheal and erythema diameters were larger in UF dog (6.02 mm × 16.7 mm) and in AP dog (5.96 mm × 16.0 mm) versus conventional dog (3.71 mm × 10.1 mm), with statistical significance ($p < 0.05$) for each comparison. No significant difference was noted between wheal and erythema diameter with UF dog versus AP dog. UF dog achieved a higher maximum wheal diameter (27 mm) versus AP dog (19 mm) and conventional dog (20 mm). UF dog also achieved a higher maximum erythema diameter (50 mm) versus AP dog (45 mm) and conventional dog (40 mm). UF dog and AP dog both had qualitatively higher test positivity versus conventional dog (24.2% and 23.5% respectively, compared to 16.3%), with these differences approaching statistical significance.

DISCUSSION AND CONCLUSION

To our knowledge, this is the first publication that detailed real-world experience with SPT to UF dog at a large academic center. The results in our study population suggest that UF dog yields positive SPT results at a rate similar to AP dog, whereas both UF dog and AP dog yield positive SPT results more frequently than did conventional dog. In addition, we show that both UF dog and AP dog demonstrate larger maximum wheal sizes when compared with conventional dog. Finally, UF dog achieves the largest maximum wheal and erythema size when compared with both AP dog and conventional dog. Given that this study did not include allergen challenges, no conclusive statements can be made from the data herein about the ability of each test to discriminate clinical allergy versus sensitization alone. In addition, it is unclear whether UF dog achieved the highest maximum wheal and erythema size due to a potentially higher content of Can f 1 and Can f 3 or if these results could be indicative of the presence of cross-reactive allergens to which the patient is sensitized. Therefore, UF dog could potentially yield higher sensitivity but lower specificity when compared with AP dog and conventional dog. Future study could include a comparison of cat and other mammal dander sensitization with that of UF dog, AP dog, and conventional dog to explore this concept of cross-reactivity further. Our data were limited by the lack of power to establish primary outcome statistical superiority or noninferiority among the extracts, and we suggest the need for larger studies to confirm our findings. However, our descriptive results align with reported quantities of major allergens across the different dog allergen extracts and suggest that practicing allergists can be reassured about comparable SPT performance of UF dog and AP dog.

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