# Characterization of Commercial Dog Allergen Extracts, Including New Ultrafiltered Dog Extract

**Shannon Brown, M.S.** and Melissa Austin, M.S. (Jubilant HollisterStier, Spokane, WA)

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## BACKGROUND

There are several FDA-approved dog allergen extracts indicated for allergy diagnostics and immunotherapy: conventionally-processed dog extracts from HollisterStier, Stallergens Greer, and ALK-Abello; Acetone Precipitated (AP) Dog Hair and Dander extract (HollisterStier); and a new Ultrafiltered (UF) Dog Hair and Dander extract (HollisterStier). Because of their unique manufacturing processes, both AP and UF Dog extracts are highly concentrated compared to the conventionally-produced dog extracts. While dog allergen extracts are not standardized products, measurements of Can f 1 major allergen content have been used to assess the strength of dog extracts.

## PURPOSE

The study objective was to characterize the available dog allergen extracts using commercially available and in-house analytical methods.

## METHODS

The following commercially available (US-licensed) dog allergen extracts were obtained from their manufacturers and analyzed using the following quantitative and qualitative methods.

- 1:100 w/v AP Dog Hair and Dander, HollisterStier (concentrated format)
- 1:650 w/v UF Dog Hair and Dander, HollisterStier (concentrated format)
- 1:10 w/v Dog Hair and Dander, HollisterStier (conventional format)
- 1:20 w/v Dog Epithelia, Stallergens Greer (conventional format)
- 1:20 w/v Dog Epithelium, ALK-Abello (conventional format)

Total protein was quantified using a commercially-available Bradford Assay (Thermo Scientific).

Can f 1 major allergen was quantified using a commercially-available sandwich ELISA (InBio). Reference standards were calibrated back to the original ST-CF1 standard to maintain consistency with data in published literature.

Can f 3 allergen (albumin) was quantified using a sandwich ELISA developed in-house. Reference standard: InBio, NA-CF3-1. Antibodies: Anti-Dog Can f 3, Bethyl Laboratories, A40-113.

Allergen profiles were qualitatively compared using standard SDS-PAGE and Western Blotting techniques and a plasma pool from dog-sensitive patients.

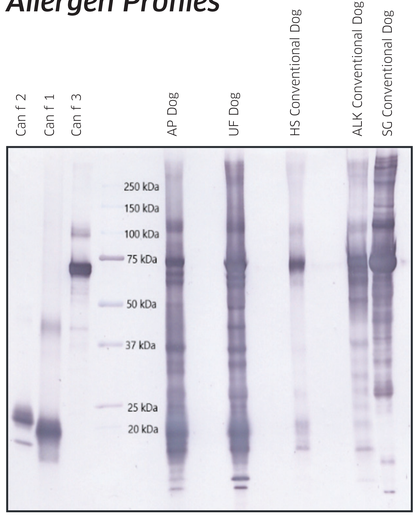
Compositional analysis was completed using Mass Spectroscopy (service provided by InBio). Protein and allergen content was identified via peptide mapping of sample spectra to allergen sequences provided by Uniprot and the International Union of Immunological Societies.

## RESULTS

| | Quantitative Assessment (µg/mL) | | | Qualitative Compositional Analysis Allergen Presence by Mass Spec | | | |
|---|---|---|---|---|---|---|---|
| **Dog Extract (Mfg)** | **Can f 1** | **Can f 3** | **Total Protein** | **Can f 1** | **Can f 3** | **Can f 4** | **Can f 6** |
| AP Dog Hair and Dander (HS) | 171 | 40 | 246 | ✓ | ✓ | ✓ | ✓ |
| UF Dog Hair and Dander (HS) | 183 | 70 | 412 | ✓ | ✓ | ✓ | ✓ |
| Dog Hair and Dander (HS) | 5-10 | 2 | 23 | ✓ | | | |
| Dog Epithelia (SG) | < 1 | 300 | 186 | | ✓ | | |
| Dog Epithelium (ALK) | 2 | 5-10 | 47 | ✓ | | | |

*Quantitative values are representative for each product; typically this is an average of several lots, if available. There is batch to batch variability around the values presented.*

### Allergen Profiles




## DISCUSSION

Other dog allergens recognized by the WHO/IUIS, including Can f 2, Can f 5, Can f 7, and Can f 8, were not detected by Mass Spec in any of the samples.

For protein profiling, HS and ALK conventional dog extracts were first concentrated before loading on the gel. This extra step was required in order to visualize the allergen bands because these extracts are too dilute, as provided. Samples of purified Can f 1, Can f 2, and Can f 3 allergens (InBio) were included for reference.

## CONCLUSIONS

Concentrated extracts (AP Dog and UF Dog) contain up to 35 times more critical Can f 1 major allergen and moderate amounts of Can f 3 allergen compared to conventionally-produced extracts. The concentrated extracts make it possible to easily achieve the 15 µg Can f 1 probable effective dose for immunotherapy.