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Specific allergen concentration of WHO and FDA reference preparations measured using a multiple allergen standard

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To the Editor:

Allergen measurements require well-defined allergen standards. Allergists rely on these measurements for dosing patients on immunotherapy with the aim of achieving maintenance doses of 5 to 20 μg of specific allergen that have been associated with clinical efficacy.[1] Allergists need to know that allergen measurements made by manufacturers are consistent and can reliably be used in clinical practice. Allergen measurements are widely used in the indoor air quality industry to assess exposure in homes, the workplace, schools, and commercial buildings. They are routinely used for assessing health risks associated with allergen exposure, for assessing the efficacy of allergen avoidance procedures, and for developing new allergen control products.[2]

Measurements of allergens by ELISA rely on standards of known allergen concentration, but few national or international allergen standards exist. The World Health Organization/ International Union of Immunological Societies (WHO/IUIS) Allergen Standardization Committee initiated a program to develop purified allergen standards for calibration of *in vitro* measurements. This initiative was funded by the European Union to develop certified reference materials for allergenic products ("Development of Certified Reference Materials for Allergenic Products and Validation of Methods for their Quantification," acronym CREATE). The aims of CREATE were to develop international reference materials with verifiable allergen content.[3,4]

Our goal was to apply the principles of allergen standardization developed in CREATE to other purified allergens. We developed a single "universal" allergen standard (UAS) for use in ELISA and in a fluorescent multiplex array for indoor allergens.[5] Purified proteins are essential in multiplex systems to reduce nonspecific interactions. Eight purified allergens (Der p 1, Der f 1, Der p 2, Fel d 1, Can f 1, Rat n 1, Mus m 1, and Bla g 2) were combined in the UAS. The protein concentration of the purified allergens was determined by amino-acid analysis, in keeping with CREATE. A detailed validation of the UAS and comparison with previous ELISA standards will be published elsewhere.[6] Here, we report the concentration

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of specific allergens in WHO/IUIS and US Food and Drug Administration (FDA) reference preparations using the single multiallergen standard.

Specific allergen concentrations of WHO/IUIS and FDA reference preparations were determined by using ELISA (Table I). The WHO/IUIS *Dermatophagoides pteronyssinus* standard 82/518 has been widely used as a standard for measurements of allergen exposure with a reported concentration of 12.5 μg Der p 1 per ampoule.[7] A value of 7.2 μg Der p 1 per ampoule was obtained by using the UAS, which is similar to estimates of 5 μg Der p 1 per ampoule reported previously.[8,9] The WHO/IUIS dog hair standard has an assigned potency of 100,000 IUs per ampoule and contained 20.4 μg Can f 1 per ampoule as determined by using the UAS.

There are no published reports of the specific allergen concentrations of US FDA reference materials. The 10,000 allergy units/mL *D pteronyssinus* references had similar concentrations of Der p 1 and Der p 2 in both the E8 and E10 standards (range, 20.3–27.6 μg/mL) (Table I). The 10,000 allergy units/mL *D farinae* references (E9 and E10) contained comparable levels of Der f 1 to the levels of Der p 1 seen in the *D pteronyssinus* references. It was not possible to use the Der p 2 standard in the UAS for the calibration of Der f 2 in *D farinae* references. The Der p 2 standard in the UAS underestimated the amount of Der f 2 by ~8-fold (data not shown). The Der f 2 concentration of 10,000 allergy units/mL *D farinae* references was determined by using a purified in-house Der f 2 standard (Table I). The Der f 2 concentrations of the E9 and E10 *D farinae* references were very similar. It was reassuring that the allergen levels in E8 and E9 (previous mite standards) were broadly similar to those of the current E10 standard. Overall, Group 1 and Group 2 allergen levels were comparable, and in some cases almost identical, in the FDA references for both mite species.

The FDA assigns potency to cat allergenic products based on the Fel d 1 content determined by radial immunodiffusion. Allergenic products containing 10 to 19.9 FDA units/mL of Fel d 1 have an assigned potency of 10,000 bioequivalent allergy units/mL.[10] The Fel d 1 values in units of 2 of the FDA references (E4 and E5 hair) were outside of the recommended range. The Fel d 1 concentrations in micrograms per milliliter of the FDA cat references (E4 and E5) determined by ELISA using the UAS were consistent with the results obtained by radial immunodiffusion (Table I).

Our data confirm that the Der p 1 level of 12.5 μg per ampoule assigned to the WHO/IUIS *D pteronyssinus* standard (National Institute for Biological Standards and Control 82/518) is an overestimate and that the actual value is in the range of 5 to 7 μg per ampoule.[8,9] The data show that 1 unit Can f 1 in the WHO/IUIS dog hair extract (National Institute for Biological Standards and Control 84/685) corresponds to 5 ng Can f 1 and that 1 FDA unit Fel d 1 is ~1 μg Fel d 1. Values obtained by using previous standards have been applied to many studies involving thresholds or guidelines for exposure levels that result in allergic sensitization or symptom exacerbations.[6] At this point, we do not believe that revision of such guidelines in the absence of further epidemiologic data would be worthwhile. Rather, future studies should incorporate purified allergen standards into the exposure assessment to generate a body of data based on standards that fulfill the CREATE principles.

Dosing of immunotherapy based on measurements of specific allergens plays an increasing role in allergy practice.[1] Although several US allergen manufacturers routinely measure specific allergens in their immunotherapy products, these measurements are not based on a national standard and may not be directly comparable. Our data provide the specific allergen concentrations in mite and cat allergen reference preparations used by the FDA for potency testing of all US standardized allergenic products. This provides a mechanism for standardizing allergen measurements in US products that allergists use for immunotherapy.

The results suggest that the approach of using multiallergen standards could be extended to other sources, for example, pollens, molds, and foods, where purified allergens are available. It is critically important that regulatory agencies generate purified natural or recombinant allergen standards that researchers and companies can use as reference preparations. Two of the allergens used in CREATE, Bet v 1 and Phl p 5, are being formulated as biological reference preparations by the European Directorate for the Quality of Medicines.[4] ELISA tests for each allergen are also being validated to produce certified ELISA products that can be used for analytical purposes. Once completed, the biological reference preparations will be incorporated into the European Pharmacopoeia and will become international standards for licensing of allergenic products. Our data demonstrate the feasibility of applying CREATE principles to other allergens and underline the need for validated purified allergens and assays to be developed and maintained by standardization agencies and regulatory authorities.

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TABLE I

Allergen concentration of reference standards measured by ELISA using the UAS

	Units	Allergen	Concentration
WHO-IUIS standards [*]			
Dermatophagoides pteronyssinus 82/518	100,000 IU/ampoule	Der p 1	7.2 μg/ampoule
Dog hair 84/685	100,000 IU/ampoule	Can f 1	20.4 μg/ampoule [†]
FDA standards [‡]			
D pteronyssinus E8	10,000 AU/mL	Der p 1	20.3 μg/mL
D pteronyssinus E10	10,000 AU/mL	Der p 1	27.6 μg/mL
D pteronyssinus E8	10,000 AU/mL	Der p 2	23.4 μg/mL
D pteronyssinus E10	10,000 AU per mL	Der p 2	23.5 μg/mL
D farinae E9	10,000 AU/mL	Der f 1	19.9 μg/mL
D farinae E10	10,000 AU/mL	Der f 1	29.2 μg/mL
D farinae E9	10,000 AU/mL	Der f 2 [§]	19.2 μg/mL
D farinae E10	10,000 AU/mL	Der f 2 [§]	15.0 μg/mL
Cat hair E4	10,000 BAU/mL or 7.0 Fel d 1 units/mL	Fel d 1	5.4 μg/mL [‖]
Cat pelt E4	10,000 BAU/mL or 14.8 Fel d 1 units/mL	Fel d 1	15.6 μg/mL [‖]
Cat hair E5	10,000 BAU/mL or 22.0 Fel d 1 units per mL	Fel d 1	24.8 μg/mL [‖]

BAU, Bioequivalent allergy unit.

[*] Mean of duplicate determinations.

[†] Based on these data, 1 IU Can f 1 = ~5 ng Can f 1.

[‡] Mean data from measurements by 2 operators on 3 separate occasions. The values for Fel d 1 units in the cat hair and pelt standards were determined by radial immunodiffusion by the FDA (data kindly provided by Cherry Valerio, FDA Center for Biologics Evaluation and Research).

[§] Determined using a separate in-house natural Der f 2 standard with a concentration of 1.25 mg/mL by amino acid analysis.

[‖] 1 FDA unit Fel d 1 = ~1 μg Fel d 1.