

Quality Control of House Dust Mite Extracts for Allergen Immunotherapy

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Dear Editors,

Casset et al. [1] have recently investigated the allergen composition of commercially available *Dermatophagoides pteronyssinus* extracts from different European manufacturers. Specifically, this study concluded that (1) commercial extracts available from different manufacturers contain varying amounts of Der p 1 and Der p 2 major allergens, and often lack detectable Der p 5, 7, 10 and 21 minor allergens, and (2) such variations in allergen composition and content can affect diagnostic skin test results.

Whereas aqueous allergen extracts have been commonly used for decades for immunotherapy and diagnostic purposes, a well-known challenge for manufacturers is to guarantee a consistent quality for those extracts administered to humans. As pointed out by Casset et al. [1] and others [2], this is specifically difficult for house dust mite extracts, as they contain high endogenous levels of proteases which may degrade the allergens under poor storage conditions. Also, the allergen composition (i.e. the spectrum of major and minor allergens) is known to vary depending upon both the nature of the source materials used to prepare the extracts (e.g. mite bodies vs. whole mite cultures) and the culture process [3, 4]. This challenge is made even more complex when considering that an

ideal product for house dust mite immunotherapy should combine both *D. pteronyssinus* and *D. farinae* extracts.

In this context, we would like to draw attention to the integrated and multi-pronged approach that we have implemented at Stallergènes, to ensure pharmaceutical-grade quality, as well as batch-to-batch consistency of mite extracts intended for human administration. As summarized in table 1, multiple quality checks are routinely performed in compliance with regulatory guidelines [5] on both the source materials (i.e. cultures obtained from *D. pteronyssinus* and *D. farinae*), the aqueous allergen extracts used as active substances, and the finished products (which encompass both diagnostic pricks, solutions for subcutaneous or sublingual immunotherapy, or sublingual tablets).

To ensure extract consistency, source materials are obtained from cultures of each of the two mite species in a well-controlled medium combining wheat germ, yeast and synthetic amino acids (the latter in proportions which recapitulate the composition of the human stratum corneum) [6]. Those culture conditions have been rigorously established for each of the mite species to support optimal growth as well as high allergenic activity and major allergen content [6]. Our source materials are

prepared from whole mite cultures and thus comprise both bodies and fecal particles. The rationale supporting this approach is to obtain extracts whose allergen composition and content mimick natural exposure conditions. The characterization of such *D. pteronyssinus* and *D. farinae* source materials using a combination of proteomic approaches (i.e. 2D gel analysis and mass spectrometry-based protein sequencing, as well as immunoblotting with allergen-specific antibodies) confirmed unambiguously the presence of a broad spectrum of both major and minor allergens in these cultures (table 1). On a quantitative basis, we also confirmed that allergens belonging to groups 1, 2, 7, 10, 13 and 20 were among the 50 most abundant proteins present in *D. pteronyssinus* source materials [6]. Importantly, such defined culture conditions have as of today been proven to ensure batch-to-batch consistency over several hundreds of independent industrial lots, with only a limited variability (i.e. $\pm 30\%$) in terms of allergenic activity as well as group 1 and group 2 contents, which is well acceptable for such complex biological products.

In addition, both allergen extracts and finished products are also tested for allergenic activity (by ELISA inhibition) and major allergen content (using commercial

Table 1. Quality control of house dust mite extracts during the various manufacturing steps

Source materials	Allergen extracts (drug substance)	Finished products
– Development of standardized culture conditions in a pharmaceutical-grade culture medium (Stalmite™) for both <i>D. pteronyssinus</i> and <i>D. farinae</i> – Batch release according to allergenic activity (IR/ml) – Batch release according to quantification (µg/g protein) of Der p 1, Der p 2 content (<i>D. pteronyssinus</i>) or Der f 1, Der f 2 content (<i>D. farinae</i>) – Confirmation by immunoblot and mass spectrometry analysis of the presence of minor allergens (groups 3–10, 13–15, 18, 20 and 21 for <i>D. pteronyssinus</i> and 3, 5, 7, 9, 13, 14 and 20–22 for <i>D. farinae</i>) – Batch release following comparison of protein profiles (electrophoretic methods) with IHRS	– Batch release according to allergenic activity (IR/ml) – Batch release according to quantification (µg/g protein) of Der p 1, Der p 2 content (<i>D. pteronyssinus</i>) or Der f 1, Der f 2 content (<i>D. farinae</i>) – Batch release following comparison of protein profiles (electrophoretic methods) with an IHRS	– Batch release according to allergenic activity (adjusted in comparison with an IHRS) – Major allergen content (groups 1 and 2) – Sensitivity and specificity studies in human patients (for pricks) – Clinical studies, postmarketing studies and pharmacovigilance (for subcutaneous and sublingual immunotherapeutic products)

IHRS = In-house reference standard.

ELISAs) with acceptance criteria set up for these parameters. A well-characterized allergen extract (with its potency measured by titrated skin reactivity evaluated in patients) is used as an in-house reference standard to determine the total allergenic activity of each commercial extract intended for diagnosis or immunotherapy.

Lastly, a critical step in documenting the quality of mite finished products is

their evaluation in the context of multiple clinical studies. For example, sensitivity and specificity studies have been conducted in mite-allergic patients with both *D. pteronyssinus* and *D. farinae* pricks. Also, the safety and efficacy of our mite extracts used for subcutaneous or sublingual immunotherapy have been established by dedicated clinical studies in large cohorts of patients [7, 8] and confirmed by practi-

tioners who have used these products for many years.

In summary, we recognize the need of ensuring the consistency of house dust mite extracts used for diagnosis and immunotherapy purposes. Applying pharmaceutical quality standards to each of the steps needed to develop and manufacture such allergen extracts is, in our opinion, the best guarantee to achieve this goal successfully.

References

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