

How Allergen Extracts Are Made—From Source Materials to Allergen Extracts

Mite allergen extracts and clinical practice

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ABSTRACT

Objective: To provide physicians, researchers, and other interested health care professionals with information about how mite source materials and allergen extracts are manufactured, including the critical process parameters that can affect the final composition of allergenic extracts available for clinical use.

Data Sources: A PubMed search was performed using focused keywords combined with relevant regulatory documents and industry guidelines.

Study Selections: The information obtained through literature and specialized books was evaluated and combined with the personal expertise and experience of the authors.

Results: *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* are the primary species responsible for allergen sensitizations and allergy symptoms in genetically predisposed individuals. Storage mites belonging to the families Glycyphagidae, Echimyopodidae, and Acaridae can also be relevant sources of indoor mite allergens. The cultivation and purification processes used to produce mite raw materials play a critical role in the final composition of mite allergen extracts. Mite extract standardization in the United States is based on total allergenic activity with respect to a single national standard, whereas in Europe consistency is ensured by in-house standards and international references. Because of the limitation of allergen avoidance and pharmacotherapy for patients with severe allergic rhinitis and asthma, house dust mite subcutaneous immunotherapy or sublingual immunotherapy can be an invaluable treatment option for them.

Conclusion: Differences in manufacturing processes and extract standardization approaches may lead to differences in extract quality and potency. Physicians should be aware of these potential sources of mite extract variability. Use of well-standardized house dust mite extracts would be critical for success in the diagnosis and treatment of house dust mite allergy.

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Introduction

House dust mites (HDMs) are a predominant source of indoor allergens throughout the world.¹ HDMs are present in human dwellings, where they feed on human skin scales and other organic debris, and are especially abundant in mattresses, sofas, carpets, and other porous materials. HDMs cause allergic rhinoconjunctivitis and allergic asthma and contribute to atopic eczema and other allergic skin diseases.² For example, up to 50% of individuals with asthma are sensitized to mites.³ Mite allergens are found in the indoor environment throughout the year. Although exposure to mite allergens is considered to be perennial, symptoms caused by mite allergens may be more prevalent at certain times of the year,

depending on the geographic location and the fluctuations in temperature and relative humidity (RH) throughout the year in non-air-conditioned houses.

House dust has been considered a causative factor for allergic symptoms for almost a hundred years. However, it was not until 1920 that mites, which thrive in domestic environments, were linked to allergic diseases.^{4,5} In the 1960s, various surveys identified high numbers of the genus *Dermatophagoides* in homes, which suggested their potential relevance as a source of indoor allergens contained in house dust. Since then, other domestic mite species, which sensitize and cause allergic symptoms, have been described. Different allergens contained in various species have been purified, sequenced, and cloned (Table 1).

Mite allergen extracts are essential to diagnose and treat mite allergy. To produce allergen extracts, mites are first cultured and grown in large quantities under specific and controlled conditions in appropriate culture media. Proper conditions to grow mites are

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Table 1Taxonomy, Distribution, and Number of Allergens Included in the IUIS Database for the Mite Species That Have Been Described as Allergenic^a

Order	Suborder	Superfamily	Family	Species	Distribution	Allergens identified	No. of allergens in IUIS
Trombidiformes	Oribatida (Hyporder: Astigmata)	Acarioidea	Acaridae	<i>Acarus siro</i>	Worldwide	Yes	1
				<i>Aleuroglyphus ovatus</i>	Worldwide	Yes	
				<i>Thyreophagus entomophagus</i>	Worldwide	No	
				<i>Tyroborus lini</i>	Worldwide	No	
				<i>Tyrolichus casei</i>	Worldwide	No	
				<i>Tyrophagus putrescentiae</i>	Worldwide	Yes	8
				<i>Suidasia pontifica</i> (= <i>medanensis</i>)	Worldwide	Yes	
				<i>Chortoglyphus arcuatus</i>	Worldwide	Yes	1
				<i>Blomia kulagini</i>	Europe, Asia	Yes	
				<i>Blomia tjbodas</i>	Europe	No	
		Glycyphagoidea	Chortoglyphidae	<i>Blomia tropicalis</i>	Pantropics and subtropics	Yes	13
				<i>Austroglycyphagus malaysiensis</i>	Asia	No	
				<i>Glycyphagus domesticus</i>	Worldwide (high latitude)	Yes	
				<i>Gohieria fusca</i>	Worldwide	Yes	
				<i>Lepidoglyphus destructor</i>	Worldwide (high latitude)	Yes	
			Pyroglyphidae	<i>Dermatophagoides farinae</i>	Worldwide	Yes	28
				<i>Dermatophagoides microceras</i>	Northern hemisphere	Yes	
				<i>Dermatophagoides pteronyssinus</i>	Worldwide	Yes	
				<i>Dermatophagoides siboney</i>	Caribeann	Yes	
				<i>Euroglyphus maynei</i>	Cosmopolitan	Yes	
			Glycyphagidae	<i>Malayoglyphus intermedius</i>	Worldwide	No	5
				<i>Pyroglyphus</i> (= <i>Hughesiella</i>) <i>africanus</i>	Pantropics and subtropics?	No	
				<i>Sturnophagoides brasiliensis</i>	Pantropics and subtropics	No	
				<i>Cheyletus eruditus</i>	Worldwide	No	
		Analgoidea	Pyroglyphidae				
		Prostigmata	Cheyletoidea	Cheyletidae			

Abbreviation: IUIS, International Union of Immunological Societies.

^aTaxonomy is based on Zhang Z-Q, Fan Q-H, Pesic V, et al. Order Trombidiformes Reuter, 1909. *Zootaxa*. 2011;3148:129–138.

required to guarantee a suitable allergenic composition of the final product. Immunochemical characterization of each lot is essential to demonstrate lot-to-lot consistency of mite extracts.

More than 95% of allergen immunotherapy practiced in the world is administered in the United States and Europe, and mite immunotherapy represents approximately 50% of the total volume of marketed vaccines mainly of the genus *Dermatophagoides* (Laboratorios LETI, S.L., Madrid, Spain, unpublished data, 2015).

The purpose of this article is to provide physicians, researchers, and other interested health care professionals with information about how mites are cultured and processed and how allergen extracts are manufactured to diagnose and treat individuals sensitized to mites. The article also compares the different available mite allergen extracts.

Classification of Clinically Relevant Mite Species and Allergens

The main species of HDM belong to the family Pyroglyphidae and include *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Euroglyphus maynei* (Table 1). These mites are abundant worldwide, and differences in their morphologic characteristics and biology can influence their distribution. For example, *D farinae* is more common than *D pteronyssinus* and *E maynei* in dry regions.

Other clinically relevant mites are the so-called storage mites (SMs), primarily species belonging to the Glycyphagidae, Acaridae, and Echimyopodidae families. Individuals exposed to SMs in occupational settings or through consumption of food contaminated with these mites can become sensitized to them and experience allergic symptoms. The most studied species belonging to the Glycyphagidae and Acaridae families are *Lepidoglyphus destructor*, *Glycyphagus domesticus*, *Tyrophagus putrescentiae*, and *Acarus siro*. *Blomia tropicalis*, a member of the Echimyopodidae family, can be abundant in both agricultural environments and house dust in tropical and subtropical areas. Other species of

Blomia, including *Blomia kulagini* and *Blomia tjbodas*, have been identified in temperate regions of the world.⁶

HDMs and SMs have different life cycles that influence the natural dynamics of their populations, which affects their capacity to be cultured. HDMs have lower fecundity compared with that of SMs and a longer generation time; they thrive in stable environments with a constant food source, even surviving under unfavorable conditions into adulthood. In contrast, SMs are fast-maturing species with high fecundity and increase their populations quickly under optimal conditions. However, when the conditions deteriorate, they fail to survive, resulting in a high mortality rate.⁷

Eighty-two mite allergens derived from 10 species have been identified to date. They have been classified into 36 groups according to the similarity of their amino acid sequence and biochemical functions (Table 2). Many mite allergens are enzymatically active (eg, peptidase, glycosidases, and transferases). Others mite allergens are lipid-binding proteins or structural proteins. Although many are described as major allergens, not all of them contribute equally as a cause of IgE-mediated disease. Therefore, some authors use the term *serodominance* to describe the clinical relevance of each allergen.⁹ The serodominant allergens for HDMs are groups 1, 2, and 23, and the minor allergen groups include 3, 4, 5, 6, 7, 8 through 11, 13, 15 through 18, 20, and 21. The allergenicity of other groups of allergens (14, 22, 24–33) has not been determined to date. However, this classification should be put into appropriate context because allergens considered as minor could be clinically relevant, depending on geographic variations, environmental conditions, or allergic manifestations. For example, group 11 is considered to be relevant in atopic dermatitis.¹⁰

Source materials to prepare mite allergen extracts are obtained from inactivated mite cultures and can include different mite components (eg, fecal pellets, mite bodies, mite parts, egg cases, and skin casts). Different mite allergens vary in origin. Many are digestive enzymes present in salivary glands and gut cells that

Table 2Mite Allergen Groups Included in the IUIS, Biochemical Name, and Function of Species of Mites^a

Group	Molecular weight, kDa	Biochemical	Mite species	Localization into the mite	Localization into culture fractions	Prevalence of major or minor allergen
1	24–39	Cysteine protease	DP, DF, DM, EM, BT	Gut	Feces or bodies	Major
2	14	ML domain protein	DP, DF, EM, BT, LD, GD, TP	Gut or other cells	Feces or bodies	Major
3	25	Trypsin	DP, DF, EM, BT, TP	Gut	Feces or bodies	Major or minor
4	57	Amylase	DP, EM BT	Gut	Feces or bodies	Minor
5	15		DP, BT, LD	Gut		Major or minor
6	25	Chymotrypsin	DP, DF, BT	Gut	Feces or bodies	Minor
7	25–31	Similar lipopolysaccharide-binding protein, bactericidal permeability increasing family	DP, DF, LD		Feces or bodies	Major or minor
8	26	Glutathione-S-transferase	DP, DF, BT	Other cells	Bodies	Major
9	30	Collagenolase	DP	Other cells	Feces or bodies	Major
10	37	Tropomyosin	DP, DF, BT, LD, CA, TP	Muscle	Bodies	Minor
11	96	Paramyosin	DP, DF, BT	Muscle	Bodies	Major or minor
12	14		BT	Other cells		Major
13	15	Fatty acid binding protein	DF, BT, LD, TP, AS	Other cells	Bodies	Minor
14	177	Apolipoprotein, lipid transfer protein	DP, DF, EM	Other cells	Bodies	Major
15	63–105	Chitinase	DP, DF	Gut	Feces or bodies	Major
16	55	Gelsoline, viline	DF	Other cells	Bodies	Minor
17	53	EF hand protein, calcium-binding protein	DF	Other cells		Minor
18	60	Chitin binding	DP, DF	Gut	Feces or bodies	Major
19	7	Péptido antimicrobiano	BT	Gut		Minor
20	40	Arginine kinase	DP, DF		Bodies	Minor
21	16	Group 5 homologue, hydrophobic binding?	DP, DF, BT	Gut	Bodies	Major
22	17	MD-2-like protein, lipid binding?	DF		Feces or bodies	
23	8	Peritrophin	DP	Gut	Feces or bodies	Major
24	13	Ubiquitinol-cytochrome c reductase binding protein	DP, DF	Other cells		Major
25	34	Triosephosphate isomerase	DF	Other cells?	Feces or bodies	Major
26	18	Myosin alkali light chain	DF	Other cells?	Bodies	Minor
27	48	Serpin	DF	Gut	Feces or bodies	Minor
28	70	Heat shock protein	DF, TP	Gut or other cells	Feces or bodies	Major
29	16	Cyclophilin	DF	Gut or other cells	Feces or bodies	Major
30	16	Ferritin	DF	Other cells	Bodies	Major
31	15	Cofilin	DF	Other cells	Bodies	Minor
32	35	Secreted inorganic pyrophosphatase	DF	Other cells	Bodies	Minor
33	52	α -tubulin	DF	Other cells	Bodies	Minor
34	18	Troponin C, calcium-binding protein	TP			Minor
35	52	Aldehyde dehydrogenase	TP			Major
36	14	Profilin	TP			Major

Abbreviations: AS, *Acarus siro*; B, body fraction; BT, *Blomia tropicalis*; CA, *Chortoglyphus arcuatus*; DF, *Dermatophagoides farinae*; DM, *Dermatophagoies microceras*; DP, *Dermatophagoides pteronyssinus*; EM, *Euroglyphus maynei*; F, fecal fraction; GD, *Glycyphagus domesticus*; IUIS, International Union of Immunological Societies; LD, *Lepidoglyphus destructor*; TP, *Tyrophagus putrescentiae*.

^aLocalization of the allergens into the mite is based on Henmar et al.⁸ Presence in mite culture fractions is based on Müsken et al.⁶ and Henmar et al.⁸ Prevalence of sensitization is based on the IUIS allergen database.

concentrate in fecal pellets. Other allergens, such as structural proteins, are present in muscle cells. Consequently, the concentration of specific allergens in a given allergen extract depends on the mite source materials used to produce it. For example, HDM fecal extracts are rich in group 1, 3, 6, 18, and 23 allergens. In addition, these extracts contain other clinically relevant allergens, including those classified in groups 2 through 4, 7, 9, 15, 22, 25, and 27 through 29. However, the levels of group 8, 10 through 14, 16, 20, 21, 26, and 30 through 33 allergens in fecal pellet extracts are low. In contrast, extracts derived from whole mite bodies contain all mite allergen groups in different proportions.¹¹ Therefore, fecal and whole body extracts have selective antigenic and allergenic profiles, as well as diverse enzymatic activity.¹²

Mite Cultures to Prepare Allergen Extracts

In addition to the type of source material used to prepare mite allergen extracts, other parameters can have an effect on mite cultures and contribute to the variability of the final source material used to prepare mite allergen extracts. This is illustrated by the heterogeneity of the allergen content and composition of marketed products, even those produced by the same manufacturer.¹³ Therefore, it is necessary to monitor and optimize the processes associated with the production of mite source materials to the best

possible extent to ensure the quality and consistency of mite allergen extracts.

An outline of the mite production process is illustrated in Figure 1.

Culture Medium

HDMs were initially cultured using their natural food source, human skin scales.¹⁴ Subsequently, alternative culture media were developed, including animal skin scales, dried daphnia, ox liver, fish food flakes, dog food, rodent chow, wheat germ, and fungal cultures,¹⁵ usually with yeast as a supplement.¹⁶ The manufacturers of mite allergen extracts currently use a mix of different food sources to culture mites, which include pork liver, brine shrimp eggs, wheat germ, or yeast.¹⁷ Another approach is to use vitamin¹⁸ or amino acid supplements,¹⁹ which resemble the composition of the human stratum corneum. European and US guidelines^{20,21} indicate that the mite culture media should not contain any components that could potentially be allergenic. The presence of human or animal products in the culture medium needs to be appropriately justified to ensure that it does not contain any potential pathogens; γ -radiation can be used to treat the culture media to support their use.

Numerous studies indicate that the mite diet directly influences the growth rate of mite populations and the allergenic composition

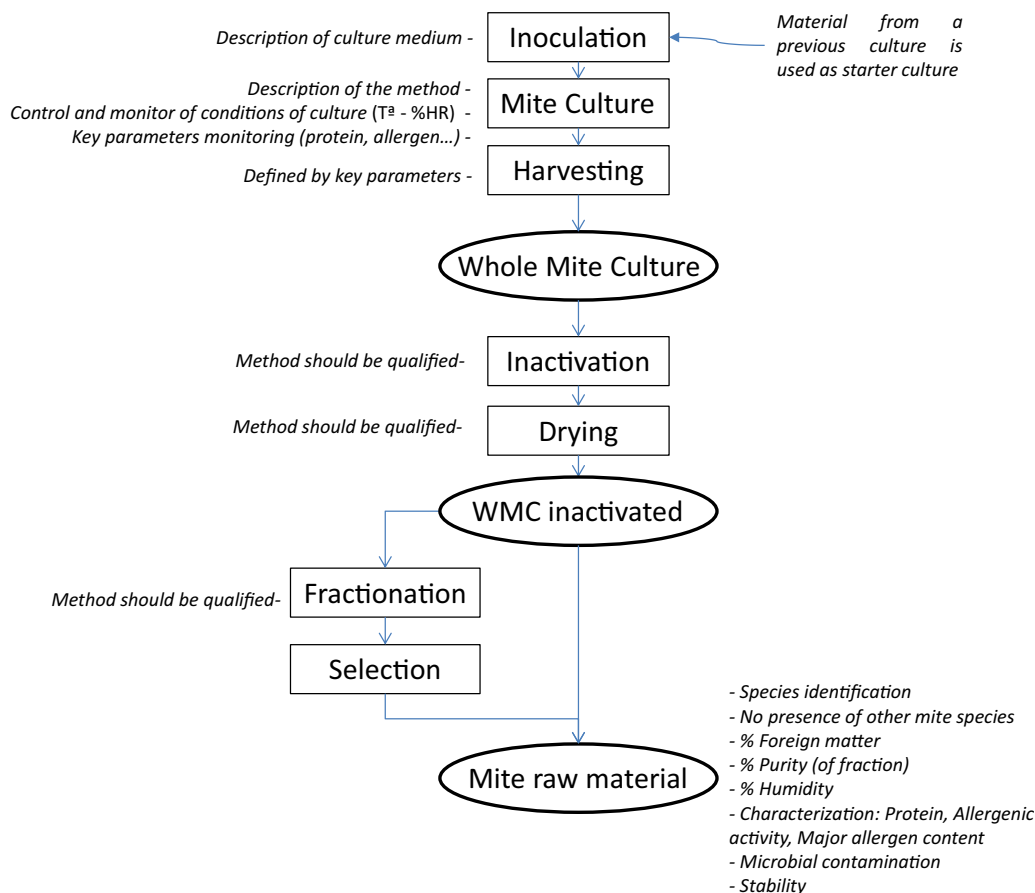


Figure 1. Flowchart of steps involved in the production of mite source material.

of the mite cultures.²² Therefore, it is essential to use a proper medium to grow mites.

Culturing Method and Critical Parameters

The most common method to culture mites is to inoculate flasks that contain growth medium with starter cultures. The flasks should be appropriately ventilated but also adequately sealed to prevent mites from escaping and to maintain axenic cultures. Precautions should be taken to avoid contamination by other mite species, especially when they are being cultured in the same facility. This can be accomplished by culturing different mite species in separate rooms or areas within a facility.

Although some mite species are cultured under unique conditions for optimal growth, general conditions (eg, 20°C–30°C and 70%–80% RH) are adequate to grow HDMs and most SMs. Higher temperatures (>30°C) and RH (>80% RH) can accelerate the growth of mite populations but also favor fungal growth, which may be detrimental to mite survival. Ideally, particular culture parameters, especially RH²³ and temperature,²⁴ should be determined for each mite species because they directly influence the growth of the mite population and the allergen content of the final allergen extract.

Mite cultures have a typical growth pattern. The initial phase is characterized by a latency period followed by an exponential growth period, resulting in a maximum population of mites. The final phase consists on a rapid decrease in the mite population with a high mortality rate. The immunologic characteristics of the culture, including the types and levels of particular allergens²⁵ and enzymatic activity,²⁶ vary, depending on the phase. Hence, the culture conditions and the time to harvest the mites should be

specified for each mite species. Key parameters for monitoring mite populations and deciding when to harvest include the protein content, allergenic activity, and/or major allergen levels.

Inactivation: Drying

Before mite cultures are harvested, they have to be inactivated to retain their immunologic properties and kill live mites. The most common process used to do so is freezing the cultures below –20°C for at least 24 hours. Usually, inactivated mite cultures are dried under controlled conditions to reduce their moisture content. Whatever the case, the methods used to inactivate and dry the mites should be described and controlled.

Purification: Obtaining Fractions

Because a mite culture is a mixture of mite bodies, eggs, cast skins, fecal pellets, and residual materials from the culture medium, different culture fractions can be obtained using various techniques (Fig 2). The method used by most manufacturers is mechanical sieving⁸ with different pore sizes to separate culture components based on particle size. The objective of the procedure is to obtain fractions enriched with particular types of mite material. In this way, fractions composed primarily of mite bodies (90–350 µm) and fecal particles (<50 µm) can be obtained and saved for subsequent extraction. Other methods to separate different fractions include the use of air classifiers, which separate materials according to their size and density, or the heat-escape method, which collects living mites escaping from cultures exposed to low heat.¹⁵ Another approach is to use the whole mite culture as a source material,²⁷ with the idea that this extract resembles the natural exposure to

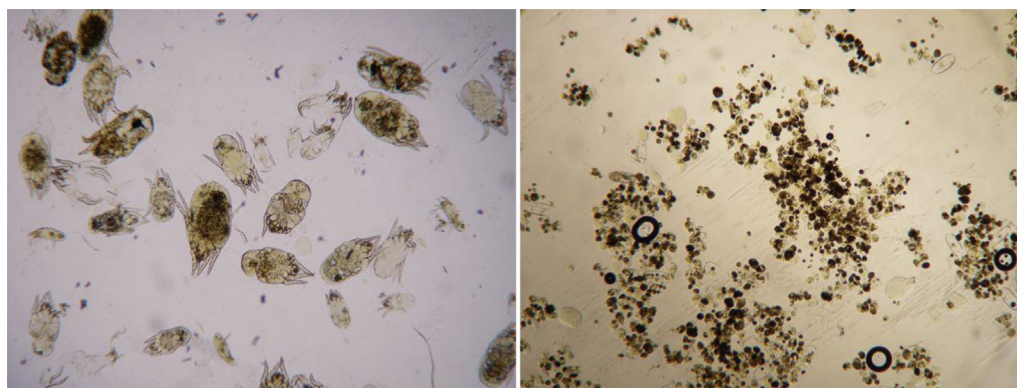


Figure 2. Mite culture fractions. Left, Mite bodies enriched fraction. Right, Mite fecal particles enriched fraction.

all types of mite materials typically present in the environment. This approach requires the use of culture media free of potential contaminating allergens.

The selection of different mite fractions is a key parameter to properly prepare mite allergen extracts. Taken together, the currently available data support the use of both mite bodies and fecal pellets as appropriate raw material to produce allergen extracts covering the full spectrum of sensitizing mite allergens.^{28,29}

Characterization of the Mite Raw Material

Mite species should be identified by appropriately trained individuals knowledgeable about mite morphology and taxonomy. The fraction(s) of the mite culture used, the quality control methods

used, and the acceptance criteria for the amount of foreign material and purity of the raw material should be established. The US Food and Drug Administration (FDA) indicates that no more than 1% of detectable foreign material should be present in the raw material,³⁰ whereas the European Pharmacopoeia²⁰ does not set specific criteria for foreign material but indicates that there should be no foreign species present.

Manufacturing Mite Allergenic Extracts for Diagnosis and Treatment: Standardization

As previously discussed, the first step to prepare mite allergen extracts is to obtain appropriate mite raw materials. This process implies extracting and purifying the allergens from the raw

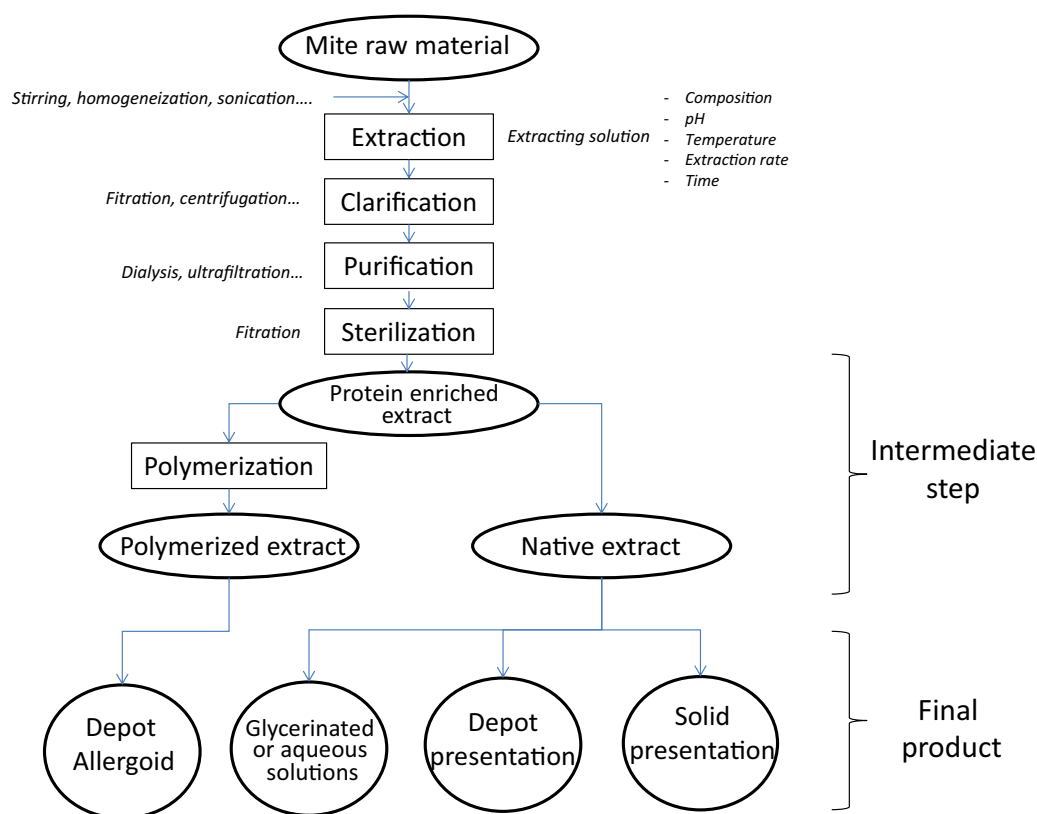


Figure 3. Manufacturing process of mite allergenic vaccines. From the mite culture to the final products, different steps are required for the production of allergenic extracts. The selection of the composition of the raw material, the intermediate step producing native or polymerized allergenic products, and the final formulation of the product in glycerinated solutions, in solid presentation or the addition of adjuvants, determine the characteristic of the vaccine for allergen immunotherapy.

material and removing contaminants and irrelevant substances using physicochemical methods.³¹ The entire manufacturing process needs to be constantly monitored and controlled for quality assurance of the final product (Fig 3).

From Raw Materials to Allergen Extracts

Because mite allergen extracts contain potent enzymatic proteins, the manufacturing process should include appropriate measures to minimize potential allergen degradation to the best possible extent.

Mite raw materials are typically extracted using an aqueous fluid after disrupting mite bodies and fecal pellets by stirring, homogenization, or sonication, which facilitates the release of allergens into the solution. This process increases the protein content of the final extract. Subsequently, clarification (filtration, centrifugation) and purification steps (dialysis, ultrafiltration, chromatography) are used to remove nonallergenic substances, including nucleic acids, carbohydrates, lipids, and salts. A final filtration step through a 0.2- μ M filter renders the extract sterile.

The composition of the extracting solution,³² extraction rate, pH, temperature, and extraction time³³ are parameters to consider. For example, the temperature during the extraction process should be maintained between 2°C and 8°C to reduce the enzymatic activity. In addition, the extraction time should be minimized. When preparing allergen extracts for in vitro use, the addition of enzyme inhibitors during the manufacturing process can be used to maintain the allergenicity and stability of the extract. However, this strategy is not recommended when the final product is going to be prepared directly for human use without additional purification steps to remove the added compounds.

The resulting protein-enriched extracts are commonly prepared as aqueous solutions containing 50% glycerin or 0.01% to 0.05% human serum albumin to stabilize the allergen extracts. A preservative, such as 0.3% to 0.5% (vol/vol) phenol is used to suppress microbial growth in the extracts, resulting in products that can be used for diagnosis and treatment.

In the United States, only standardized 50% glycerinated *D farinae* and *D pteronyssinus* extracts are licensed and available for clinical use. However, in Europe, aqueous allergenic extracts are exclusively prepared for in vivo diagnostic use. For subcutaneous immunotherapy, mite protein-enriched extracts are freeze-dried to preserve their shelf-life. Some European companies also polymerize mite allergen extracts to reduce their allergenicity.³⁴ The polymerization process produces long chains of high-molecular-weight allergens (>300 kDa), which results in a decrease in IgE reactivity of the allergen extract while maintaining its immunogenicity. When the freeze-dried material is reconstituted, solutions can be adsorbed with aluminum hydroxide or mixed with other adjuvants, such as monophosphoryl lipid A.

Polymerized extracts, as used in Europe, require more specific methods to evaluate their allergenic composition, including mass spectrometry, because their major allergen cannot be measured by conventional immunologic methods, such as the enzyme-linked immunosorbent assay. New formulations of mite allergenic extracts in tablets for sublingual immunotherapy (SLIT) have been developed to treat allergic rhinoconjunctivitis and allergic asthma.³⁵ The qualitative allergenic composition of allergen extracts is similar regardless of whether the allergens are used for diagnosis or treatment.

Standardization of Mite Allergen Extracts

Mite allergen extracts are biologically standardized in the United States based on the erythema size produced by serial dilutions of an extract administered intradermally in a population of individuals highly sensitized to mites. This method, called

ID₅₀EAL (intradermal dilution for 50 mm sum of erythema), is used to determine the potency of mite extracts that will serve as reference preparations. Mite extracts produced by licensed manufacturers are compared with the references using a validated IgE enzyme-linked immunosorbent inhibition assay. The relative potency value obtained by a parallel-line bioassay analysis is used to assign the potency of each lot of standardized mite extract.³⁶ In Europe, biological standardization of mite allergen extracts is based on the wheal size, as determined by skin prick testing. Additional adjustments in the manufacturing process of mite allergen extracts are sometimes needed to achieve final preestablished potency in biological units.³⁷

Other differences between Europe and the United States exist. For example, in the United States, the potency of mite allergen extracts is labeled in terms of allergy units (AU), whereas in Europe, the allergenic activity reported varies among manufacturers. Some European manufacturers are beginning to include major allergen testing in their standardization protocols. In the United States, the Center for Biologics Evaluations and Research (CBER) of the FDA provides the reference extracts to the manufacturers and authorizes product release. Thus, standardized allergen extracts produced by different manufacturers in the United States are qualitatively similar and consistent. In Europe, consistency is ensured mainly by using in-house standards and international references. The potency units depend on each individual manufacturer and their in-house standards, and the product release depends on the regulations existing in different countries. Therefore, the extracts produced by different European manufacturers are not usually interchangeable.

Differences have been observed between the biological potency of European and US standardized mite extracts. For example, it has been reported that European standardized diagnostic extracts derived from *D pteronyssinus* have a relative potency, approximately 50% lower than US standardized extracts.^{38,39} This observation is relevant because patients are selected for allergen immunotherapy based on skin test results, and the differential sensitivity and specificity of the diagnostic extracts used could result in an incorrect diagnosis and/or treatment.

Less prevalent mite extracts, such as certain SM species, are usually manufactured and labeled in Europe on the basis of total protein concentration or the amount of freeze-dried material. These nonstandardized mite extracts are usually marketed as named-patient products.

Regulatory Aspects

Allergen extracts have been mandated to undergo the regular registration procedures required for drugs or vaccines for years. During the past few years, several grass and various ragweed pollen allergen vaccines have been developed for SLIT purposes. The evolution of mite allergen vaccines is following a similar trend.

In the United States, CBER/FDA regulates the manufacture of allergen extracts by reinforcing current good manufacturing practices and ensuring that products are consistently produced and controlled according to quality standards. In Europe, the publication of the guideline for allergen extracts by the European Medicines Agency (EMA)²¹ in 2009, the transposition of specific requirements to the European Pharmacopoeia in 2012²⁰ (new monographs are expected to become effective in 2017), and the incorporation of European directives into national laws have had a significant effect on the practice of allergen immunotherapy. Consequently, the number of commercially available allergen extracts, mainly for diagnosis, is decreasing, and many products are voluntarily being withdrawn by the manufacturers.⁴⁰ In addition, the EMA is also working with the Pediatric Investigation Plan,

which requires allergen manufacturers to conduct clinical trials in children with at least one of their marketed allergen extracts.⁴¹

Probably one of the most debated aspects of mite immunotherapy is related to the mixture of different allergen extracts, or multiallergen immunotherapy, especially those related to heterogeneous mixtures that combine mite extracts with fungi, cat, or even pollen allergen extracts. In the United States, the preparation of mixtures that contain different allergenic extracts, including those from different taxonomic groups, are prescribed, although current immunotherapy practice parameters and recommendations discourage mixing extracts that contain high concentrations of proteases, such as those derived from fungi and insects.⁴² In Europe, the EMA proposed recommendations for allergen mixtures based on homologous grouping of the allergens.⁴³ Regarding mites, the only homologous group defined to date exclusively includes the genus *Dermatophagoides*. The use of other mite species needs to be justified.

Although the use of multiallergen vaccines is rare in northern European countries, many allergic individuals are polysensitized or polyallergic to a wide variety of allergens. In contrast, in southern countries, where the polysensitized population is much higher, multiallergen immunotherapy is frequently prescribed. In these cases, regulatory agencies are treating mixtures of different homologous groups as named-patient products, and the European guidelines recommend that the stability of mixtures used in these cases be investigated before their use.

Clinical Aspects

Because HDMs are taxonomically related, different species contain homologous allergens with structural similarities, which cross-react. Mite allergens possess both shared and species-specific determinants. Thus, mite allergic individuals may be cosensitized to multiple mite species in part because of cross-reactivity. Although a high level of cross-reactivity exists among several *Dermatophagoides* species, only limited cross-reactivity has been described between HDMs and SMs.⁴⁴ For example, the correlation of human IgE reactivity between Blo t 1 and Der p 1 was low in sera from individuals with asthma,⁴⁵ Recombinant Lep d 2 also has limited IgE cross-reactivity to the homologous allergens in *Dermatophagoides* and *B tropicalis*.

HDMs also cross-react with allergens derived from other invertebrates, such as those from cockroaches, *Sarcoptes scabiei* mites, crustaceans, and mollusks.⁴⁶ Mite group 10 allergens, which are tropomyosins, are responsible for this phenomenon.⁴⁷ For example, individuals allergic to the *Dermatophagoides* Species may experience allergic symptoms after eating crustaceans and mollusks.

Both skin prick tests and in vitro tests to measure specific IgE are used to determine allergic sensitization. Although specific IgEs for some dust mite allergen components can be measured, mainly in European countries with a research purpose,^{48,49} skin prick test, or in vitro test to measure specific IgEs for standardized *D pteronyssinus* and *D farinae* are primarily used to diagnose HDM allergy.

The main treatment options for HDM allergy are environmental control and allergen immunotherapy. HDM allergen avoidance is recommended as a basic strategy for mite allergies. Although allergen avoidance alone will not treat HDM allergy, it is an important measure to reduce allergen exposure and provide a significant benefit to patients with HDM allergy, when well implemented.^{50,51}

HDM subcutaneous immunotherapy (SCIT) and SLIT are safe and effective in reducing mite-induced allergy symptoms and medication use in patients with allergic rhinitis and asthma.^{52–54} HDM SLIT is a convenient way to induce tolerance in HDM allergic

patients and has a better safety profile compared with SCIT. However, compared with SLIT, HDM SCIT would be more practical in HDM allergic patients who are also sensitized with other environment allergens. HDM SCIT also has efficacy in the prevention of asthma development in children with allergic rhinitis. Double-blind, placebo-controlled clinical trials provide robust evidence to support the safety and efficacy of HDM sublingual tablets in allergic rhinitis patients with or without asthma.⁵⁵ HDM SLIT has been used in Europe for more than 30 years. A major US-based pharmaceutical company has announced that the FDA has accepted for review the biologics license application for their HDM SLIT tablet based on their 2 successful phase 3 clinical trials. A HDM SLIT tablet will be available in the United States once the FDA approves this product. In contrast to promising results from HDM SLIT tablet products, clinical efficacy of HDM SLIT-liquid products is inconclusive because of the lack of knowledge about effective dosing.

In an effort to reduce systemic allergic reactions during immunotherapy while maximizing immunogenicity and clinical efficacy, several methods have been developed.⁵⁶ Some of these have been used in clinical trials, such as allergoids, recombinant allergens, allergen-derived peptides, DNA-encoding allergens, immunostimulatory sequences or CpG motifs, peptides, or use of plants as vectors to deliver allergens.⁵⁷ These products are in the line of precision medicine, targeting precise epitopes of HDM and effectively inducing regulatory T-cell and B-cell responses. These products are in an investigational stage mainly in Europe, and the application of the products is not only for HDM but also other environmental allergens. Wide clinical application in allergy practice would take some time.

Conclusions and Recommendations

Mites are a main source of indoor allergens, which sensitize and cause allergic symptoms in genetically predisposed individuals throughout the world. Several factors are responsible for the diagnostic and therapeutic value of the mite allergen extracts available on the market. These factors include the appropriate selection of the mite species used to produce the extracts, the mite raw materials, and the manufacturing procedures used.

A number of mite allergens exist. Many of them are classified into groups according to their homologous characteristics and biological functions, which largely depend on phylogenetic proximity. This factor is responsible for differential levels of cross-reactivity among different mite genera and families. Cross-reactivity is the main parameter that should be considered to select appropriate mite species to prepare clinical relevant allergen extracts for diagnosis and treatment. The regulatory control of allergen extracts in the United States and Europe differs. These regulations are responsible for the type and number of mite allergen extracts that are commercially available in both areas of the world. Although standardized mite allergen extracts only include *D pteronyssinus* and *D farinae* extracts, mite extracts derived from other clinically relevant genera and families are also produced for diagnostic purposes, particularly in Europe.

This process is responsible for the final composition of manufacturing an allergen extract. Standardized mite allergen extracts are essential to support the safety and efficacy of allergen immunotherapy, both SCIT and SLIT. Undoubtedly, this aspect should improve the quality and consistency of the mite allergen extracts available to the practicing allergist. In that sense, the regulatory guidelines and directives are implemented from the raw material to the final product. Because of the limitation of allergen avoidance and pharmacotherapy for patients with severe allergic rhinitis and asthma, HDM SCIT or SLIT can be a critical treatment option for them.

References

- [1] Platts-Mills TAE, de Weck AL. Dust mite allergens and asthma—a worldwide problem. *J Allergy Clin Immunol*. 1989;83:416–427.
- [2] Langley SJ, Goldthorpe S, Craven M, et al. Relationship between exposure to domestic allergens and bronchial hyperresponsiveness in non-sensitized, atopic asthmatic subjects. *Thorax*. 2005;60:17–21.
- [3] Calderón MA, Linneberg A, Kleine-Tebbe J, et al. Respiratory allergy caused by house dust mites: what do we really know? *J Allergy Clin Immunol*. 2015;136:38–48.
- [4] Kern RA. Dust sensitization in bronchial asthma. *Med Clin N Am*. 1921;5:751–758.
- [5] Dekker H. Asthma und Milben. *Munchener Med Wochenschr*. 1928;75:515–516.
- [6] Müsken H, Fernández-Caldas E, Marañón F, Franz JT, Masuch G, Bergmann KC. In vivo and in vitro sensitization to domestic mites in German urban and rural allergic patients. *J Invest Allergol Clin Immunol*. 2002;12:177–181.
- [7] Colloff MJ. Development, life histories and population dynamics. In: Colloff MJ, ed. *Dust Mites*. Collingwood, Ontario, Canada: CSIRO Publishing; 2009.
- [8] Henmar H, Frisenette SMT, Grosch K, et al. Fractionation of source materials leads to a high reproducibility of the SQ house dust mite SLIT-tablets. *Int Arch Allergy Immunol*. 2016;169:23–32.
- [9] Thomas WR. Hierarchy and molecular properties of house dust mite allergens. *Allergol Int*. 2015;64:304–311.
- [10] Banerjee S, Resch Y, Chen KW, et al. Der p 11 is a major allergen for house dust mite-allergic patients suffering from atopic dermatitis. *J Invest Dermatol*. 2015;135:102–109.
- [11] Batard T, Baron-Bodo V, Martelet A, et al. Patterns of IgE sensitization in house dust mite-allergic patients: implications for allergen immunotherapy. *Allergy*. 2016;71:220–229.
- [12] Carnés J, Boquete M, Carballada FJ, et al. Enzymatic activity in body and fecal extracts of the storage mite *Chortoglyphus arcuatus*. *Int Arch Allergy Immunol*. 2008;145:207–212.
- [13] Cassat A, Mari A, Purohit A, et al. Varying allergen composition and content affects the in vivo allergenic activity of commercial *Dermatophagoides pteronyssinus* extracts. *Int Arch Allergy Immunol*. 2012;159:253–262.
- [14] Spieksma FT, Miyamoto T, Oshima S, Mizuno KJ. Cultivation of house-dust mites. *Allergy*. 1969;43:151–154.
- [15] Colloff MJ. Methods in house dust mite ecology and biology. In: Colloff MJ, ed. *Dust Mites*. Collingwood, Ontario, Canada: CSIRO Publishing; 2009.
- [16] Andersen A. Nutritional value of yeast for *Dermatophagoides pteronyssinus* (Acari: Epidermoptidae) and the antigenic and allergenic composition of extracts during extended culturing. *J Med Entomol*. 1991;28:487–491.
- [17] Fernández-Caldas E. Towards a more complete standardization of mite allergen extracts. *Int Arch Allergy Immunol*. 2013;160:1–3.
- [18] Chan TF, Ji KM, Yim AK, et al. The draft genome, transcriptome, and microbiome of *Dermatophagoides farinae* reveal a broad spectrum of dust mite allergens. *J Allergy Clin Immunol*. 2015;135:539–548.
- [19] Batard T, Hrabina A, Bi XZ, et al. Production and proteomic characterization of pharmaceutical-grade *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* extracts for allergy vaccines. *Int Arch Allergy Immunol*. 2006;140:295–305.
- [20] European Pharmacopoeia Commission. Allergen products. In: *European Pharmacopoeia*. Essex, England: Worldwide Book Services; 2014:1063.
- [21] European Medicines Agency. *EMA Guideline on Allergen Products: Production and Quality Issues*. London, England: European Medicines Agency; 2008. CHMP/BWP/304831/2007.
- [22] Avula-Poola S, Morgan MS, Arlian LG. Diet influences growth rates and allergen and endotoxin contents of cultured *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* house dust mites. *Int Arch Allergy Immunol*. 2012;159:226–234.
- [23] Arlian LG, Confer PD, Rapp CM, Vyszenski-Moher DL, Chang JC. Population dynamics of the house dust mites *Dermatophagoides farinae*, *D. pteronyssinus*, and *Euroglyphus maynei* (Acari: Pyroglyphidae) at specific relative humidities. *J Med Entomol*. 1998;35:46–53.
- [24] Yella L, Morgan MS, Arlian LG. Population growth and allergen accumulation of *Dermatophagoides pteronyssinus* cultured at 20 and 25°C. *Exp Appl Acarol*. 2011;53:103–119.
- [25] Cardona G, Eraso E, Serna LA, Guisantes JA, Martínez J. Analysis of the allergen expression of *Blomia tropicalis* and *Blomia kulagini* (Astigmata: Glycyphagidae) cultures. *J Med Entomol*. 2004;41:1068–1072.
- [26] Cardona G, Guisantes J, Eraso E, Serna LA, Martínez J. Enzymatic analysis of *Blomia tropicalis* and *Blomia kulagini* (Acari: Echimyopodidae) allergenic extracts obtained from different phases of culture growth. *Exp Appl Acarol*. 2006;39:281–288.
- [27] Moingeon P, Batard T, Nony E, Hrabina M, Frati F. Quality control of house dust mite extracts for allergen immunotherapy. *Int Arch Allergy Immunol*. 2013;161:285–286.
- [28] Tovey ER, Chapman MD, Platts-Mills TA. Mite faeces are a major source of house dust allergens. *Nature*. 1981;289:592–593.
- [29] Colloff MJ. Dust mite allergens. In: Colloff MJ, ed. *Dust Mites*. Collingwood, Ontario, Canada: CSIRO Publishing; 2009.
- [30] US Food and Drug Administration. *Additional Standards for Miscellaneous Products (21CFR680.1)*. Silver Spring, MD: US Food and Drug Administration; 2015.
- [31] Esch RE. Allergen source materials: state-of-the-art. *Arb Paul Ehrlich Inst Bundesinstitut Impfstoffe Biomed Arzneimittel Langen Hess*. 2009;96:5–11.
- [32] Prester L, Kovacic J, Macan J. Comparison of buffers for extraction of mite allergen der p 1 from dust. *Arh Hig Rada Toksikol*. 2012;63:293–300.
- [33] Siebers R, Luey B, Crane J, Fitzharris P. The effects of temperature and buffer on the extraction of Der p 1 from dust. *J Allergy Clin Immunol*. 1997;100:580.
- [34] Iraola V, Gallego MT, Taulés M, Carnés J. Measurement of immunoglobulin E interaction with allergen extracts by surface plasmon resonance biosensor analysis. *Ann Allergy Asthma Immunol*. 2013;111:228–229.
- [35] Bahceciler NN, Babayigit HA, Galip N. A milestone in house dust-mite-allergen immunotherapy: the new sublingual tablet S-524101 (Actair). *Expert Rev Vaccines*. 2014;13:1427–1438.
- [36] Slater JE, Esch RE. Preparation and standardization of allergen extracts. In: Adkinson NF, Bochner BS, Burks W, et al, eds. *Allergy: Principles and Practice*. 8th ed. Philadelphia, PA: Mosby; 2014:470–481.
- [37] Carnés J, Iraola V, Gallego M, Leonor JR. Control process for manufacturing and standardization of allergenic molecules. *Curr Allergy Asthma Rep*. 2015;15:37.
- [38] Larenas-Linnemann D, Matta JJ, Shah-Hosseini K, Michels A, Mösges R. Skin prick test evaluation of *Dermatophagoides pteronyssinus* diagnostic extracts from Europe, Mexico, and the United States. *Ann Allergy Asthma Immunol*. 2010;104:420–425.
- [39] Larenas-Linnemann D, Esch R, Plunkett G, et al. Maintenance dosing for sublingual immunotherapy by prominent European allergen manufacturers expressed in bioequivalent allergy units. *Ann Allergy Asthma Immunol*. 2011;107:448–458.
- [40] Klimek L, Hoffmann HJ, Renz H, et al. Diagnostic test allergens used for in vivo diagnosis of allergic diseases are at risk: a European Perspective. *Allergy*. 2015;70:1329–1331.
- [41] Klimek L, Werfel T, Vogelberg C, Jung K. Authorised allergen products for intracutaneous testing may no longer be available in Germany: allergy textbooks have to be rewritten. *Allergol J Int*. 2015;24:84–93.
- [42] Cox L, Nelson H, Lockey R, et al. Allergen immunotherapy: a practice parameter third update. *J Allergy Clin Immunol*. 2011;127(1 suppl):S1–S55.
- [43] Lorenz AR, Luttkopf D, May S, et al. The principle of homologous groups in regulatory affairs of allergen products—a proposal. *Int Arch Allergy Immunol*. 2009;148:1–17.
- [44] Fernández-Caldas E, Puerta L, Caraballo L. Mites and allergy. *Chem Immunol Allergy*. 2014;100:234–242.
- [45] Cheong N, Soon SC, Ramos JD, et al. Lack of human IgE cross-reactivity between mite allergens Blo t 1 and Der p 1. *Allergy*. 2003;58:912–920.
- [46] Sidenius KE, Hallas TE, Poulsen LK, Mosbech H. Allergen cross-reactivity between house-dust mites and other invertebrates. *Allergy*. 2001;56:723–733.
- [47] Witteman A, Akkerdaas J, Leeuwen J, et al. Identification of a cross-reactive allergen (presumably tropomyosin) in shrimp, mite and insects. *Int Arch Allergy Immunol*. 1994;105:56–61.
- [48] Melioli G, Compalati E, Bonini S, Canonica GW. The added value of allergen microarray technique to the management of poly-sensitized allergic patients. *Curr Opin Allergy Clin Immunol*. 2012;12:434–439.
- [49] Thomas WR, Smith WA, Hales BJ, Mills KL, O'Brien RM. Characterization and immunobiology of house dust mite allergens. *Int Arch Allergy Immunol*. 2002;129:1–18.
- [50] Nurmatov U, van Schayck CP, Hurwitz B, Sheikh A. House dust mite avoidance measures for perennial allergic rhinitis: an updated Cochrane systematic review. *Allergy*. 2012;67:158–165.
- [51] Woodcock A, Forster L, Matthews E, et al. Control of exposure to mite allergen and allergen-impermeable bed covers for adults with asthma: Medical Research Council General Practice Research Framework. *N Engl J Med*. 2003;349:225–236.
- [52] Nelson HS, Makatsori M, Calderon MA. Subcutaneous immunotherapy and sublingual immunotherapy: comparative efficacy, current and potential indications, and warnings: United States Versus Europe. *Immunol Allergy Clin North Am*. 2016;36:13–24.
- [53] Larenas-Linnemann D. Sublingual immunotherapy in children: complete and updated review supporting evidence of effect. *Curr Opin Allergy Clin Immunol*. 2009;9:168–176.
- [54] Nelson HS. Update on house dust mite immunotherapy: are more studies needed? *Curr Opin Allergy Clin Immunol*. 2014;14:542–548.
- [55] Nolte H, Maloney J, Nelson HS, et al. Onset and dose-related efficacy of house dust mite sublingual immunotherapy tablets in an environmental exposure chamber. *J Allergy Clin Immunol*. 2015;135:1494–1501.
- [56] Chapman MD, Briza P. Molecular approaches to allergen standardization. *Curr Allergy Asthma Rep*. 2012;12:478–484.
- [57] Nguyen TH, Stokes JR, Casale TB. Future forms of immunotherapy and immunomodulators in allergic disease. *Immunol Allergy Clin North Am*. 2011;31:343–365.