Application Note
LPS-free House Dust Extract

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Introduction: House dust

What is house dust?

Many people sneeze or snuffle in dusty areas. The components of house dust that can induce allergic reaction include molds, pet and human dander and cockroach waste. However, the dust mite - found in ordinary house dust - plays the most important role in all the sniffling and sneezing. The dust mite is a microscopic, spider-like insect which is found in homes. It is primarily in carpets, mattresses and upholstered furniture and thrives in humid and warm conditions. The dust mite feeds on shed scales from human skin! The waste products secreted by these mites are highly allergenic. These waste products continue to induce allergic symptoms even after the mite that drop out them has died. The house dust mite (*Dermatophagoides pteronyssinus* in Europe and *Dermatophagoides farinae* in North America), sometimes shortened by allergists to HDM, is a cosmopolitan guest in human habitation. They are considered to be one of the most common reasons of asthma worldwide.

Asthma is characterized by recurrent wheezing, breathlessness, chest tightness, and coughing. Research in the past decade has revealed the importance of inflammation of the airways in asthma and clinical treatment to decrease chronic inflammation. Asthma is combined with production of IgE to common environmental allergens including house dust mite, animal dander, cockroach, fungal spores, and pollens.

Scientific history

The expression "house dust mites" has been applied to a large number of mites detected in association with dust in dwellings. The first permanent structures for houses date back to 6,000 to 5,000 B.C., but it was not until the late 1600s that scientist became interested in the dust of houses. The pyroglyphids are parasites associated with birds and/or mammals. Kern (1921) discovered house dust to give positive cutaneous reactions in sensitive patients. Cook (1922) and Coa (1922) also detected that dust extracts gave positive skin reactions in over 30% of the individuals tested. Voorhorst et al. (1964) and Oshima (1964) first published their insights that mites were recognized to contribute to the house dust allergy problem.

Habitat of dust mite

The dust mite lives in the modern environment of fully-carpeted, double-glazed, draft-proof homes, and is comfortable at 25 °C and 75% relative humidity. The mites are particularly common in bedding and carpets.

The mite universally lives on shed human skin cells, which are pre-digested by the fungus Aspergillus repens. An average person sheds about 1.5 grams of skin a day (approximately 3-4.5 kg per year), which is enough to feed roughly a million dust mites. Further, dust mites in bedding derive moisture from human breathing, perspiration, and saliva.

Organisms found in house dust

This picture shows the important allergen-producing organisms and relative quantity of their body parts and by-products detect in one gram of house dust.
Why does house dust cause allergic reactions?

House dust is a mixture of many substances. Their concentration varies from home to home, depending on the type of furniture, building materials, presence of pets, moisture and other factors. A speck of dust may contain fabric fibers, human skin particles, animal dander, microscopic creatures called mites, bacteria, parts of cockroaches, mold spores, food particles and other debris. Of these, animal dander, house dust mites, and cockroaches are the most common culprits. People may be allergic to one or more of these substances, and, if exposed to the dust, will have an allergic reaction.\(^7\)

Allergy to foreign proteins in house dust is extremely widespread. Despite the fact that immunotherapy with house dust extract has been utilized for over sixty years, environmental control is still infrequently applied as a therapeutic measure.\(^8\)

The house dust mite is one of the most significant allergens, implicated in allergic asthma, rhinitis, conjunctivitis and dermatitis. The protein responsible for the allergic reaction is DerP1, a protease digestive enzyme discovered in mite feces.

Measures to control house dust mites:
- Vacuuming carpeted areas regularly, preferably with a HEPA filter-equipped vacuum cleaner
- Regular damp dusting of surfaces
- Replacement of carpets with vinyl flooring
- Covering of mattresses and pillows with impervious materials
- Daytime internment of children's plush toys in a freezer
- Use of chemicals to kill mites (acaricides)
- Use of fungicides to kill Aspergillus
- Use an FDA approved air filtration & cleaning system [especially with high-efficiency particulate air (HEPA + filtration)]
- Reduce ambient humidity below 70% to inhibit growth of Aspergillus

Though these techniques can help to decrease the level of house dust mites, attempts to eradicate homes completely have yet to be successful. Immunotherapy, in the form of injections of the allergen into patients, has been successful for some in much the same way that "allergy shots" have been helpful for sufferers of hayfever.\(^9\)

Chemical control

No pesticides are presently named for house dust mites. However, two non-pesticide products, Acarosan and Allergy Control Solution are obtainable for treatment of house dust mites and their allergens. The active component of each is benzyl benzoate and tannic acid. Benzoic acid esters, such as benzyl benzoate, are very effective acaricides in both laboratory and field evaluations. Health risks seem to be slight as benzoates are rapidly metabolized in the body to hippuric acid, which is secreted in the urine. Most acaricidal studies for house dust mite control have been transacted in Europe. Before pesticide recommendations are made in the United States, approval will be needed by the Environmental Protection Agency (EPA).\(^10\)

For this information we can not give any guarantee!
For more details we ask to look into the indicated literature.
House dust and immune system

Endotoxin (Lipopolysaccharide, LPS) and allergen exposure have been explored in the context of asthma for more than a century. Endotoxin is a potent immune-stimulatory component of the bacterial cell wall of all Gram-negative bacteria. As such, endotoxin is ubiquitous in our environment. Endotoxin exposure has been well demonstrated to underlie "Monday Asthma" or byssinosis in cotton workers, and has since emerged as a frequent cause of asthma-like symptoms in a wide range of occupational settings. Asthmatics are particularly sensitive to inhaled endotoxin, and inhalation induces both immediate and sustained airflow obstruction. The paradox of endotoxin exposure is that higher levels of exposure in early life might mitigate the development of allergy and persistent asthma. With endotoxin exposure being significantly higher in homes with animals and in farming households, where allergy and asthma are less likely to develop, endotoxin and other microbial exposures in early life may keep allergen sensitisation and asthma from developing by promoting Th1-type immune development. These observations, consistent with the "Hygiene Hypothesis" of allergy and asthma, are an encouraging glimpse of the potential for early immune modulatory approaches to asthma therapy and prevention. 

Sensitization to cockroach allergens (CRA) has been implicated as a major cause of asthma, especially among inner-city populations. Endotoxin from Gram-negative bacteria has also been investigated for its role in attenuating or exacerbating the asthmatic response. A study made by Remick and colleague’s shows that HDE containing high levels of cockroach allergens and endotoxin collected from different sources can induce an asthma-like response in murine model. 

House dust extract-activated bone marrow-derived dendritic cells were found to produce IL-6 and IL-12 in a concentration-dependent manner and to increase their expression of CD40, CD80, CD86, and MHC class II molecules. Furthermore, correlations were seen between the relative bioactivities of house dust extracts and their endotoxin levels. The results from Horner and colleague’s shows that house dust extracts elicit Toll-like receptor-dependent dendritic cell responses. 

Several components in house dust extract can bind IgE, these interactions are not all immunologically specific. Non-immune interactions may conceivably participate to the biological effects of house dust extract, they are actually found to interfere with the assay of allergen-specific IgE, especially when the serum under investigation has a high IgE yield. By alteration of the incubation medium, non-immune interactions can be prevented to a large extent. The immunological interactions between IgE and components in house dust extract have been investigated using twenty extracts, both in inhibition-type assay and in direct insolubilization assay. *D. pteronyssinus*-related allergens were found to be present in almost all extracts, however, not all house dust specific IgE reacted with mite-related allergens. It is clear, however, that no single figure can represent a completely reliable measure for the biological potency of a house dust extract. 

For more details we ask to look into the indicated literature.

On the following page you find the protocol “LPS removal from house dust extract with EndoTrap® HD”.

EndoTrap® red is also suitable for removing LPS from house dust extract. For which system you decide, depends only on your buffer composition. It is also possible that you dilute your house dust extract in the equilibration buffer of EndoTrap® - enriched with NaCl*- as in the protocol presented below:

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*A high salt concentration reduces non-specific binding. If you want to work with high salt conditions we recommend EndoTrap® HD! To see with which EndoTrap® system YOU obtain better results simply try out our testing kits EndoTrap® HD 1/1 and EndoTrap® red 1/1.
Protocol
LPS removal from house dust extract with EndoTrap® HD*

Prearrangement:
- Dust was collected from the kitchen of the homes of asthmatic children using a vacuum cleaner with a dust collector (Indoor Biotech.). The dust was extracted in PBS overnight at 4°C on a rotator and centrifuged at 1000g for 10 min at 4°C to remove particulate matter.
- Dilute 100 µl HDE (not heat inactivated) in 300 µl EndoTrap® HD equilibration buffer (enriched to a salt concentration of 400 mM NaCl [normally only 150 mM NaCl]).

Preparation and Activation:
1. Remove the top cap first from the EndoTrap® column. This prevents air bubbles from being soaked up. Next, remove bottom cap and place the column in a suitable holder. Allow storage solution to drain completely from column [appr. 8 min.].
2. Fill up the column two times to the edge of the column with regeneration buffer (RB)* [this corresponds to appr. 2x 3 ml or 6 column volumes of RB] and let the column drain out completely. [appr. 12 min.]
3. Fill up the column two times to the edge of the column with equilibration buffer (EB)¹ [this corresponds to appr. 2x 3 ml or 6 column volumes of EB] and let the column drain out completely. [appr. 12 min.]
4. Apply sample onto the column, once all sample enters the column (sinks in the resin), re-cap the column and incubate for 45 min at room temperature.
5. In order to elute the house dust extract, remove the top cap, apply an extra 1 ml equilibration buffer (EB), remove the bottom cap, let the column drain out and collect completely the flow through (= fraction 1).*
6. In order to be sure to elute the whole sample, apply an extra 1 ml equilibration buffer (EB), let the column drain out and collect completely the flow through (= fraction 2).*

Regeneration and Storage
1. Fill up the column two times with equilibration buffer (EB) [this corresponds to 2x 3 ml or 6 column volumes of EB] and let the column drain out completely. [appr. 12 min.]
2. If you want to store the column, close the column with the bottom cap and apply 1 ml of regeneration buffer (RB) supplemented with 0.02% sodium azide and store at 4°C.
   OR:
   If you want to regenerate the column, make sure you start with step 2 of “Activation and Endotoxin removal”.

* This protocol was kindly provided to us by Ms. Sudha Natarajan, University of Michigan, Department of Pathology, on 08.07.2005.

* Hyglos recommends collecting fractions, so that the sample solution is not too strongly diluted. Contains the first fraction too less from your “protein” sample, you have the possibility to look if your “protein” is also still in the second fraction. Gladly you can also collect your sample at once.

* Regeneration buffer “HD” (RB): 20 mM HEPES, 1 M NaCl, 2 mM EDTA, pH 7.5
¹ Equilibration buffer “HD” (EB): 20 mM HEPES, 150 mM NaCl, 0.1 mM CaCl₂, pH 7.5
An effective method for removing endotoxin contamination from a house dust extract, while retaining high allergen levels.

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Removal of LPS contamination from in vivo stimuli is an important consideration in animal models. In our model of allergic asthma, the asthmatic response is induced by a house dust extract (HDE) that contains large amounts of LPS and cockroach antigens (CRA) Bla g1 and Bla g2. LPS contamination is responsible for increased neutrophil influx and cytokine production in the asthmatic lung. Therefore, LPS removal is necessary in order to study the antigen specific immune response. Unfortunately, existing methods of LPS removal also deplete allergens to undetectable levels. We assayed the ability of two systems, DetoxyGel (Sigma) and EndoTrap® (Hyglos) to deplete LPS, while retaining CRA levels in the HDE. Application of the HDE to the DetoxyGel column resulted in 94% depletion of LPS, however 99.9% of Bla g1 and 48% Bla g2 were also removed. The EndoTrap System removed 90% of the LPS, but also removed ~60% of both Bla g1 and Bla g2. The intact extract contains 18.21ug/mL endotoxin and 16.65ug/mL CRA, while the EndoTrap treated extract contains 1.75ug/mL LPS and 6.86ug/mL CRA. Since the EndoTrap system leaves a measurable and consistent amount of Bla g1 and Bla g2 in the HDE, this method is preferred given that appropriate levels of CRA can be measured and calculated after LPS removal for in vivo administration.

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For further congress information:

Hyglos comment: Performance characteristics of the column mode of EndoTrap®

How you should work with a re-usable real flow through system:

EndoTrap® is a real flow through system. That means that you can fill in your sample without break. Unfortunately you can only put 4 ml at once into the column. If you want, you can refer a funnel from us, which makes it possible to fill in directly 20 - 25 ml. We recommend regenerating the column after each cleaning step (one cleaning step means that you have to put through your working volume one time). That means that you have to regenerate the column (indicated in our package inserts) after all of your sample volume was put through. Afterwards you can fill in your sample volume again to reduce further the LPS contamination (sometimes you have to repeat the cleaning step several times to get your final desired LPS concentration). You can calculate that you can reduce your LPS contamination during each cleaning step for approximately two log steps [e.g. from starting contamination of 10,000 EU to 100 EU to 1 EU during two cleaning steps]). However, parameters such as pH, ionic strength, temperature, contact time, etc. might have to be optimized for each application to obtain maximum endotoxin removal with minimum loss of product. Various EndoTrap® applications are in development and will be presented on our web side. Nonetheless you can reach our technical support by email or phone who will be glad to assist you in case you have special questions or in case of special applications.
Publications regarding the topic:


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References:

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