# Prevalence of Specific IgE to Dust and Storage Mites in Patients from Buildings with Moisture Damage Using Pharmacia- and DPC-Specific IgE Immunoglobulin Assays

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**Abstract:** The interpretation of the results of *in vitro* tests for allergy to different kinds of mites may be challenging for clinicians due to the methodological differences between commercial tests.

We assessed the presence of specific IgE-antibodies to house dust and storage mites in the serum of 24 patients with respiratory symptoms, and in 24 healthy controls, using two methods: the Pharmacia UniCAP System® (today Thermo Fisher Scientific. Allergy. Phadia AB) and DPC Alastat assays®. We also tested a storage mite mix reagent from Pharmacia. The presence and characterisation of the mites in the dust samples were assessed using microscopy. The Pharmacia experimental storage mite mix was positive for 11 out of 12 patients in a Pharmacia positive specific test. In the patient sera, Pharmacia detected 61 positive specific responses, whereas DPC detected 24. This indicates a significant analytical difference between the methods.

The presence of identifiable mites or mite allergens in dust found by microscopy might confirm the IgE response. Combining the specific IgE test for the house mite Dermatophagoides pteronyssinus and a storage mite mix test, including the mites Acarus siro, Glycophagus domesticus, Lepidoglyphus destructor and Tyrophagus putrescentiae, can produce a cost-effective estimate of a suspected mite sensitisation case (IgE response).

**Keywords:** Storage mite, house dust mite, mite exposure, allergy tests, mouldy buildings.

## 1. INTRODUCTION

Asthma and other respiratory diseases caused by sensitisation to mites are common health problems all over the world. Indoor allergens are risk factors for asthma, and *Dermatophagoides pteronyssinus* in particular is a dominant allergen for asthma [1-4] In addition to house dust mites, since the early 1900s, storage mites have been associated with both occupational and non-occupational syndromes [5-9]. Storage mites have long been known to cause occupational sensitisation among, for example, farmers, greenhouse and grain workers and bakers [10-15]. In addition, they have frequently been found in moisture-damaged buildings, where mould growth supplies a suitable nutrition medium [16,17].

Assessing the presence of mite-specific IgE in the serum of sensitised people aids in the diagnosis of mite allergy and leads to further sanitation procedures. However, commercial analytical methodologies differ in analytical sensitivity and specificity. This is clearly shown by international external quality assessment surveys such as the United Kingdom National External Quality Assessment Scheme (UKNEQAS Ltd, Sheffield, UK). The aim of our study was to evaluate the prevalence of IgE antibodies to four house dust and five storage mite species in a group of 24 patients with respiratory symptoms, by comparing the DPC and Pharmacia methods for specific IgE in serum. We also tested the usefulness of a new experimental Pharmacia IgE-ImmunoCap® storage mite mix reagent. The serum IgE results were compared to those of skin prick tests. In addition we demonstrated the presence of mites in dust samples from homes and workplaces by microscopy, using the Acarex® test.

## 2. MATERIALS AND METHODS

### 2.1. Patients

Over a one-year period, we chose 24 ambulatory hospital patients (2 men and 22 women) with respiratory symptoms and indoor air problems at work or at home for the study. Their mean age was 42.2 years (median 43.5 y) and age range 7–62 years. Seven (29.2%) were smokers, 8 (25%) ex-smokers and 11 (45.8%) non-smokers. The staff physicians at the Clinic of Indoor Air Health Problems, Skin and Allergy

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Hospital, Helsinki University Central Hospital examined the general health of the patients. Clinical assessment included medical history and current symptoms, a physical examination, pulmonary function tests, chest and maxillary radiographs, a complete blood cell count, skin prick tests (SPT) to common allergens including mites, and the determination of specific serum IgE antibodies to house dust and storage mites. All patients with a positive SPT (> 2 mm) to some of the storage mites (*Acarus siro, Lepidoglyphus destructor, Tyrophagus putrescentiae*) and some patients positive to only the house dust mites (*Dermatophagoides pteronyssinus, Dermatophagoides farinae*) were included in the study.

### 2.2. Controls

The control group consisted of 23 healthy, symptom-free individuals, 9 men and 14 women, with no known indoor air problems. Their mean age was 42.7 years (median 46.8 years) and age range 20–60 years.

## 3. ANALYTICAL METHODS

### 3.1. Serum specific IgE determinations

Serum samples were stored at -20 $^{o}$C until they were analysed for mite-specific IgE antibodies. We determined the serum IgE to 9 mite antigens in all 24 patients: *Dermatophagoides pteronyssinus* (d1), *Dermatophagoides farinae* (d2), *Dermatophagoides microceras* (d3), *Acarus siro* (d70), *Lepidoglyphus destructor* (d71), *Tyrophagus putrescentiae* (d72), *Glycophagus domesticus* (d73), and *Euroglyphus maynei* (d74). The IgE antibody measurements were performed using a kinetic enzyme-labelled immunoassay, according to the instructions of the manufacturer of AlaSTAT® (Diagnostic Products Corporation, Los Angeles, CA). The reaction rate was directly related to the allergen-specific IgE concentration, which is expressed in IU/ml (kU/L). An IgE concentration of over 0.35 IU/ml was regarded as positive. We used a positive human serum control containing IgE specific for *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae,* and a negative control with no detectable allergen-specific IgE. The method was calibrated against the WHO IgE 2$^{nd}$ International Reference Preparation (IRP 75/502).

Using a Pharmacia RoboCAP-AutoCAP® analyser and the Pharmacia CAP System Specific IgE FEIA®, we also determined the above mite-specific IgE antibodies (Pharmacia Diagnostics AB, Uppsala, Sweden, today Thermo Fisher Scientific. Allergy. Phadia AB) and DPC Alastat assays®). We used reagent caps d1, d2, d3, d70, d71, d72, d73, and d74. The method was calibrated against the WHO IgE 2$^{nd}$ IRP 75/502 and controlled by the positive Pharmacia Specific IgE Control® containing d1 and d2, and the Pharmacia Quality Club Specific IgE Control Survey®. The cut-off limit of the method was 0.35 kU/l. Results of > 0.35 kU/l were regarded as positive. The precision of the Pharmacia method was 15.6 CV % at 0.43 kU/L, and 10.8 CV % at 4.03 and 18.4 kU/L. We also tested a semi-quantitative experimental storage mite CAP Mix containing *Acarus siro, Lepidoglyphus destructor*, *Glycophagus domesticus* and *Tyrophagus putrescentiae*.

We performed SPTs using two house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, Soluprick SQ, 10 Hep, ALK, Denmark), three storage mites (*Acarus siro* 1:100 w/v, *Lepidoglyphus destructor* 100 BU/ml, *Tyrophagus putrescentiae* 100 BU/ml) and both positive (histamine dihydrochloride, 10 mg/ml) and negative (solvent) control solutions. The patients were classified as positive if the allergen caused a weal of 3 mm or more in diameter and if the control solution produced the expected reaction. The purity of storage mite allergen extracts is not known. The culture medium of mites, for example, may influence the allergen content.

### 3.2. Dust sampling, mite analysis, and the Acarex test

We asked the patients to collect dust samples according to a strict dust sampling procedure from their home and/or work environment. Dust for the mite sample was collected in a clean vacuum cleaner bag by vacuuming approximately 1 m$^2$ of the patient's floor or bed for two minutes. After filtering stones, hairs and scraps, the dust samples were transferred into sealable bags. We collected workspace samples from the room that was suspected to be causing health symptoms; from the surfaces of floors, carpets and furniture. Each patient sent one to six dust samples for mite analysis. The samples were stored at -25 $^{o}$C before analysis. We analysed a total of 43 dust samples.

From each sample, we took two sub-samples of 25mg to 50 mg of dust for counting and identifying the mites. The sub-samples were distributed into small Petri dishes with 2.5 ml lactic acid and three to five drops of 1% lignin pink, and incubated at +55 ˚C–60 ˚C for 24 to 48 hours. The mites were extracted under a stereo-microscope at low magnification (x 30–40),

**Table 1: Immunoglobulin E Antibody Levels to Nine Mites in Studied Patients (n= 24 (Patients) and Controls (23)**

| Mite | Sensitisation (DPC) Patients > 0.35 kU/l n (%) | Sensitisation (Pharmacia) Patients > 0.35 kU/l n (%) | Sensitisation (DPC) Controls > 0.35 kU/l n (%) | Sensitisation (Pharmacia) Controls > 0.35 kU/l n (%) |
|---|---|---|---|---|
| *Dermatophagoides pteronyssinus* | 8 (33) | 10 (42) | 0 (0) | 4 (15) |
| *Dermatophagoides farinae* | 5 (21) | 10 (42) | 1 (4) | 3 (12) |
| *Dermatophagoides microceras* | 1 (4) | 9 (38) | 1 (4) | 4 (15) |
| *Acarus siro* | 0 (0) | 10 (42) | 1 (4) | 2 (8) |
| *Lepidoglyphus destructor* | 3 (13) | 10 (42) | 1 (4) | 2 (8) |
| *Tyrophagus putrescentiae* | 2 (8) | 11 (46) | 0 (0) | 2 (8) |
| *Glycophagus domesticus* | 3 (13) | 6 (25) | 0 (0) | 0 (0) |
| *Euroglyphus maynei* | 2 (8) | 4 (17) | 1 (4) | 1 (4) |

mounted in Heinze PVA medium, and subsequently counted and identified under a microscope (x 40–400). The results are presented as number of mites per gram of dust. When no mites were found, we used the detection limit (DL) divided by two as the sample value.

The Acarex test (Allergopharma Joachim Ganzer KG, Germany) was used according to the manufacturer's instructions. Dust was obtained by a household vacuum cleaner using a new dustbag. Four square metres of surfaces such as beds or carpets were sampled for eight minutes, and eight square metres of hard surfaces were sampled for eight minutes. The dust was sieved through a 200-mesh metal sieve to remove large particles such as hair or sand. We mixed 140 mg of fine dust with 1100μl of test reagent (methanol-potassium hydroxide). After ten seconds, we wetted a diazo dye-containing test strip and optically read the result after one minute against an orange colour code. The results were reported as negative, mild (+), moderate (++) or heavy (+++). This test detects the guanine content in the faeces of mites and other members of the *Acari* genus. The cut-off limit was 0.6 mg guanine/g dust.

### 3.3. Statistical analysis

We analysed the data using the *Analyse-it* statistical package (Method evaluation edition, clinical laboratory 1.73; Analyse-it Software Ltd, USA). We used the Pearson correlation and Deming regression analysis programmes.

### 3.4. Ethics

The Ethics Committee of the Departments of Dermatology and Allergic Diseases approved the study protocol. All the patients gave their informed consent.

## 4. RESULTS

### 4.1. Prevalence of mite-positive patients and controls

Table **1** summarises the prevalence of elevated (> 0.35 kU/l) specific serum IgE-Ab as an indicator of the patients' sensitisation to mites. In 24 patients, DPC obtained 16 positive house dust mite results (*D. pteronyssinus, D. farinae, D. microceras, E. maynei*), and Pharmacia found 33. This indicates a two-fold difference between the analytical sensitivity of the methods. The same tendency applied to the storage mites. DPC found no positive results for *Acarus siro*, a common mite, but Pharmacia found ten. On an individual level, DPC found that seven patients were sensitised to house dust mites alone; Pharmacia found the same result for ten patients. DPC found that four patients were positive to only storage mites; Pharmacia found 12 positive cases. DPC found four positive cases for both categories, and Pharmacia found ten. In the control group, four people with no clinical symptoms showed positive results to some mites with no history of exposure (Table **2**).

The DPC method detected sensitisation to the four house dust mites (*Dermatophagoides pteronyssinus, Dermatophagoides farinae, Dermatophagoides microceras* and *Euroglyphus maynei*) in 33%, 21%, 4% and 8% of the patients, respectively, and to four storage mites (*Acarus siro, Tyrophagus putrescentiae, Lepidoglyphus destructor* and *Glycophagus domesticus*) in 0%, 8%, 13% and 13% of the patients, respectively (Table **1**, Table **3**). The Pharmacia method detected sensitisation to the same four house dust mites in 42%, 42%, 38% and 17% of the patients

**Table 2: Controls: Comparison between Pharmacia and DPC**

| Patient | Mite | Pharmacia | DPC |
|---|---|---|---|
| 1-20 | ***Dermatophagoides pteronyssinus*** | N | N |
| | ***Dermatophagoides farinae*** | N | N |
| | ***Acarus siro*** | N | N |
| | ***Lepidoglyphus destructor*** | N | N |
| | ***Tyrophagus putrescentiae*** | N | N |
| | ***StMix*** | N | |
| 21 | ***Dermatophagoides pteronyssinus*** | P | N |
| | ***Dermatophagoides farinae*** | P | N |
| | ***Acarus siro*** | P | N |
| | ***Lepidoglyphus destructor*** | P | P |
| | ***Tyrophagus putrescentiae*** | P | N |
| | ***StMix*** | P | |
| 22 | ***Dermatophagoides pteronyssinus*** | P | N |
| | ***Dermatophagoides farinae*** | P | N |
| | ***Acarus siro*** | P | N |
| | ***Lepidoglyphus destructor*** | P | N |
| | ***Tyrophagus putrescentiae*** | P | N |
| | ***StMix*** | P | |
| 23 | ***Dermatophagoides pteronyssinus*** | | |
| | ***Dermatophagoides farinae*** | P | N |
| | ***Acarus siro*** | P | N |
| | ***Lepidoglyphus destructor*** | N | N |
| | ***Tyrophagus putrescentiae*** | N | N |
| | ***StMix*** | N | N |
| | | N | |

Abbreviations: N= negative result, P= positive result.

*StMix: Acarus siro, Glycophagus domesticus, Lepidoglyphus destructor, Tyrophagus putrescentiae*

*Cut-off levels: SPT > 4 mm, Specific Ige and StMix > 0.4 ku/l*

respectively, and sensitisation to the same four storage mites in 42%, 46%, 42% and 25% of the patients, respectively (Table **1**, Table **3**). The DPC method revealed that 38% (n= 9) of the patients had specific IgE antibodies to one or several house dust or storage mites; the corresponding percentage for the Pharmacia method was 46% (n=11). Of the controls, 8% showed mite sensitisation by the DPC method, and 15% by the Pharmacia method.

### 4.2. Analytical compatibility between DPC and Pharmacia routine mite IgE methods

We found no correlation with any of the tested mite species in the patient group.

### 4.3. Compatibility of Pharmacia® Storage Mite Mix and Pharmacia Specific IgE®

Table **4** summarises the diagnostic sensitivity and specificity of the Pharmacia Storage Mite Mix Cap. The Mix was positive in 11 out of 12 storage mite-positive patients (91%). *Tyrophagus* was positive (0.7 kU/L) in Patient 7 (Table **3**) but was not detected by the Mix or SPT. SPT was positive for *A. siro,* but the specific IgE was negative. Microscopy detected mites in this patient's house dust (Table **4**). In the control group, the Mix of one person, who had positive specific IgE to *Acarus*, Lepidoglyphus, and *Tyrophagus,* was also positive. A correct negative Mix result was obtained in a person found positive to *D. pteronyssinus*, *D. farinae* and *D. microceras*, and negative to *Acarus*, *Lepidoglyphus, Glycophagus* and *Tyrophagus*. Both controls were clinically symptomless and could not specify any episode of exposure to mite-sustaining conditions. All other controls were negative to all mite species as well as to the Mix, providing a specificity of 100%.

**Table 3: Summary of Specific IgE, Prick Tests and Dust Analyses of each Patient**

| Patient no | Mite | SPT | Phar-macia | DPC | Microscopy | Patient no | Mite | SPT | Phar-macia | DPC | Microscopy |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1-6 | Dp | N | N | N | Negative | 16 | Dp | N | P | P | Negative |
| | Df | N | N | N | | | Df | N | P | P | |
| | As | N | N | N | | | As | N | P | N | |
| | Ld | N | N | N | | | Ld | N | P | N | |
| | Tp | N | N | N | | | Tp | P | P | N | |
| | StMix | | N | | | | StMix | | P | | |
| 7 | Dp | N | N | N | Positive | 17 | Dp | N | N | N | Positive*) |
| | Df | N | N | N | | | Df | N | N | N | |
| | As | P | N | N | | | As | N | N | N | |
| | Ld | N | N | N | | | Ld | N | N | N | |
| | Tp | N | P | N | | | Tp | N | N | N | |
| | StMix | | N | | | | StMix | | N | | |
| 8 | Dp | N | P | P | Positive | 18 | Dp | N | P | P | Positive |
| | Df | N | P | P | | | Df | N | P | P | |
| | As | P | P | N | | | As | P | P | N | |
| | Ld | N | P | P | | | Ld | P | P | P | |
| | Tp | N | P | N | | | Tp | P | P | N | |
| | StMix | | P | | | | StMix | | P | | |
| 9 | Dp | N | P | N | Negative | 19 | Dp | N | N | N | Positive |
| | Df | N | P | N | | | Df | N | N | N | |
| | As | N | P | N | | | As | N | N | N | |
| | Ld | N | P | N | | | Ld | N | N | N | |
| | Tp | N | P | N | | | Tp | N | N | N | |
| 10 | StMix | | P | | | | StMix | | N | | |
| | Dp | N | N | N | Positive | 20 | Dp | N | P | N | Positive |
| | Df | N | N | N | | | Df | p | P | N | |
| | As | N | N | N | | | As | P | P | N | |
| | Ld | N | N | N | | | Ld | P | P | N | |
| | Tp | N | N | N | | | Tp | P | P | N | |
| 11 | StMix | | N | | | | StMix | | P | | |
| | Dp | P | P | P | Negative | 21 | Dp | N | P | N | Positive |
| | Df | P | P | P | | | Df | P | P | N | |
| | As | P | P | N | | | As | P | P | N | |
| | Ld | P | P | P | | | Ld | N | P | N | |
| | Tp | P | P | P | | | Tp | P | P | N | |
| 12 | StMix | | P | | | | StMix | | P | | |
| | Dp | N | P | P | n.a. | 22 | Dp | N | N | P | Negative |
| | Df | P | P | P | | | Df | N | N | N | |

***Table 3 continued…***

| Patient no | Mite | SPT | Phar-macia | DPC | Microscopy | Patient no | Mite | SPT | Phar-macia | DPC | Microscopy |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | As | P | P | N | | | As | N | N | N | |
| | Ld | P | P | N | | | Ld | N | N | N | |
| | Tp | P | P | N | | | Tp | P | P | P | |
| | StMix | | P | | | | StMix | | P | | |
| 13 | Dp | N | N | P | Positive | 23 | Dp | N | N | N | Negative |
| | Df | N | N | N | | | Df | na | N | N | |
| | As | N | N | N | | | As | P | N | N | |
| | Ld | N | N | N | | | Ld | N | N | N | |
| | Tp | N | N | N | | | Tp | N | N | N | |
| | StMix | | N | | | | StMix | | N | | |
| 14 | Dp | P | P | N | Positive | 24 | Dp | N | P | N | Negative |
| | Df | N | P | N | | | Df | N | P | N | |
| | As | N | P | N | | | As | P | P | N | |
| | Ld | N | P | N | | | Ld | P | P | N | |
| | Tp | N | P | N | | | Tp | P | P | N | |
| | StMix | | P | | | | StMix | | P | | |
| 15 | Dp | N | N | N | Positive*) | | | | | | |
| | Df | N | N | N | | | | | | | |
| | As | N | N | N | | | | | | | |
| | Ld | N | N | N | | | | | | | |
| | Tp | N | N | N | | | | | | | |
| | StMix | | N | | | | | | | | |
| | | | | | | | | | | | |

Dermatophagoides pteronyssinus (Dp), Dermatophagoides farinae (Df), Acarus siro (As), Lepidoglyphus destructor (Ld), Tyrophagus putrescentiae (Tp).

Storage Mite Mix (StMix): Acarus siro, Glygophagus domesticus, Lepidoglyphus destructor, Tyrophagus putrescentiae.

Cut-off levels: SPT > 4 mm, Specific Ige and StMix < 0.4 kU/l.

Dust microscopy: > 100 mites/g dust,

*) non-identifiable fragments.

**Table 4: Sensitivity and Specificity of Pharmacia Storage Mite Mix (StMix) IgE Reagent**

| Patients | Specific IgE | StMix | | |
|---|---|---|---|---|
| Positive | 12 | 11 | 91% | Sensitivity |
| Negative | 12 | 13 | 92% | Specificity |
| N | 24 | 24 | | |
| | | | | |
| Controls | Specific IgE | StMix | | |
| Positive | 1 | 1 | 100% | Sensitivity |
| Negative | 22 | 22 | 100% | Specificity |
| N | 23 | 23 | | |
| | | | | |
| All tested | Specific IgE | StMix | | |
| Positive | 13 | 12 | 92 | Sensitivity |
| Negative | 34 | 35 | 97 | Specificity |
| N | 47 | 47 | | |
| | | | | |

**Table 5: Results of Acarex test versus number of mites per g of settled dust (n= 54)**

| | Mites<LD | LD-100 | 100–500 | > 500 |
|---|---|---|---|---|
| Acarex 0 | 35 | 14 | 1 | 0 |
| Acarex +1 | 2 | 1 | 0 | 0 |
| Acarex +3 | 0 | 0 | 0 | 1 |

LD= limit of detection.

### 4.4. Comparison of serum-specific IgE and SPT

No systematic tendency was evident between the IgE and SPT results (Table **3**). The Acarex test was positive in three cases (Table **5**).

## 5. DISCUSSION

House dust mites cause allergic symptoms and diseases among both children and adults, and exacerbate the symptoms of asthma. Specific IgE antibodies to mites have shown to be significantly related to an increased risk of adult-onset asthma [18]. The significance of storage mites as symptom-provoking agents and a cause of allergic diseases is not very well documented in work environments other than farming, greenhouse work and the food processing industry. Co-sensitisation to storage mites may occur in patients sensitised *to Dermatophagoides pteronyssinus* [19].

Therefore, reliable immunological tests for assessing both groups of mites are an essential aid to diagnosis.

The differences in the detection of specific mite IgE-Ab by the Pharmacia/Phadia and DPC analytical systems are probably dependent on the allergen coupling principle used for the allergen extract reagent. DPC uses a liquid polymer as the carrier of the allergen molecules; Pharmacia uses a cellulose sponge. The exposure and concentration of the allergen molecules and epitopes exposed to the serum IgE antibodies of the patient will therefore be sterically different. The nativity of the manufactured mite allergen extracts may also differ. This divergence can be clearly seen in the international quality control surveys of UKNEQAS. For correct diagnosis, it is therefore essential to consider symptoms, physical surroundings and clinical and laboratory tests as a whole. Sensitisations shown by an SPT and immunological reactivity *in vitro* do not always correlate [19]. Moreover, dust analysis by Acarex or microscopy (sv dock) provides considerably little additional information when SPT and specific IgE disagree.

The mite extracts contained over 30 different allergens. Some were common to all mites, some species specific [20]. The cross reactivity between house dust mites was substantial [19]. The storage mite families showed less uniformity [21]. Testing IgE for all available mite species is costly. The good performance of the tested experimental Storage Mite Mix therefore suggests that the use of a combination of the Mix and the *D. pteronyssinus* test for surveying the possibility of mite allergy is highly effective. However, this product is presently not commercially available. As far as we know, no comparisons like those carried out in this study have been published earlier. This study shows that Storage Mite Mix would be useful for clinicians.

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