

Quantification of house-dust-mite populations

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Since the proposal that allergen from the mite *Dermatophagoides pteronyssinus* is identical to the house-dust allergen supposed to cause asthma and allergy, interest in the abundance, species diversity, and frequency of mites in the home has expanded. Surveys and ecologic studies have confirmed the ubiquity of mites in house dust, especially mites of the family Pyroglyphidae, but most do not give an accurate estimate of population size or mite exposure. These are still important issues today in attempts to quantify the exposure to house-dust mite that constitutes a risk, to assess mite-eradication trials, and to determine fluctuations in mite population. The problems encountered with common methods of house-dust sampling, mite extraction, and measuring house-dust allergen are discussed.

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The distribution of the house-dust mite in different habitats in the home is well documented (1). The main areas used for sampling dust are usually bedding, floors, and soft furnishings. There are several issues which the investigator needs to consider when attempting to quantify mites from these habitats, because the ease with which mites are extracted will be influenced by the nature of the habitat and the composition of the house dust. The appropriate choice of units for expressing mite density and estimates of population size is also an important consideration.

Most studies have expressed mite density and estimates of population size by unit weight of house dust because it is easy to standardize; however, the composition of house dust will vary according to the source. Dust from bedding and soft furnishings is likely to include more fibrous and lighter material than dust from floors, which may include heavier particulate material such as sand and grit. Therefore, comparing mite density per unit weight of dust from different habitats or even similar habitats may be misleading and underestimate the population in areas where dust density is higher. Measurements of mite density per unit weight of dust also does not take into account the total weight of dust collected. This can overemphasize the importance of a few mites living in a low-dust environment, whereas high mite abundance in dusty habitats will be underemphasized.

In a comprehensive review of house-dust-mite ecology, Colloff (2) suggests that expressing mite density per unit area, and not per unit weight, may be more appropriate for studies involved with repeated measurements of mite population density, where alterations to the dust load can provide misleading data. Examples include mite-eradication trials, where the use of acaricidal powders may artificially increase the weight of dust, thus having a diluting effect on mite density, and population-fluctuation studies, where heavy sampling may artificially deplete the amount of dust and confound estimates of the size of mite populations.

A further consideration for house-dust sampling is the topography of a habitat. The surface features and uniformity of a habitat may vary, and this will influence the microclimate and thus the distribution and abundance of mites. It is an important consideration, if only part of a habitat, for example, part of a floor or mattress, is to be sampled, that the area sampled is representative of the whole area under study.

Methods of house-dust sampling

Vacuum sampling

Vacuum sampling is a widely used method to obtain dust samples from habitats in the home. The efficiency of removing mites from a habitat

Table 1. House-dust-sampling protocols used in vacuum sampling

Authors	Bedroom floor	Living-room floor	Mattress
van der Heide et al. (4)	Total area	Total area	—
Hill et al. (5)	1 m ² , 2 min	1 m ² , 2 min	Whole upper surface, 4 min
Fletcher et al. (6)	1 m ² , 2 min	1 m ² , 2 min	1 m ² , 2 min
Dotterud et al. (7)	—	—	Half upper surface and vertical side, 1.5 min
Wickmann & Korsgaard (8)	—	—	Whole upper surface, 3 min

compared to brushing was shown by Abbott et al. (3), who found significantly fewer mites in brushed samples of dust for both the mattress and carpet than in vacuum-sampled dust.

Most survey studies attempt to standardize their sampling procedure when visiting different homes. This usually involves sampling the same region of a habitat with a defined area and sampling for the same amount of time. There are still clear differences in approach between investigators, and there is often insufficient descriptive information to allow proper comparison (Table 1). A study by van der Heide et al. (4) sampled dust from the total area of the bedroom and living-room floors; however, Hill et al. (5) and Fletcher et al. (6) both sampled a 1 m² area of the bedroom and living-room floors for 2 min (Table 1). Hill et al. (5) specified that the floor area sampled was adjacent to the bed in the bedroom and in the living room adjacent to where the child in the study sat. Fletcher et al. (6) did not specify the area from where the samples were taken. Hill et al. (5) and Fletcher et al. (6) both investigated the mattress; however, while Fletcher et al. (6) used the same sampling procedure, Hill et al. (5) sampled the whole upper surface area of the mattress over a time period of 4 min. In other studies, Dotterud et al. (7) sampled half of the upward-facing surface and vertical side of the mattress and the inside of the bed over a time period of 1.5 min, and Wickmann & Korsgaard (8) had the whole upper mattress surface sampled in a time period of 3 min (Table 1). In the Wickmann & Korsgaard (8) study, the dust samples were collected from the mattress by the child's parents with the family's own vacuum cleaner.

Most studies attempt to standardize vacuum sampling by using the same equipment each time and thereby avoiding differences in the efficiency of dust collection due to the power of the suction or design of the vacuum. A variety of different models, including conventional and filter cylinder and upright cleaners and hand-held models, and manufacturers are cited in the literature (5, 6, 9, 10); however, little work has been done to compare performance. Hegarty et al. (9) compared four filter vacuum cleaners and a conventional upright

vacuum cleaner fitted with either a standard vacuum cleaner bag or one with a pore size of 0.1 µm. Comparisons were made in pairs; the conventional upright vacuum cleaner fitted with the standard or reduced pore size bag, the "beat/sweep" action of an upright filter cleaner compared with the vacuum-alone action of a cylinder cleaner, and two cylinder filter cleaners compared to determine whether different cylinder cleaners varied in efficiency. They found no statistical difference in the weight of dust obtained using the different bags in the conventional cleaner and similarly no difference between two models of cylinder filter cleaners. However, the "beat/sweep" action upright filter cleaner retrieved a greater weight of dust than any other cleaner from eight of nine homes tested with all the cleaners.

A criticism of vacuum sampling is the variable extraction efficiency of live and dead mites. Colloff (2) highlights this when arguing whether mite density per unit weight or mite density per unit area is more appropriate for the expression of mite densities in dust samples taken by vacuum sampling. While the number of dead mites and allergen in the form of faecal material can be related to the quantity of dust obtained from the substrate, the number of live mites extracted from the dust by vacuuming cannot. The discrepancy in efficiency of collection of live and dead mites is due to the live mites' ability to cling to the substrate, actively resisting attempts of the vacuum to remove them (11). Bischoff et al. (12) suggest that vacuum sampling a 1 m² area in 2 min may remove only approximately 10% of the house-dust mites present; more frequently, only 2–5% may be removed.

Controlling for vacuum sampling error is a further problem for the investigator. In a study assessing steam treatment for the eradication of mites, Colloff et al. (13) controlled for sampling error by using two adjacent floor carpet areas, sampling each area before treating one and then sampling both areas after treatment. The reduction of dust-mite allergen content in the steam-treated carpet was significantly greater than the reduction of dust-mite allergen content in the control, a result which was attributed to the vacuum sampling. The use of adjacent areas as comparable samples is

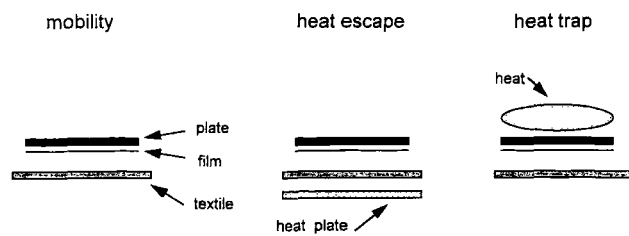


Fig. 1. Mobility and heat-escape sampling from textile.

restricted to large textiles – for example, carpets – because it is assumed that there is a uniformity in the distribution and abundance of mites. This uniformity is not observed in the mattress. Using an *in situ* core-sampling technique, Hay (14) found a patchy distribution of *Dermatophagoides pteronyssinus* within a single mattress, a result which he attributed to the behavioural response of the mite to local changes in temperature and humidity. Fletcher et al. (6) controlled for sampling variation by using paired samples and rejecting the pair if the measured mite-allergen content differed by more than 5%.

Repeat sampling of an area by vacuum sampling may introduce serious bias by depleting dust, which may take weeks to replace, and over this time, the dust composition can change and the mite population dynamics alter. In the course of a clinical trial of mite eradication, there can be additional problems with the use of dry powder or wet vacuuming treatments which alter the dust load of the habitat. In a recent clinical trial of Acarosan powder (5% benzyl benzoate), an attempt to minimize these problems was made by using a “zigzag” vacuum sampling pattern and expressing mite-allergen content as a surface-area-related measurement (10). The “zigzag” vacuum-sampling pattern method, originally described by Colloff (15), results in the repeat sampling of a general area with minimum risk of sampling error, as exactly the same area is not covered twice.

Mobility and heat-escape sampling

The ability of live mites to cling to the substratum can lead to an underestimate of the mite population present. Repeated vacuum cleaning has shown that dust quantities and mite numbers do decrease with increased vacuum cleaning, but that this method fails to remove all live mites (11). Methods of sampling live mites have been developed that take advantage of the mites' natural mobility and behavioural response to local changes in air humidity and temperature. An increase in temperature linked to a fall in relative air humidity will cause the mites to seek a more agreeable environment.

The mobility test involves a surface area to be

covered with adhesive film and weighted with a heavy plate to ensure even contact between the film and the substratum (Fig. 1). The film is left undisturbed for 24 h, preferably in the dark, and can then be peeled off and the mites counted directly under light microscopy (11). Under the favourable climatic conditions of temperature (25°C) and relative air humidity (75%), approximately 30% of the total mites seeded on various carpet fabric were shown to be recoverable, although under less favourable conditions of relative air humidity (25%), this figure was reduced to 8% (11, 16).

The heat-escape method is a further dimension to the mobility test, involving the application of heat on the back of the textile object (Fig. 1). A gradual increase in temperature causes a directional movement of the house-dust mites away from the source of the heat toward the adhesive film. Bischoff et al. (16) found that up to 65% of mites released on a carpet were recoverable by this method. An estimate of the total mite population in a carpet fragment was proposed by de Boer & Kuller (17), a method involving extrapolating the total number of mites from repeated consecutive heat-escape procedures. Confounding factors of the heat-escape method which lower the recovery of mites include the increasing complexity of the textile structure or condensation on the adhesive film. However, considerably higher estimates of live mite density are always achieved than with vacuum sampling (12).

A modification of the heat-escape method is described by Colloff (2) for use when it is not possible to access textiles on both sides. It involves a heat trap on the surface of the textile that encourages mites to move toward the heat. The heat source is mild enough not to decrease humidity but warm enough to increase the mites' mobility.

The choice between vacuum-sampling or heat-escape methods will depend on the purpose of the study. The heat-escape method provides a more accurate estimation of actual live mite populations, a result which would be important if estimating the rate of mite recolonization after mite-eradication treatment; however, for a clinical trial investigating mite-allergen exposure, a vacuum dust sample may be more relevant.

Methods of extracting mites from house dust

A variety of methods for extracting live and dead mites from house dust have been developed (reviewed by Wharton [18]). The most common in use today are flotation and suspension. The heat-

escape method is limited to detecting living mites, with only a small proportion of dead mites reaching the adhesive film by mechanical disturbance. In both the flotation and suspension methods, as the dust composition increases in complexity, mites became trapped in the dust material. For this reason, some investigators have sieved the dust before extraction in order to remove the fibrous material that can trap the mites during the extraction process. Sieving the dust results in more easily manageable samples and has been used to standardize mite density per unit weight.

Flotation

Flotation involves exploiting the difference in density of the mites from the aqueous media in which the dust sample is submerged. Hart & Fain (19) submerged 100 mg of dust in 70% ethanol for a minimum of 4 h, decanted the supernatant, and resuspended the dust material in distilled water, which was then poured into a glass Petri dish. The mites float to the surface to be relatively easily removed. The efficiency of this method appears to depend on the composition of the dust sample and the vigilance of the investigator. Colloff (2) found a geometric mean extraction for 33 samples of house dust of only 27% (range 3–88%). The method appeared to be efficient for samples containing little or no fibrous material; however, mites were noted to stick to the walls of vessels when supernatants were decanted. The flotation method still has advantages over other extraction procedures; in particular, the simplicity of the method. Large numbers of samples can be processed in a short time, and provided all samples are treated in the same way, comparisons of relative mite exposure or assessment of mite-eradication treatment can successfully be made.

Suspension

Suspension extraction methods involve wetting and dispersing dust samples in a suitable media – for example, saturated sodium chloride with detergent, or 90% lactic acid with heating – which can then be stained to distinguish between dust material and mites. The suspension can be examined in one dish or decanted through a series of dishes until all the fluid has been examined (2). Sodium chloride suspension has proven to have an advantage over lactic acid suspension, as it does not kill the mite and an accurate assessment between live and dead mites can thus be made (20). Where a sampling procedure or extraction method results in killing the mites, an assessment of live and dead mites present at the time of sampling can be inferred

from the observed “intact” (presumed live) and “damaged” (presumed dead) mite bodies (20).

Studies on the age structure and dynamics of house-dust-mite populations have shown for *D. pteronyssinus* that half or more of the mites in a dust sample may be represented as eggs (21). The contribution eggs make to the allergen pool has still to be determined; however, they can be readily visualized in a lactic acid suspension, staining heavily upon addition of lignin pink (21).

Alternative quantitative measures

Specific assays for house-dust-mite allergen

The identification, purification, and characterization of two major allergens, Der p 1 and Der p 2, from *D. pteronyssinus* (22, 23) led to various immunochemical assays being developed for their detection, including RAST inhibition, the radio-immunoassay (RIA), and the enzyme-linked immunosorbent assay (ELISA) (24). These assays provide an alternative means of quantifying house-dust mites with respect to assessing mite exposure and avoid the difficulty of extracting, identifying, and counting mites.

The most common assay in use today is the two-site monoclonal antibody-based ELISA (25, 26). Samples are assayed against standards to determine an accurate measurement of allergen content. Exposure to more than 2 µg Der p 1/g of dust during infancy has been proposed as a significant risk factor for sensitization, and exposure above 10 µg Der p 1/g of dust during infancy is associated with the development of asthma by the age of 11 years and hospital admission (27, 28).

Relating mite-allergen levels to mite density is not simple because the faecal allergen pool is stable and represents past populations of mite as well as current (29). It has been suggested that 10 µg/g of dust is approximately equivalent to 500 mites/g of dust; however, mite-allergen levels provide no information on species diversity and the relative mite density of the various mite species present (24).

Specific assays for house-dust-mite allergen have mainly been used on vacuumed samples of dust; however, it does not necessarily follow that dust concentrations of allergens reflect airborne concentrations of allergens which are inhaled. Price et al. (30) found a significantly higher concentration of allergen above a wool carpet than for both synthetic carpets and hard floors, a fact which could not be explained by a greater allergen concentration in the floor dust.

A few studies have attempted to sample and quantify airborne allergens occurring as a consequence of natural activity. Oliver et al. (31) found

18/45 airborne samples taken at 2 l/min with a Casella air sampler attached to the pillow overnight had detectable levels of Der p 1, and two samples also had detectable Der p 2. With the same sampler used in the same way, Custovic et al. (32) found that only 2/32 samples taken from the pillow of asthmatic subjects had detectable levels of Der p 1 or Der p 2. In the same study, Custovic et al. (32) found that the levels of Der p 1 and Der p 2 in vacuumed dust samples taken from the bed correlated with the clinical activity of asthma. It is clear that current techniques for airborne sampling need to be improved before they can be used as an accurate means of assessing house-dust-mite exposure.

Indirect assays for house-dust-mite allergen

A simple indirect measurement of house-dust-mite allergen was developed by Bischoff & Schirmacher (33), who measured the amount of guanine, a nitrogenous excretory product of mites, in a simple colour reaction by fixing it to an azo dye with a red colour. The test is semiquantitative, because it detects guanine produced by any mite species and also cross-reacts with xanthine (34, 35). Comparing pure mite cultures with house dust, Hallas et al. (35) found that 63% of the guanine detected in house dust originated from non-house-dust-mite sources. Despite this, comparisons between measurements of guanine and of Der p 1 and Der f 1 by RIA and ELISA have shown positive correlations (36, 37).

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