

Anti-mite Covers: Potential Criteria for Materials Used Against Dust Mites

Abstract There are many types of material used to construct bedding encasing for dust mite and allergen protection, but relatively few have been evaluated for their anti-mite properties. This study surveyed the anti-mite/allergen properties and physical characteristics of covers with a view towards establishing potential parameters for evaluating the efficacy of such encasings. Fifty-three covers advertised as mite-proof were randomly purchased from local markets found in ten different countries. All fabrics were evaluated for their ability to block the movement of allergens and live mites. Air permeability, dust leakage, and thread count, where applicable, were also measured. The major finding was that plastic and the tightly woven covers completely prevented mite penetration while exhibiting > 99 % allergen impenetrability. The film-coated fabrics, the mixed type, and the acaricidal-coated non-woven were successful in blocking allergens, but were commonly observed to allow some mite penetration. For the tightly woven fabrics, dust leakage and air permeability were minimal, while thread count was uniformly high. Cotton bed sheets were completely penetrable by mites and allergens. Overall, allergen impenetrability was negatively associated with dust leakage, thread count, and air permeability. Thread count also exhibited inverse relationships between dust leakage and air permeability. Dust leakage and air permeability were positively associated. The optimal characteristics for effective encasings include tightly woven covers with allergen impenetrability > 99 %, resistance to live mite penetration, dust leakage of < 4 %, air permeability between 2 and 6 cm³/sec/cm², a thread count $\geq 246/\text{in}^2$, and a pore size of 2–10 μm .

Key words Allergen/mite-proof encasements, fabric/material characteristics

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House dust mites (HDMs) are microscopic animals about 300 μm in size that are invisible without the aid of a microscope or magnifying glass. They are typically found in household dust and predominantly reside in bedding [1], especially pillows and mattresses which are their major habitat for breeding and colonization. HDMs thrive on human skin scales, bacteria, and other organic matter. Eventually the fully decomposed particles become fecal droppings. Der p1, the marker allergen found in mite feces [2], readily induces allergic symptoms in susceptible allergic patients when inhaled. Over the long term and without adequate protection, asthma may develop. Reducing exposure to mite allergens often leads to improvement of clinical symptoms which is the easiest and most practical method to implement and often is prescribed in parallel with clinical treatment. The most common methods for controlling allergens involve physical measures such as covering bedding with mite and allergen occlusive covers, the regular laundering of blankets and bed linen, and removing carpets and replacing them with vinyl flooring [3]. Of these measures, encasement of bedding with impermeable covers is widely used [4–6], and in some countries is indispensable as an initial strategy.

However, a difficulty with this approach is the uncritical acceptance of all covers as being equally effective in preventing mite/allergen penetration, perhaps, because there has been remarkably little information or research on anti-mite covers. Attention must be focused on the type of materials used in the construction of these encasing products which operate according to different principles and mechanisms in the eradication of mites. For example, tightly woven fabrics are derived from twisted cotton or synthetic fibers perpendicular to each other and having either a regular, systematized bi- or tri-axial weaving pattern [7]. Such a construction allows little space for mites to penetrate and if its pore size is less than 10 μm , it will prohibit allergen penetration [8]. On the other hand, non-woven fabrics are made from spun-bonded hot filaments laid down randomly, fused together at their cross points and further bonded by heat and pressure [7]. Consequently, manufacturers suggest not washing this fabric as frequent washing would deteriorate and loosen the fibers, thereby creating access points for mites. Because of this disorganized, non-systemized disarray of synthetic fibers, no consideration is given to pore size in its manufacture. Plastic is an effective barrier [9, 10] because it is pore-free, but since it prohibits the passage of air, it is uncomfortable. Some covers have a bonded layer of film or membrane adhered to one side of a loosely woven or non-woven cloth. Acaricides which eradicate mites upon contact have also been incorporated directly into encasements. Recent developments in fiber- and nano-technology hold promise as an effective delivery system for depositing acaricides directly into the fibers of bedding materials. In one study [11], the anti-mite inorganic compound, ceramite, was introduced into the structure of spun polypropylene

non-woven fibers and when inserted into the bedding of mite allergic patients resulted in a statistically significant decrease in mite guanine concentration. Guanine is the major nitrogenous excretory product of arachnids found in house dust, and it is directly correlated with specific mite allergens. In a second study [12], eugenol-loaded chitosan nanoparticles having potent anti-fungal and anti-dust mite activity were dip-coated onto cotton fabrics. This resulted in greater HDM mortality depending on the percentage concentration and the duration of exposure.

A recent study [13] which tested for mite penetration revealed that every single tightly woven cover was completely successful in this regard. It was hypothesized that if certain physical characteristics of this type of cover were known, it might prove beneficial in establishing criteria for the successful prevention of mite and allergen penetration into bedding materials. Therefore, the purpose of this research was to survey additional samples of encasing products in terms of their ability in blocking the passage of live mites and their allergens using the biochemical technique of enzyme-linked immunosorbent assay, ELISA, for the quantification of allergen presence. A secondary purpose was to study the association between certain physical characteristics of bedding fabrics with a view towards establishing promising potential criteria for effective encasements.

Methods

Fabric samples

Fifty-three mite-proof covers (pillowcases or pillow protectors) claiming to be "anti-mite" were obtained from ten different countries and were classified into eight categories according to fabric texture and the information disclosed on their packaging: (a) tightly woven ($n = 30$), (b) film/membrane-coated woven ($n = 4$), (c) acaricidal-coated woven ($n = 1$), (d) non-woven ($n = 10$), (e) membrane-coated non-woven ($n = 3$), (f) acaricidal-coated non-woven ($n = 1$), (g) plastic ($n = 1$), and (h) a combination of woven and non-woven ($n = 3$). For comparative purposes, three regular cotton bed sheets, procured locally, were also studied for mite and allergen penetration, resulting in a total of 56 fabrics being tested.

The fabric samples were divided into two sets: a mite/allergen penetration set and a physical examination set. The first set was assessed by the heat escape method, ELISA, and for dust leakage. These tests were performed in the Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. The second set was sent to the Thailand Textile Institute (THTI) for examination of nine non-mite physical characteristics of the fabrics, i.e., air permeability, thread count, fabric type, fabric weight, fabric thickness, structure weave, warp yarn, welf yarn, and density. However, only air per-

meability and thread count are reported in the present study. All assessments were completed without knowledge of the results of any other evaluations.

House dust mites

The *Dermatophagoides pteronyssinus* mites used in this study were maintained at the Siriraj Dust Mite Center for Services and Research (SDMC), Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. The mites were separated from their culture media by a mite isolator; a specially designed apparatus which forces the mites down and away from the food source.

Heat escape method [14]

This method evaluated the ability of the material to block the penetration of live mites within a short period. An average of ten adult mites of either gender selected under a stereomicroscope was placed in contact with either the outer or inner surfaces of a test fabric stretched over a 50 ml beaker filled three-quarters with water. A 60 W light bulb was then placed 10 cm above the fabric and illuminated for 15 min. Observation of mite behavior was made continuously throughout this period. Mites would try to penetrate the fabric to escape the heat. If the fabric was porous, mites could then easily pass through. The experiments were replicated three times for both sides, resulting in six samples/fabric. If a single mite penetrated any fabric, it was considered as a penetration. The percent penetration was calculated from the number of penetrated fabrics divided by the total number of fabrics tested in that particular category.

Enzyme-linked immunosorbent Assay [14]

The measurement of mite allergens (Der p1) is determined by two-site monoclonal antibody based-ELISA (Indoor Biotechnologies, USA). Dust samples are incubated in a 96-well microtiter plate precoated with a specific capture antibody. The Der p1 in the sample binds with the specific antibody, thereby becoming immobilized. The microwells are washed several times, after which an enzyme conjugate is added. The antibody of the enzyme conjugate binds with the immobilized antigen (Der p1) to form a sandwich of antibody-antigen-antibody/enzyme bound to the microwell. The amount of antigen-antibody complex bound to the microwell is directly proportional to the amount of mite allergens in the dust sample. The higher the concentration of mite allergens in the dust samples, the greater is the intensity of the emitted color (detectable signal), allowing quantification of the antigen in the antigen/antibody complex. This method was applied to evaluate the efficacy of the various fabrics in blocking mite allergens.

Briefly, artificial dust was pooled from a mixture of 5 g house dust and 0.1 g of *D. pteronyssinus* spent mite medium, and then homogenized by sieving successively three times through a 500 μm mesh. In order to determine the concentration of mite allergens in the initial dust, ten randomly chosen aliquots of dust were assayed for the concentration of the major mite allergen, Der p1. Five grams of the dust containing the known amount and concentration of Der p1 were then placed between two layers of the test fabric which was vacuumed through the top surface for 2 min. The retrieved dust was weighed and assessed for its Der p1 concentration. The percentage of allergen impermeability of the fabric and the percentage of dust leakage through the fabric were calculated according to the following formulas:

$$\% \text{ Permeability} = \frac{\left[\begin{array}{l} \text{Total amount of Der p1 in the} \\ \text{dust recovered from the encasing} \end{array} \right]}{\left[\begin{array}{l} \text{Total amount of Der p1 in the} \\ \text{dust added to the encasing} \end{array} \right]} \times 100 \quad (1)$$

The total amount of Der p1 (μg) was obtained by multiplying the allergen concentration ($\mu\text{g/g}$ of dust) by the total amount of dust (g) as indicated in the numerator above. Allergen impenetrability was simply obtained by subtracting the % Permeability from 100 %. The percentage of dust leakage was determined by:

$$\% \text{ Dust leakage} = \frac{\text{Weight of the retrieved dust}}{\text{Weight of the initial dust}} \times 100 \quad (2)$$

Thread count

Thread count represents the coarseness or fineness of a fabric. It was performed according to the International Organization for Standardization (ISO), Direct Counting (Method A) [15] and was defined as the number of horizontal (warp or lengthwise) and vertical (weft or widthwise) threads in 1 in² of the fabric [16]. Thus, only woven fibers could be analyzed for thread count. Each fabric sample was measured five times at a minimum distance of 2 cm.

Air permeability

This is defined as the rate of air flow passing perpendicularly through a known area under a standard differential pressure of 0.5 inches of water between the two surfaces of the test samples. From the rate of air flow, the air permeability of the fabric is determined and expressed in SI units (Système Internationale d'unités) as cubic centimeters per second per square centimeter of fabric ($\text{cm}^3/\text{s}/\text{cm}^2$). Air permeability was tested according to the American Society for Testing and Materials (ASTM) D737 protocol [17] using a Frazil Auto Air Permeability Tester (Model AP-360D). Each fabric sample was tested ten times.

Table 1 The total number (N) and percentages of fabric samples showing mite penetration by the heat escape method and the mean allergen impenetrability percentages with their standard deviations.

Types of fabrics	Heat escape		Allergen impenetrability %		
	N	# Fabrics (%)	N	Mean	SD
Plastic	1	0	1	100	–
Film-coated non-woven	3	2 (67)	3	99.9	0
Film-coated loosely woven	4	4 (100)	1	99.9	–
Non-woven	10	5 (50)	8	95.6	8.6
Tightly woven	30	0	27	99.0	3.8
Woven mixed with non-woven	3	2 (67)	3	98.5	1.5
Acaricidal-coated non-woven	1	1 (100)	1	97.5	–
Acaricidal-coated loosely woven	1	1 (100)	1	0	–
Regular bed sheet	3	3 (100)	3	37.7	53.0

Statistical methods

Computer analysis using SPSS 13 was employed for all descriptive measures including all means, standard deviations, standard errors, confidence intervals, and Pearson's product moment correlation coefficients.

Results

The percentage of fabrics showing mite penetration by the heat escape method is presented in Table 1. There were two covers (tightly woven and plastic) showing no penetration (0 %); the woven mixed with the non-woven covers and the film-coated non-woven covers showed some penetration (67 %); and four covers (film- or membrane-coated woven, acaricidal-coated non-woven, acaricidal-coated loosely woven, and the regular bed sheets) exhibited 100 % penetration. Fifty percent of the non-woven covers were found to be penetrable by dust mites.

Table 1 also presents the degree of allergen impenetrability in terms of mean percentages for the number of samples in each category. In evaluating the ELISA protocols for allergen impermeability, six of the nine categories had an average greater than 97 %. However, when the raw data (not given) were examined, the situation became clearer as 39/48 covers fell above 97 %, including the one plastic cover, all four of the film-coated covers (three non-woven and one loosely woven fabrics), five non-woven samples, 25 of the tightly woven covers, two of the mixed type, one acaricidal-coated non-woven cover, and one of the regular bed sheets. Thus, the acaricidal-coated loosely woven cover, three of eight non-woven covers, two of the tightly woven covers, one of three mixed type covers, and two of the regular bed sheets offered no or only minimal to some protection from allergen infiltration. The 99 % CI for the tightly

woven fabrics extended from 97.09 % to 100.91 %. For the 25 tightly woven samples above 97 %, the allergen impenetrability was at or above 99.7 %.

Table 2 presents the data for dust leakage and air permeability. For dust leakage, seven of the categories had an average less than 5 %, the exceptions being the acaricidal-coated loosely woven cover and the regular bed sheets. Of the tightly woven samples (99 % CI, 0.15–3.51 %) whose average was 1.83 %, 25 of them had leakage of 2.2 % or less. For the eight non-woven samples whose average was 4.32 %, five were less than 1.15 %. The plastic cover had absolutely no dust leakage, while two of the regular bed sheets and the acaricidal-coated loosely woven fabric had dust leakage > 85 %.

For air permeability, four of the fabric types had an average pressure differential less than 5 cm³/s/cm², with the remainder being much larger. For the tightly woven samples (99 % CI, 1.96–6.08 cm³/s/cm²) whose average was 4.02 cm³/s/cm², 18 were less than this value and greater than zero. For the non-woven fabrics, four of the six had a zero air flow, while the remaining two were greater than 90 cm³/s/cm², thus spuriously inflating the value of the mean. In addition, the plastic cover and three of the film-coated fabrics (loosely woven and non-woven) all had zero air flow.

Only woven fabrics could be assayed for thread count. The values of the mean thread counts per square inch with their standard deviation (SD) and N in each category were as follows: (a) 26 tightly woven samples had a mean of 265.6 with a SD of 38.6 (99 % CI, 246.07–285.13); (b) three woven samples mixed with non-woven fibers had a mean of 213.7 with a SD of 9.0; (c) the one acaricidal-coated loosely woven sample had a mean of 125.0, while (d) two regular bed sheets exhibited a mean of 180.5 and a SD of 0.7. Twenty-four of the 26 tightly woven samples had a thread count greater than 238. None of the other six samples from the remaining three categories approached this value.

Table 2 The number and means with their standard deviations of samples showing the percentage of dust leakage and the air permeability index.

Types of fabrics	Dust leakage %			Air permeability (cm ³ /s/cm ²)		
	N	Mean	SD	N	Mean	SD
Plastic	1	0	—	1	0	—
Film-coated non-woven	3	0.6	0.2	2	0	0
Film-coated loosely woven	1	0.1	—	2	0.4	0.5
Non-woven	8	4.3	6.2	6	38.4	61.3
Tightly woven	27	1.8	3.4	27	4.0	4.2
Woven mixed with non-woven	3	2.0	1.6	3	28.8	18.3
Acaricidal-coated non-woven	1	3.8	—	1	72.8	—
Acaricidal-coated loosely woven	1	100	—	1	140.6	—
Regular bed sheet	3	62.6	52.6	2	52.4	1.5

Figures 1–3 show the results of correlational analyses depicted as straight-line associations and the Pearson correlation coefficients between the important non-mite parameters. All correlations were statistically significant at $p = 0.0001$ or better. Figure 1 depicts in Panel A a negative correlation between allergen impenetrability and air permeability ($r = -0.61$), and in Panel B a positive association with thread count. Thus, as allergen impenetrability increased, the air permeability decreased, and as thread count increased, so did the degree of non-penetration by allergens. Figure 2

shows an almost perfect inverse relationship ($r = -0.9995$) in Panel A between dust leakage through the fabrics and allergen impenetrability, but a positive relationship with air permeability in Panel B ($r = 0.59$). However, this latter correlation may have been spuriously affected by the two outlier points (51, 99) and (140, 100). Therefore, and within limits, it is suggested that an increase in air permeability may have been accompanied by a greater amount of dust leakage. Similarly, high dust leakage was associated with high allergen penetration (or low allergen impenetrability).

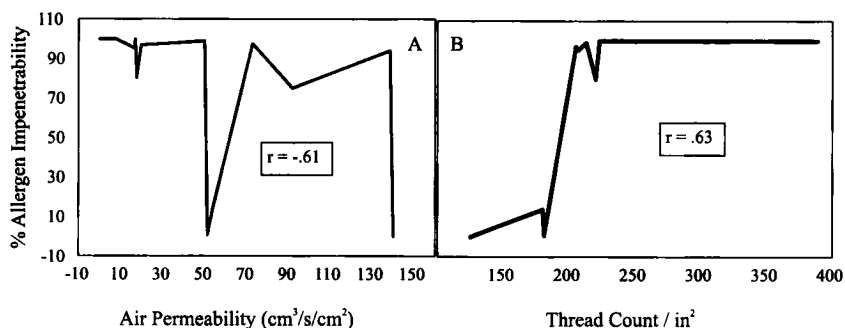


Figure 1 The correlation between the percentage of allergen impenetrability with both air permeability and thread count depicted as a straight line association.

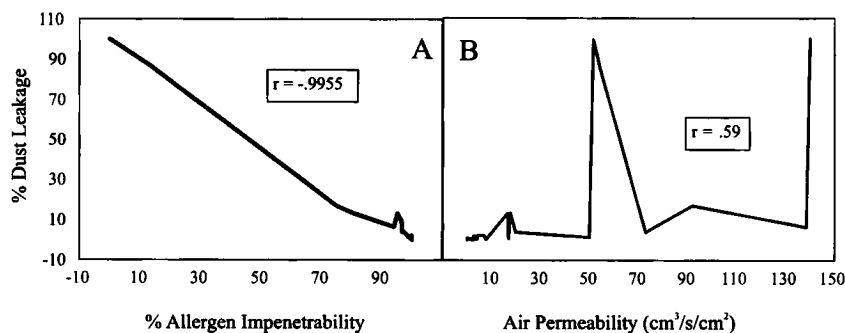
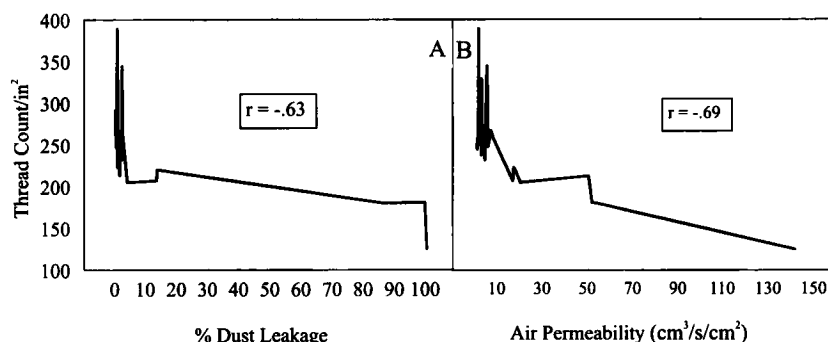


Figure 2 The correlation between the percentage dust leakage and percentage allergen impenetrability and air permeability represented as a straight line association.

Figure 3 The correlation between the thread count and percentage dust leakage and air permeability described as a straight line relationship.



The two negative correlations in Panels A (-0.63) and B (-0.69) of Figure 3 suggest that by increasing thread count, dust leakage and air permeability were considerably decreased.

Discussion

The purpose of this study was to survey and evaluate encasing materials from different countries which claimed to be mite-proof in their ability to block the passage of live mites and their allergens. A secondary purpose was to suggest likely characteristics that all successful encasements should possess. While tightly woven covers and plastic are clearly superior over other types of covers in preventing mite penetration [13, 18], the question remained as to their effectiveness in doing the same against allergens. Most clinical encasing studies [19–23] reporting a significant reduction in mite allergen levels do not describe the materials used in the construction of the covers. The one clinical study [24] that specifically mentions using tightly woven mite allergen-impermeable covers did not measure the level of mite allergens in bedding, but did report a significant reduction in symptom load in mite allergic individuals. The results of the present study indicate that the physical characteristics of tightly woven covers could suggest promising parameters for all encasing products against HDMs and their allergens.

In addition to preventing mite penetration by providing a physical barrier to the excursion of live mites from the external environment through the strata of bedding fabrics, all anti-mite covers should provide almost complete allergen exclusion. Allergens must be prevented from entering or if produced by resident mites from exiting the impermeable cover and becoming airborne. These results indicate that live mites do not penetrate tightly woven fabrics from either the outside or the inside, which confirms an earlier result using the Siriraj Chamber method [13].

The allergen impenetrability data suggest that most of the tightly woven fabrics as well as plastic, the film-coated

woven and non-woven samples afforded 99 % or greater insulation against aeroallergens. Therefore, a useful minimal cut-off for the tightly woven fabrics is 99 %. In this regard, it should be noted that regular bed sheets do not offer any protection from live mites and allergens, and do not qualify as potential covers.

The upper limit of the CI for dust leakage of the tightly woven samples (3.51 %) may be considered as another component of our criteria list. However, it is possible that high levels of dust may contain low levels of allergens, and consequently, the dispensed aeroallergens may be inconsequential. In general, dust leakage through these samples did not seem to be a major problem with the exception of two of the regular bed sheets and the acaricidal-coated loosely woven fabric.

Since the number of horizontal and vertical threads per square inch of fabric represents the coarseness or fineness of a fabric, thread count is another physical characteristic playing a role in the efficacy of anti-mite covers. In this study, the mean thread count of 26 tightly woven fibers was decidedly higher than the remaining six non-tightly woven samples (187.8). The criterion of greater than 246 thread count/in² was chosen as the lower limit of the CI offering greater than 99 % protection from aeroallergens based on a frequency count of the tightly woven samples exceeding this mark.

The venting of air and water vapor through the planar surface of the cover provides a modicum of comfort derived from the prevention of sweating induced by heat accumulation [24] that other covers (e.g. plastic) do not exhibit [25]. The mean air permeability for the tightly woven samples was low, but not zero, which contrasted sharply with the non-woven fabrics, the three mixed type samples, the regular bed sheets, and both acaricidal-coated fabrics. McLaughlin et al. [26] have suggested that finely woven fabrics with pore sizes of 10 and 20 microns, which impeded only 5 % of air flow, were effective in blocking ~95 % Der p1. If the pores sizes were further reduced to 6 and 2 microns, air flow was impeded by 40 and 74 %, respectively, but still blocked 98 % of dust allergens. If air permeability is increased, there is a greater chance of dust

and its components from penetrating into the cover. The current data indicate that the tightly woven samples, in general, exhibit a unique combination of air porosity and mite/allergen retardation. Hence, it is recommended that the criteria for this variable be the exact 99 % confidence limits.

We found a significant inverse relationship between thread count and air permeability as well as dust leakage suggesting that within limits, the higher the thread count, the less space will be allocated for air and dust penetration. The three highest thread counts of the tightly woven fabrics with their respective air permeability scores were 389 and 1.57, 344 and 5.26, and 329 and 2.80, which suggests that for these samples, the lack of "breathability", which is a comfort issue, might be sacrificed for increased mite and allergen protection. The correlational data indicate that the higher the thread count, the greater the allergen impenetrability. This, however, is a moot point insofar as 24 of the 26 tightly woven samples all had allergen impenetrability scores between 99.7 % and 99.9 %, but their thread counts varied from a low of 232 to a high of 389. Thus, while all tightly woven samples are not equal in thread count, the percentage of allergen impenetrability they offer is for all intents and purposes, equal. Furthermore, if the number of fibers in the thread count was increased, dust leakage would decrease; and as allergen impenetrability increases by any and all of the means cited above, there is less chance of dust with its inherent components penetrating any type of fabric.

It is not unreasonable to assume that the structural characteristics and efficacy ratings of various covers, with the possible exception of plastic, will be substantially changed after continued use and repetitive launderings over time. A recent study [13] did show that after four months of use, two brands of non-woven covers exhibited worn and matted surface threads, easily providing access points for mite penetration. To our knowledge, no experimental, non-clinical study has evaluated the structural integrity of tightly woven covers for withstanding mite and allergen penetration after repeated washings and use over a long period of time. However, a number of studies have recorded allergen decrements as a result of impermeable encasements alone after nine weeks [5], six months [27, 28], and one year [22, 23], which suggests that the structural integrity of these encasements was not compromised.

From all of the above, it may be concluded that plastic and tightly woven covers do indeed block mite penetration and prevent the leakage of allergens, while other types of covers are sufficiently lacking in this regard. Therefore, it is suggested that the following criteria, which have been rounded off to whole numbers, are optimal for the choice of an effective encasing product against house dust mites and their allergens: (a) the potential candidate must prohibit mite penetration and exhibit an allergen impermeability of 99 % or better; (b) Vaughan et al. [8] recommend the fabric should have a pore size between 2 to 10 μm ; (c) dust leakage

through the fibers of the fabric should be less than 4 %; (d) air permeability should be greater than 2 and less than 6 $\text{cm}^3/\text{s}/\text{cm}^2$; and (e) a thread count greater than 246/in² would be sufficient. All the randomly collected covers used in this study were then screened by these five criteria. It was found that 41 % of the fabrics did not satisfy these basic requirements.

It is our concerted opinion that tightly woven covers are the only type of fabric fulfilling all of these requirements, at the present time. This information may be useful for manufacturers to produce such medical textiles and also have relevance for allergists and dust mite sensitive allergic patients as an initial approach in allergen and mite avoidance procedures. Moreover, it should be provided as packaging information for consumers.

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