Research Note

New wet sieving method for isolating house dust mites

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Abstract: A new wet sieving method for isolating house dust mites from dust was developed by modifying the Furumizo method. In five successive sievings of samples collected by vacuum cleaners, the average proportion of mites isolated in the first three sievings to the total number was 98.4% (ranged from 89.5 to 100.0%) for live mites and 98.7% (90.8–100.0%) for dead mites. Recovery rates of 100 live *Dermatophagoides farinae* marked with Rhodamin B in each of four 500 mg dust samples ranged from 84–95% by three times of sieving; and of dead ones, from 91–98%. This new method was superior to the Darling solution centrifugation method, as far as the isolation efficiency was concerned, when these two methods were compared among five samples each of 0.5 g dust.

INTRODUCTION

Several techniques have been developed for the isolation of house dust mites, such as flotation, centrifugation, and a combination of flotation or centrifugation and sieving. In the flotation techniques, mites are isolated by the difference in the specific gravity between mites and dust particles. Oshima

(1964) isolated mites from dust by an ether-tetrachloride mixture. Largest number of mites were observed in a fraction with a specific gravity less than 1.50 and greater than 1.45. Mites are also floated in a saturated sodium chloride solution (Sasa *et al.*, 1970), Darling solution (Bronswijk, 1973), and 90% lactic acid (Korsgaard, 1982), each solution having a specific gravity of 1.2. But the mites are not completely isolated from the dust because the difference in the specific gravity between mites and dust is small. Spieksma and Spieksma-Boezeman (1967) added centrifugation to the flotation techniques to raise the isolation rates of mites, and Miyamoto and Ouchi (1976) also developed a similar combined method.

Spieksma and Spieksma-Boezeman (1967) reported that when the dust was sieved under a dry condition, 96.8% of the mites which slipped through a 0.5 mm-mesh sieve were retained by a 0.075 mm-mesh sieve. But, in this case, mites were hidden by small dust particles which adhered to them even after the sieving. Furumizo (1973) developed a wet sieving method in which mites were cleaned with water, and no centrifuge was required for the isolation. According to Wharton (1976), Furumizo obtained isolation rates of 96–98%.

In the present paper, a new wet sieving technique which modifies the Furumizo method is reported, and the efficiency of mite isolation is compared with that of the Darling solution centrifugation method developed by Miyamoto and Ouchi (1976), heretofore one of the most popular methods used.

DESCRIPTION OF THE METHOD

Figure 1 shows a schema of the new method. The procedure is as follows. 1) A dust sample is weighed under a dry condition. 2) The sample and 80 ml of 50% ethanol are put into a 100-ml wide-mouth jar. 3) This jar is shaken for 1 min. 4) The contents of the jar are poured through a series of 0.42 mm and 0.075 mm-mesh sieves; the sieves are then washed in running water for 30 sec. To obtain a higher isolation rate, the dust retained on the 0.42 mm-mesh sieve is put into the jar with 50% ethanol and sieved again. 5) The sample retained on

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Fig. 1 Schematic diagram illustrating the isolation process of mites.

the 0.075 mm-mesh sieve is put in 1% methylene blue for several seconds to dye the dust; mites are not dyed by this treatment. 6) The dyed sample is rinsed out to an open-ended cylinder (6 cm in diameter and 4 cm in height) which is put on a piece of filter paper (7 cm in diameter) placed in a Buchner funnel; the sample may leak out between the filter paper and the funnel if the cylinder is not used. When the sample is large, it is divided into two or three pieces of filter paper. Before sucking air from the funnel, water is poured into the cylinder from a washing bottle to disperse the dust particles uniformly on the filter paper. 7) Mites are picked up from the filter paper under a stereoscopic microscope ($\times 20$). The first six steps of this method are performed in 5 min.

I modified the Furumizo method in the following way. 1) The sample is weighed before soaking in ethanol. In the Furumizo method, it takes 24 hr to measure the volume of the sample in the sediment in 65% ethanol. When the dust stays on the surface of the ethanol, the volume can not be measured. 2) A wide-mouth jar is used instead of a metallic cloth, because some mites remain

on the metallic cloth despite frequent washings. 3) The series of sieves is washed in running water after every sieving. This raises the isolation rate of mites. 4) The sample retained on the 0.075 mm-mesh sieve is dyed. 5) The sample is collected on a piece of filter paper instead of a sorting tray. It is not easy to examine mites in suspension in the sorting tray, because some mites stay on the surface of the ethanol and the microscope focus must be frequently adjusted.

EFFICIENCY OF THE NEW METHOD

The necessary number of sievings in the new method was determined by repeated sievings of dust samples. The samples were collected from 22 houses by vacuum cleaners. The average weight of the samples and its standard errors were 459.9 ± 81.9 mg; 14 of the samples weighed under 500 mg. Each sample was sieved five times. The isolated number of mites was recorded for each of the five sievings. The cumulative proportion of mites isolated in each sieving to the total number of mites isolated in the five sievings was shown in Table 1. By the third sieving, at least 90% of the total number of mites had been isolated; the minimum value of the isolation rate increased only 5% in the 4th sieving. Therefore, three-time sievings are recommended in this method.

The isolation efficiency might be overestimated in the above test, because not all mites were isolated in those five sievings. Four 500 mg-dust samples were used to examine the recovery rates by the method. In each sample, 100-live and 100-dead *Dermatophagoides farinae* marked with Rhodamin B were released. The samples were sieved three times, and the number of isolated mites was recorded for each sieving. The recovery rates ranged 67–78% for live mites and 57–81% for dead mites at the first sieving, and 82–90 and 84–96%, respectively, after the second sieving (Table 2). The third sieving slightly increased the total recovery rates. The recovery rates were lower than the estimates of isolation rates. This is probably due to the loss of a few mites during isolation.

Estimates of the isolation rates reported by Wharton (1976) are 60–80% (Bronswijk, 1973), 70–90% (Spieksma, 1967), and 96–

Table 1 Isolation efficiency of mites in the successive sievings.

Number of sievings	Isolation efficiency (%)*					
	Live mite**			Dead mite**		
	Average	Min.	Max.	Average	Min.	Max.
1	82.3	37.4	100.0	84.4	38.2	100.0
2	95.1	78.9	100.0	96.5	79.8	100.0
3	98.4	89.5	100.0	98.7	90.8	100.0
4	99.5	94.7	100.0	99.6	96.3	100.0

* The cumulative proportion of mites which had been isolated in each time of successive sievings to the total number of mites isolated in five sievings. ** The number of samples and the average weight $\pm$ standard error were 22 and 459.9 ± 81.9 mg, respectively; among these samples, one contained no live mite.

Table 2 Recovery of marked mites in successive sievings by the new method.

Sample	No. of isolated mites							
	First sieving		Second sieving		Third sieving		Total	
	Live	Dead	Live	Dead	Live	Dead	Live	Dead
1	68	57	19	27	6	9	93	93
2	67	75	15	21	2	1	84	97
3	73	80	14	13	8	4	95	97
4	78	81	12	15	1	2	91	98

Before the first sieving, 100 live and 100 dead mites were marked with Rhodamin B and released in each dust sample.

Table 3 Comparison of mite numbers isolated by two methods.

Sample No.	No. of isolated mites	
	Darling solution centrifugation	New method
1	18	36
2	67	73
3	157	231
4	65	108
5	45	57

98% (Furumizo, 1973); Miyamoto and Ouchi (1976) estimated the isolation rates of their method to be 73.2–89.6%. Comparing with these estimates, isolation efficiency by the new method was higher.

The numbers of isolated mites by the new method were compared with those by the Darling solution centrifugation method

(Miyamoto and Ouchi, 1976). Two series of dust, 500 mg each, were taken from each of the five dust samples. One series was examined by the new method, and the other series was by the Darling solution centrifugation method. In all the tests, a larger number of mites was isolated by the new method (Table 3). The number of dead mites isolated by the new method was 1.1–2.0 times that isolated by the other method. Lower recovery rates by the latter method were ascribed to a high proportion of mites remaining in the sediment. Eleven mites were detected in the sediment of sample No. 1, and 36 in sample No. 4. Almost all the precipitated mites were completely dried, and sometimes broken.

The new method has a higher recovery efficiency and the process is simplified in comparison with the Darling solution centrifugation method. It takes less than 10 min to isolate mites by the new method but at

least 60 min by the other. Although considerably more dust particles are observed on the filter paper in the new method, dyeing the dust particles and dispersing them on the filter paper facilitate mite detection.

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REFERENCES

- Bronswijk, J. E. M. H. van (1973): *Dermatophagoides pteronyssinus* (Trouessart, 1897) in mattress and floor dust in a temperate climate (Acari: Pyroglyphidae). *J. Med. Entomol.*, 10: 63-70.
- Furumizo, R. T. (1973): The biology and ecology of the house-dust mite *Dermatophagoides farinae* Hughes, 1961 (Acarina: Pyroglyphidae). In: *Ph.D. Dissertation*, 143 pp., University of California, Riverside.
- Korsgaard, J. (1982): Preventive measures in house-dust allergy. *Am. Rev. Respir. Dis.*, 125: 80-84.
- Miyamoto, J. and T. Ouchi (1976): Ecological studies of house dust mites. Seasonal changes in mite populations in house dust in Japan. *Jpn. J. Sanit. Zool.*, 27: 251-259 (in Japanese with English summary).
- Oshima, S. (1964): Observations on floor mites collected in Yokohama. I. On the mites found in several schools in summer. *Jpn. J. Sanit. Zool.*, 15: 233-244 (in Japanese with English summary).
- Sasa, M., J. Miyamoto, S. Shinohara, H. Suzuki and A. Katsuhata (1970): Studies on mass culture and isolation of *Dermatophagoides farinae* and some other mites associated with house dust and stored food. *Jpn. J. Exp. Med.*, 40: 367-382.
- Spieksma, F. Th. M. (1967): *The House-Dust Mite Dermatophagoides pteronyssinus* (Trouessart, 1897), *Producer of the House-Dust Allergen* (Acari: Psoroptidae), x+65 pp., V Leiden.
- Spieksma, F. Th. M. and M. I. A. Spieksma-Boezeman (1967): The mite fauna of house dust with particular reference to the house-dust mite *Dermatophagoides pteronyssinus* (Trouessart, 1897) (Psoroptidae: Sarcoptiformes). *Acarologia*, 9: 226-241.
- Wharton, G. W. (1976): House dust mites. *J. Med. Entomol.*, 12: 577-621.

摘 要

室内塵ダニ検出のためのふり水洗法とその検出率

Furumizo (1973) を改良したふり水洗法の検出率を検討した。新しい方法は以下の点で Furumizo 法と異なっている。1) 試料の乾燥重量の測定。2) 試料と 50% エタノール 80 ml を振とうするための 100 ml の広口びんには金網を入れない。3) 広口びんの内容物は目の大きさが 0.42 mm と 0.075 mm のふりを通し、そのたびに 30 秒間水洗する。4) 0.075 mm のふりを 1% のメチレンブルーに数秒間漬けて試料を染める。5) 染色した試料をブフナーろうと内のろ紙の上に置いた円筒に流し込む。家庭で採取したほこり 22 試料をそれぞれ 5 回ずつふり処理し、5 回で得られた合計に対する各回の抽出率を検討したところ、3 回のふり処理で生体は平均 98.4% (89.4~100%)、死体では 98.7% (90.8~100%) の検出率が得られた。ローダミン B で染色したコナヒョウヒダニ *Dermatophagoides farinae* の生体と死体それぞれ 100 個体ずつを 500 mg のほこり 4 試料に混入し、ダニの分離を試みたところ生体で 84~95%、死体で 91~98% の検出率を得た。さらに 5 軒の家から 500 mg のほこりを 2 組ずつ取って新検出法と Darling 液遠心懸濁法の検出数を比較したところ、新検出法によるほうが多くのダニを得られた。