ORIGINAL ARTICLE

Immunoquantitative Measurement of Soybean Aeroallergen Emissions at Industrial Sites

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Abstract

Background: Asthma attacks and mortality due to inhalation of soybean antigens in Barcelona, Spain have been well documented. A new control scheme was adopted in the city to avoid the emission and dispersion of soybean dust into the atmosphere during unloading. We studied soybean allergen emission during unloading and at 3 industrial sites and compared the results obtained.
Methods: Over a period of 31 months, 628 paired air samples from 3 plants (A, B, C) involved in soybean manipulation in Barcelona harbor were collected. Samples were analyzed by a radiometric competitive inhibition assay (RCIA) and the enzyme-linked immunosorbent assay (ELISA). A Bland-Altman plot was used to compare the soybean concentrations measured by each assay.
Results: The median values for the 628 samples were 5535 U/m^3 (range, 370-18 416 751) for the RCIA and 9955 U/m^3 (range, 400-22 349 059) for the ELISA. Plant A had the lowest emission levels and the lowest Spearman rank correlation coefficient (0.409). The correlation coefficients were 0.747 and 0.794 for plants B and C. Soybean aeroallergen concentrations differed by plant. The highest variability in values was seen for plant A, which had the lowest allergen concentrations.
Conclusions: The competitive assays described are useful tools for the measurement of soybean allergen emission levels at industrial sites. These methods may be used to monitor unloading and the impact of environmental interventions.

Key words: Competitive inhibition assay. Soybean. Aeroallergen. Asthma. Emission. Risk.

Resumen

Introducción: En Barcelona se han descrito diversos episodios de asma y mortalidad debida a la inhalación de alergeno de soja. A consecuencia de este hecho se ha adoptado un nuevo sistema de control para eliminar la emisión y dispersión del polvo de soja a la atmósfera durante los procesos de descarga. El presente estudio ha sido elaborado con el objetivo de describir los métodos existentes en la actualidad para estimar la emisión de alergeno en zonas industriales y durante los procesos de descarga, con especial referencia a la comparación entre ellos.
Métodos: Durante un periodo de 31 meses, se recogieron 628 muestras ambientales de tres plantas industriales (A, B, C) implicadas en la manipulación de soja en el puerto de Barcelona. Las muestras fueron analizadas mediante un método de RCIA (ensayo de inhibición competitivo radiométrico) y un método de ELISA-inhibición. Para la comparación de la concentración de aeroalergeno de soja obtenida mediante ambos métodos se utilizó el análisis estadístico de Bland and Altman.
Resultados: Los valores medios de las 628 muestras analizadas fueron 5,535 U/m^3 (rango 370 – 18,416,751) para el método de RCIA y 9,955 U/m^3 (rango 400 – 22,349,059) para el método de ELISA-inhibición. La planta A fue la que presentó los niveles de emisión más bajos y el menor coeficiente de correlación de Spearman (0.409). Para las plantas B y C, los coeficientes de correlación fueron 0.747 y 0.794. Las concentraciones de soja diferían en cada planta. La mayor variabilidad se observó en los valores de la planta A, que era la que presentaba los valores más bajos de aeroalergeno de soja.
Conclusiones: Los ensayos competitivos descritos son útiles para la estimación de la emisión de alergeno de soja en zonas industriales. Estos métodos pueden ser utilizados para el control de estas actividades y para monitorizar el impacto de las intervenciones medioambientales.

Palabras clave: Ensayo de inhibición competitivo. Soja. Aeroalergeno. Asma. Emisión. Riesgo.

Introduction

Dust discharged into the air during unloading of soybeans from ships was identified as the etiologic agent in 26 asthma outbreaks occurring in Barcelona, Spain between 1981 and 1987 [1]. The outbreaks accounted for 958 admissions to the emergency room due to unusually severe asthma exacerbations, resulting in 20 deaths and frequent use of the intensive care unit [1,2]. Similar outbreaks have been described in other cities, notably in New Orleans [3]. The allergens involved in epidemic asthma outbreaks were identified as the isoallergens Gly m IA and Gly m IB, which are located in soybean hulls and have molecular weights of 7.0 kDa and 7.5 kDa, respectively [4,5].

Since 1987, efforts have been made to avoid the emission and dispersion of large amounts of soybean dust into the atmosphere during unloading [6]. Initial corrective measures, which were based on emission channeling and the use of standard polyester sleeve filters, reduced the dispersion of allergen into the atmosphere, with the result that no new outbreaks occurred for some years [6,7]. However, evidence of occasional elevation in environmental soybean allergen concentrations and documented persistence of soybean sensitivity in affected patients suggested that the possibility of exposure remained [8,9]. After a cluster of asthma cases was detected in 1994, a system to ensure the monitoring of allergen levels in the city was designed based on the analysis of filters obtained from air pollution control stations. In 1996, there was an outbreak of soybean-related asthma [8] and the city health authorities adopted a new control scheme. This involved ensuring airtight transportation circuits and increasing the filtration yield of emission sources at the 3 plants analyzed in this study, daily soybean aeroallergen monitoring near the harbor, and periodical measurement of emissions produced in the unloading or processing of soybean [7]. These measures have been effective in decreasing mean environmental soybean aeroallergen concentrations [7,10] and have prevented further asthma outbreaks to date.

Three immunochemical methods have been used to measure soybean aeroallergen levels in the air. The first inhibition method developed was the radio-allergosorbent test (RAST) [11], which was replaced by a radiometric competitive inhibition assay (RCIA) using specific rabbit antibodies [12]. Later, an enzyme-linked immunosorbent assay (ELISA) was implemented [13]. Both RCIA and ELISA have demonstrated good correlation and are considered effective and reproducible for the measurement of soybean aeroallergen levels. Currently, quantification of allergen levels in the city is based on the ELISA method. These assays share a potential long-term reproducibility problem because they use antibody pools of finite existence [14]. No studies compare these methods for the measurement of emission levels. We present the current methods for the study of soybean allergen emission in unloading at industrial sites, and compare the results obtained with each of them.

Materials and Methods

Unloading and Processing of Soybean

Three plants are involved in soybean manipulation in Barcelona harbor. Plant A unloads, stores, and processes soybean to produce oil and flour. Plant B unloads and stores soybean, and delivers it to Plant C for processing. All plant operations and processes take place under cover and negative pressure, and air is filtered before being emitted to the atmosphere. These operations may be summarized as follows: unloading (pneumatic), storage in silos and transfer (transportation, elevation), cleaning, drying, grinding, oil extraction, and flour drying. For filtering dust and soybean allergen, these plants use silicone resin–treated spun bond polyester filter cartridges (plant A), polytetrafluoroethylene (PTFE) resin–treated micro pore size (MPS) filter bags (plant B), and MPS polyester filter bags or MPS polyester pulse pleat filters (plant C).

Air Sampling at the Industrial Sites

The facilities for unloading, manipulation, storage, and grinding of soybean in the port of Barcelona use pneumatic transportation circuits. All foci of channeled emissions are identified. At these foci, air is expelled to maintain the low pressure in the pipes. As this air is filtered before reaching the atmosphere, the emissions thus produced are channeled emissions. The steps undertaken to measure allergen emission led to the identification of almost 40 foci of channeled emissions in the 3 plants. The level of emission at each point and the global value for each plant are measured quarterly. Under normal working conditions, no dust is detected on sampling filters using a gravimetric method (mg/m^3). Laboratory analyses provide a measurement of emissions in terms of allergen units per volume of air; with the known air volume flow rate at each focus, emissions can be estimated in allergen units per unit of time. Allergen sampling is based on EN 13248-1:2001 [14], adapted to the requirements of our analysis. Particulate matter is withdrawn isokinetically from each source and collected on a 50-mm diameter PTFE membrane filter (0.3 μ pore size) at duct temperature. Sampling time is adjusted for every source to match the required collection volume with the assay's detection and reporting parameters, 3 samples are taken from each duct and analyzed; the mean value of the 3 samples is taken as the actual emission value for the focus. For the comparison study, 6 consecutive samples were obtained from the foci at each site. Companion samples were split and sent to the laboratory for RCIA and ELISA analysis. Soybean aeroallergens were extracted from the filters in 1 mL phosphate buffered saline (0.2%) with bovine serum albumin (0.5%) and Tween 20, and kept overnight at 4ºC. The filter was then discarded and the eluates were analyzed.

Laboratory Measurement of Soybean Concentrations

Both methods have been described elsewhere [11,12]. A low-molecular-mass soybean allergen standard containing proteins with a molecular weight below 10 kDa was used [11]. The RCIA uses specific rabbit immunoglobulin (Ig) G antibodies for low-molecular-mass soybean hull–associated proteins. The ELISA-inhibition assay used a serum pool of specific IgE antibodies from individuals in the city of

Barcelona who were allergic to soybean. In new batches of the human sera pool, a level of specific IgE to soybean of 19 kU_A/L (UniCAP 100, Pharmacia AB, Uppsala, Sweden) was maintained. The standard curve was constructed from 6 data points using a 4-parameter logistic curve fit, and the percentage inhibition was calculated by comparison with the uninhibited serum pool.

Comparison Between RCIA and ELISA

Over a period of 31 months (November 2003 to June 2006), 628 paired air samples from the 3 different plants were collected: 120 from plant A, 162 from plant B, and 346 from plant C. Samples were analyzed by RCIA and ELISA. Both assays used the same extraction method and the low-molecular-mass standard (see above) as antigen and reference preparation. Both have similar sensitivities: RCIA had a detection limit of 500 U/mL and ELISA had a detection limit of 300 U/mL.

Statistical Analysis

The soybean concentrations obtained by the RCIA and ELISA methods were compared using statistical procedures described in a previous study on soybean aeroallergen levels in the city [10]. The median values of soybean aeroallergen levels were calculated and are expressed with the interquartile range. The value of the lower limit of detection was assigned to samples with undetectable levels. A 1-sample Kolmogorov-Smirnov test confirmed a non-normal distribution; therefore, a Spearman rank correlation was applied to allergen concentrations to determine the correlation between pairs of data. A Bland-Altman plot [15-17] was used to compare the soybean concentration obtained using each assay. After logit transformation of the data, the difference between the paired measurements was plotted against the mean of the measurements. The differences between both methods were expressed using a 95% normal range. The limits of this range were expressed as the mean (SD). SPSS release 11.0 for Windows (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analyses.

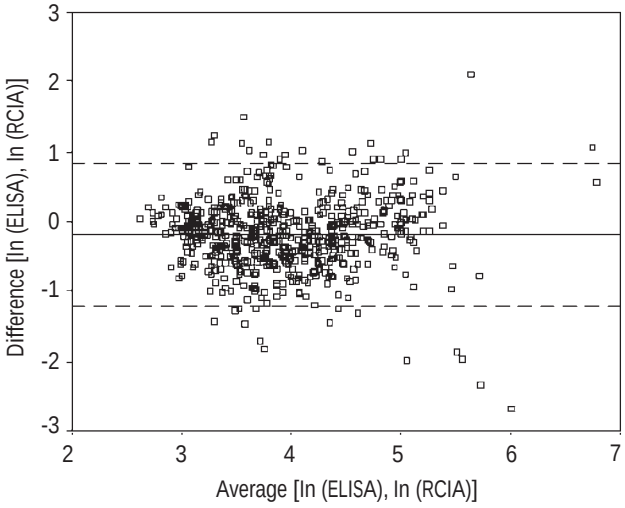

Figure 1. Log-difference plot of 628 paired samples measured by RCIA and ELISA immunoassays with mean difference (solid line) and limits of agreement (dashed lines).

Table. Soybean Aeroallergen Emission Levels at Channeled Foci by Plant and Immunoassay Method[a]

	Method	Median	Range	Spearman Correlation Coefficient
Plant A n = 120	RCIA ELISA	2530 3896	370-140 260 400-617 066	0.409
Plant B n = 162	RCIA ELISA	4565 10 028	732-18 416 751 445-7 875 690	0.747
Plant C n = 346	RCIA ELISA	9843 14 786	552-4 858 883 468-22 349 059	0.794

Abbreviations: ELISA, enzyme-linked immunosorbent assay; RCIA, radiometric competitive inhibition assay.
[a] Results are expressed in U/m^3

Results

Comparison Between RCIA and ELISA

Median levels of soybean allergens were calculated as the distributions that did not follow the normal curve. Median values for the 628 samples were 5535 U/m^3 (range, 370-18 416 751) for RCIA and 9955 U/m^3 (range, 400-22 349 059) for ELISA. Figure 1 shows the plot analysis of values in U/m^3 from the 628 air filter eluates. The differences between logarithms were –1.2050 and 0.8302 in approximately 95% of cases. The Spearman rank correlation coefficient was 0.750 ($P < .001$).

Stratified Comparisons by Plant

Correlations between assays were further examined by taking into account the plant where the airborne dust samples were taken. As shown in the Table, plant A had the lowest emission levels and the lowest Spearman correlation coefficient (0.409). The correlation coefficients were 0.747 and 0.794 for plants B and C. The impact of the sampling location on the values measured, and on the correlations between the results measured using both methods are further illustrated in Figure 2. The differences between logarithms in approximately 95% of cases were –1.3511 and 0.8841 for plant A, –1.2875 and 0.7449 for plant B, and –1.1022 and 0.8378 for plant C. The Figures show that soybean aeroallergen concentrations differed by plant. The highest variability is seen in the plotted values of plant A, which had the lowest allergen concentrations.

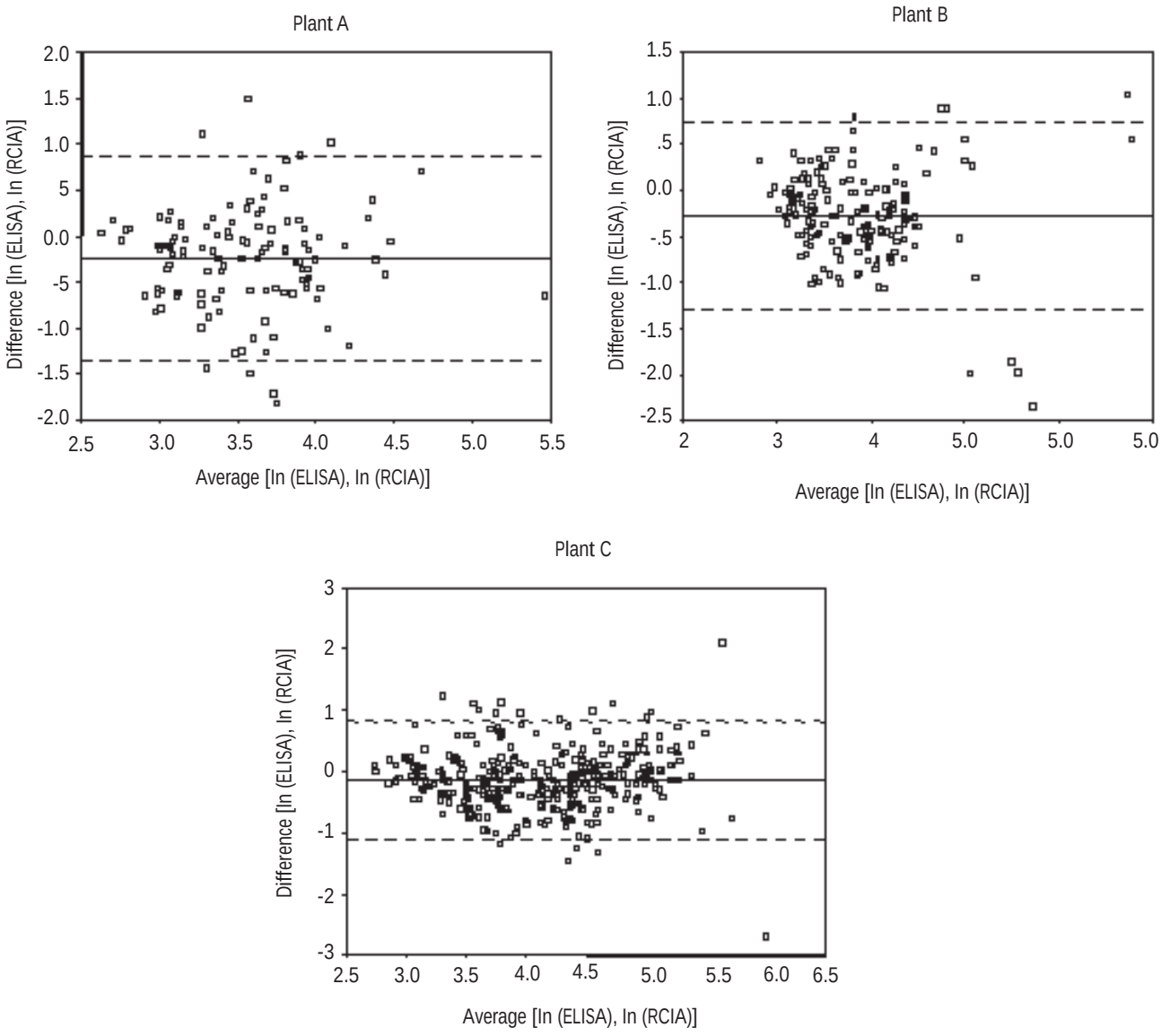

Figure 2. Log-difference plot of the samples measured in each of 3 plants by RCIA and ELISA immunoassays with mean difference (solid line) and limits of agreement (dashed lines).

Discussion

This study compared the 2 methods used to measure the emission levels of this aeroallergen in the city of Barcelona [12,13]. The results obtained provide good correlation coefficients.

The asthma outbreaks in Barcelona between 1981 and 1987 revealed the need for regular monitoring of soybean particles in the air of the city and also at the soybean unloading sites in the harbor. The new control scheme adopted by the city health authorities after the last episode of soybean-related asthma in 1996 led to a decrease in mean environmental soybean aeroallergen concentrations in the city on unloading days [7]. Today, there is no difference in these concentrations when ships are unloaded and, to date, there have been no episodes of soybean-related asthma [7,10].

Allergen values measured by the 2 immunoassays had a good correlation coefficient (0.750), which is higher than the one obtained in the comparison of these 2 methods for the measurement of soybean allergen levels in the air of the city (0.650) [13]. This higher correlation with emission levels can be explained by the fact that the range of values in emission levels is much higher than in allergen levels measured in the city, and the methods show better correlation for high values [13]. The quantitative allergen levels estimated using the ELISA method tend to be higher than those obtained with the RCIA method, although they are of a similar order of magnitude.

It is common practice to use correlation coefficients and regression lines when comparing 2 methods. This approach, however, does not measure the agreement between the methods. An alternative involves difference plot analysis [16-18], the approach used in the present study. The Bland-Altman plot in Figure 1 shows that values are grouped around the 95% limits of agreement, and in the global data these limits are acceptable for high and low values.

Soybean emission aeroallergen levels were sampling-site–dependent. The results by plant show that the correlation coefficients were similar in plants B and C, whereas plant A had a lower correlation coefficient (0.409). This result may be explained by the lower emission levels attained in plant A, in contrast with the higher agreement in plant C, which had more emission foci and the highest emission values (Table).

Differences between these immunoassays could be partially attributed to antibody specificity, as found in other studies [19,20]. Comparative studies for the measurement of other allergen levels have shown that identical air samples would yield different results depending on the assay technology used, the most important factors being the source and type of antibodies [20,21]. In the present study, the different sources of antibodies used may explain some of the differences obtained in the individual concentrations, as demonstrated in a study of another type of airborne allergens using monoclonal antibodies, rabbit polyclonals, and human sera as detection antibodies [22].

Both methods present advantages and disadvantages. ELISA has replaced the radioimmunoassay in many laboratories, as it offers comparable sensitivity without the problems of disposal and the short half-life associated with radioactive materials. However, a disadvantage of assays using human IgE antibody is the limited availability of suitable sera. In any case, in the absence of a gold standard for measuring soybean allergen in airborne dust samples, the 2 competitive assays described here are useful tools for the measurement of soybean allergen emission levels at industrial sites. The practical importance of the results is that these methods may be used for the control of such activities and to monitor the impact of environmental interventions. These conditions may arise at other sites, and whenever an outbreak of soybean-related asthma occurs, the measurement of these aeroallergens should be considered a primary tool for emission surveillance and control. Combined with industrial hygiene and epidemiology, it may result in safer and healthier environments for all.

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References

1. Antó JM, Sunyer J, Rodriguez-Roisin R, Suarez M, Vazquez L. Asthma epidemics associated with soybean dust relevant during harbor activities. N Engl J Med. 1989;320:1097-102.
2. Li JT, Swanson MC, Rando R, Ovsyannikova IG, Wentz-Murtha P, Morell F, Lopez M, Reed CE: Soybean aeroallergen around the port of New Orleans: a potential cause of asthma. Aerobiologia. 1996;12:173-6.
3. Sunyer J, Anto JM, Rodrigo MJ, Morell F. Epidemic asthma and soybean IgE antibodies. Lancet. 1989;1:179-82.
4. Rodrigo MJ, Morell F, Helm RM, Swanson M, Greife A, Anto JM, Sunyer J, Reed CE. Identification and partial characterization of the soybean-dust allergens involved in the Barcelona asthma epidemic. J Allergy Clin Immunol. 1990;85:778-84.
5. R, Varela J, Carreira J, Polo F. Soybean hydrophobic protein and soybean hull allergy. Lancet. 1995;346:48-9.
6. Anto JM, Sunyer J, Reed CE, Sabria J, Martinez F, Morell F, Codina R, Rodriguez-Roisin R, Rodrigo MJ, Roca J, Saez M. Preventing asthma epidemics due to soybean by dust-control measures. N Engl J Med. 1993;329:1760-3.
7. Villalbí JR, Plasencia A, Manzanera R, Armengol R, Antó JM, the Collaborative and Technical Support Groups for the study of soybean asthma in Barcelona. Epidemic soybean asthma and public health: new control systems and initial evaluation in Barcelona, 1996-98. J Epidemiol Community Health. 2004;58:461-5.
8. Manzanera R, Armengol R, Villalbí JR. Public health and soybean-related asthma in Barcelona. Ajuntament de Barcelona, 1999.
9. Soriano JB, Antó JM, Plasència A. Repeaters count: a sentinel method for asthma outbreaks. Thorax. 1995;50:1101-3.
10. Rodrigo MJ, Cruz MJ, García M, Antó JM, Genover T, Morell F. Epidemic asthma in Barcelona. An evaluation of new strategies for the control of soybean dust emission. Int Arch Allergy Immunol. 2004;134:158-64.
11. Swanson MC, Li J, Wentz-Murtha PE, Trudeau WL, Fernandez-Caldas E, Greife A, Rodrigo MJ, Morell F, Reed CE. Source of the aeroallergen of soybean dust: A low molecular mass from the soybean tela. J Allergy Clin Immunol. 1991;87:783-8.
12. Swanson MC, Caldas E-F, Soriano JB, Marañon F, Morell F, Reed CE. Immunoassay validation for quantification of low molecular mass asthmagenic glycoproteins associated with airborne soybean dust. J Allergy Clin Immunol. 1998;101:131.
13. Cruz MJ, Rodrigo MJ, Antó JM, Morell F. An amplified ELISA-inhibition method for the measurement of airborne soybean allergens. Int Arch Allergy Immunol. 2000;122:42-8.
14. Renström A, Gordon S, Larsson PH, Tee RD, Newman-Taylor AJ, Malmberg P. Comparison of a radioallergosorbent (RAST) inhibition method and a monoclonal enzyme-linked immunosorbent assay (ELISA) for aeroallergen measurement. Clin Exp Allergy. 1997;27:1314-21.
15. EN 13248-1:2001. Stationary source emission-determination of low range mass concentration of dust. Part 1: manual gravimetric method.
16. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;i:307-10.
17. Holls S. Analysis of method comparison studies. J Int Fed Clin Chem. 1997;9:8-12.
18. Fleiss JL. Statistical methods for rates and proportions. New York: John Wiley, 1981;212-36.
19. Gordon S, Kiernan LA, Nieuwenhuijsen MJ, Cook AD, Rosemary RD, Newman-Taylor AJ. Measurement of exposure to mouse urinary proteins in an epidemiological study. Occup Environ Med. 1997;54:135-40.
20. Renstrom A, Gordon S, Hollander A, Spithoven J, Larsson PH,

Venables KM, Heederik D, Malmberg P. Comparison of methods to assess airborne rat and mouse levels. II. Factors influencing antigen detection. Allergy. 1999;54:150-7.
21. Hollander A, Gordon S, Renström A, Thissen J, Doekes G, Larsson PH, Malmberg P, Venables KM, Heederik D. Comparison of methods to assess airborne rat and mouse allergen levels. I. Analysis or air samples. Allergy. 1999;54:142-9.
22. Swanson MC, Campbell AR, Klauck MJ, Reed CE. Correlations between levels of mite and cat allergens in settled and airborne dust. J Allergy Clin Immunol. 1989;83:776-83.

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