

An Amplified ELISA Inhibition Method for the Measurement of Airborne Soybean Allergens

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Key Words

ELISA · Inhibition · Soybean · Aeroallergen

Abstract

Background: Measurement of the soybean aeroallergen in Barcelona and other cities where soybean is unloaded is of increasing importance in controlling population exposure and evaluating the influence of such exposure on the persistence of asthma symptoms. **Objective:** The aims of the study were: (1) to standardize an amplified ELISA inhibition method for the quantification of soybean aeroallergen and (2) to compare this method to a previously described RAST inhibition method. **Methods and Results:** An amplified competitive ELISA inhibition method with a biotin-streptavidin system was carried out using a pool of sera from soybean-sensitized patients. The results were expressed as U/ml using a low-molecular-mass soybean allergen as reference standard. Reproducibility was calculated by statistically comparing the slope of the regression lines of the standard curve of 4 consecutive assays and by determining the coefficient of variation (CV) of the percent inhibition data for each

point of several independent standard curves, each from the same assay (intra-assay) and also from a separate assay (inter-assay). No significant differences in the slopes were obtained by analysis of covariance (ANCOVA) $F = 1.04$. The CV between assays varied between 4 and 22% (for the assay range used in the reference standard) and was greater than the CV within assays (5–10%). Only values with a CV(%) smaller than 20% were considered acceptable. 78.5% of the samples satisfied this criterion. The RAST inhibition and ELISA inhibition methods were compared by difference plots from the values of 338 air filter eluates. The intraclass correlation coefficient was 0.456 ($p < 0.001$). After the results of both methods were classified as lower and higher than 165 U/m³, the kappa index was 0.46 ($p < 0.001$). **Conclusions:** The data obtained in the present study are comparable to those reported from other similar immunoassays. Moreover, despite the difficulty in comparing air-sampling values from different laboratories, the kappa index may be taken to represent fairly good agreement beyond chance between both methods. All these data demonstrate that the present immunoassay is useful for measuring airborne soybean aeroallergens and can also be applied to evaluate the relationship between exposure and the development of asthma symptoms.

The first two authors have contributed equally to the design of the study and the writing of the manuscript.

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Introduction

From 1981 to 1987, 26 asthma outbreaks occurred in Barcelona. Epidemic days of acute asthma accounted for 958 emergency room admissions for unusually severe asthma exacerbations, resulting in 20 deaths and frequent admissions to the intensive care unit. Investigation of these epidemics revealed that the outbreaks were caused by soybean dust generated by unloading of soybeans in Barcelona harbor on days when the wind carried the soybean dust over the city [1–3]. Rodrigo et al. [2] demonstrated that during an epidemic the serum of patients with asthma reacted specifically to an acidic, low-molecular mass (LMM) protein (<14 kD) located principally in hulls and dust of soybeans unloaded in the harbor. Two proteins located in soybean hulls were later shown to be the main allergens responsible for this asthma epidemic [4]. Both proteins are isoallergens (Gly m 1a and Gly m 1b) with molecular weights of 7.0 and 7.5 kD, respectively, and are highly homologous with the hydrophobic soybean protein (HSP) described by Odani et al. [5].

In 1987, measures were taken to avoid the release of large amounts of soybean dust into the atmosphere during unloading. These measures consisted of fitting the silos with bag filters and regularly following up dust emissions during the soybean unloading process. This drastically reduced the spread of the dust into the atmosphere. This action produced a significant decrease in the atmospheric concentration of the dust and, as a consequence, the asthma epidemics disappeared [6]. However, low concentrations of soybean dust still persist in the atmosphere at detectable levels and despite the disappearance of the epidemics, a large number of the individuals affected were still sensitive to soybean [6].

Soybean epidemic asthma has been well documented in New Orleans, La. (USA) [7], Naples (Italy) [8] and some cities in Spain [9–11]. These studies have demonstrated the importance of reducing the emissions of soybean dust during unloading operations. Therefore it is very useful to have systems to monitor airborne soybean allergen. Measurement of soybean aeroallergen concentration is currently achieved by automated air samplers in the city [12]. The allergen is extracted from the filters in these air samplers and immunochemically assayed for its contents of soybean proteins [13, 14]. These measurements are used to assess the effectiveness of the interventions aimed at reducing allergen exposure and to examine exposure-response relationships in the asthmatic population [15].

Several different immunological methods have been used to measure occupational allergens, with the RAST and ELISA inhibition being the most used. However, ELISA methods have replaced the radioimmunoassays in many laboratories, as they offer comparable sensitivity without the problems of disposal and the short half-life associated with radioactive materials [16].

Although inhibition methods are used by the FDA to quantify skin test extracts [17] – also with other immunochemical techniques – in most cases these methods are considered semiquantitative assays with potential long-term reproducibility problems because of the use of heterogeneous antibody pools [18]. As a consequence, the comparison of absolute measurements of airborne allergens among different studies is difficult. For each antigen, therefore, assessment of the efficacy of each of these techniques is essential for the quantification of exposure data [19].

The aims of the present study were: (1) to standardize an amplified ELISA inhibition method for the measurement of soybean aeroallergen; (2) to compare this method with a previously described RAST inhibition assay [13].

Materials and Methods

Air Sampling

According to previous studies [12], large-volume automated air samplers (CAV-A/HF, MCV, SA, Barcelona, Spain) containing glass microfiber filters with a pore size of 1 µm (Whatman International Ltd., UK), installed in three districts of the city close to the harbor and changed daily facilitate the collection of samples of airborne soybean dust. The air volume used for dust collection was 55 m³/h.

Glass Microfiber Filter Elution

Soybean aeroallergens were extracted from the filters in 5 ml PBS/0.2% BSA/0.1% Tween 20, pH 7.4 overnight at 4°C, after which the filter was discarded and the eluates analyzed.

Validation of Allergen Extract

A soybean LMM allergen (soybean-LMM) containing proteins with a molecular weight lower than 10 kD was used as reference preparation for soybean aeroallergen measurement. This reference preparation was prepared following the protocol of Swanson et al. [13].

A soluble protein extraction with 0.1 M NH₄ (HCO₃) buffer, pH 7.9, was done. The eluate was subjected to double dialysis with 10- and 3.5-kD pore size membranes (Spectrapor, Iberlabo, Los Angeles, Calif., USA). Briefly, a solution of 100 ml of the soybean extract was added to the 10-kD pore size dialysis membrane. The membrane was placed posteriorly inside another 3.5-kD pore size membrane, together with 300 ml of H₂O. The extract was dialyzed against H₂O at 4°C and shaken. The fraction containing the proteins with molecular weights between 10 and 3.5 kD was later concentrated to a final volume of 2 ml.

The following studies were conducted to assess the properties of the soybean-LMM:

Protein Composition (Electrophoresis of the Extract). The extract was loaded onto a 5–17% SDS-polyacrylamide gradient gel. Antigen extract containing 20 and 25 µg of protein was treated with 5% mercaptoethanol and applied to a 3% stacking gel. Electrophoresis was carried out at 20 mA per gel until the tracing dye reached the bottom of the gel. After electrophoresis, gels were stained with Coomassie brilliant blue (Bio-Rad Laboratories, Richmond, Calif., USA).

Specificity of the Reference Preparation. In order to confirm whether this soybean-LMM maintains its allergenic properties, 32 samples from asthmatic patients with a known concentration of IgE to soybean (*Glycine max*, f14, UniCAP 100, Pharmacia AB, Uppsala, Sweden) were assayed by ELISA using the soybean-LMM standard and HSP [5] as antigens. Correlation coefficients between soybean-LMM and HSP were analyzed by the Spearman rank test.

ELISA Inhibition Assay

The soybean allergen was determined by an amplified competitive ELISA inhibition assay based on the method described by Swanson et al. [13] and modified as follows: wells of high-binding microtiter plates (Costar, Cambridge, Mass., USA) were incubated overnight at room temperature with the LMM allergen (1:1,000 v/v) in 0.2 M Na₂CO₃/NaHCO₃ buffer, pH 9.2. After three washes (0.1 M phosphate buffer/0.1% Tween 20, pH 7.4), the plates were incubated overnight at room temperature with 50 µl/well of either air sample extract or the soybean-LMM reference preparation (with an assigned value of 10⁶ U/ml) and 50 µl/well of an undiluted pool of sera from 12 allergic patients (mean age 44 years) with 19 kU/l specific IgE to soybean (UniCAP 100, Pharmacia AB, Uppsala, Sweden). The OD at this antigen concentration at 450 nm was not higher than 1.20. It should be noted that the pool of sera was obtained in large quantities prior to starting the study to ensure the availability of this reagent. After three washes, 100 µl/well of biotinylated anti-human IgE (0.5 µg/ml, clone B3102E8; SBA, Ala., USA) were incubated for 1 h at 37°C and then rinsed. Thereafter, the plates were incubated with 100 µl/well of streptavidin-peroxidase (1 µg/ml; SBA) for 1 h at 37°C and then rinsed. Thereafter, the reaction was allowed to develop with 3% H₂O₂, 3,3',5,5'-tetramethyl-benzidine for 20 min at room temperature in the dark. The reaction was stopped with 2 M H₂SO₄ and read at 450 nm with a plate reader (Titertek Multiskan Plus MKII). The standard curve was constructed from six data points using a four-parameter logistic curve fit, and the percentage inhibition was calculated by comparison with the uninhibited serum pool. The soybean concentration of the air samples was calculated as:

$$[\text{soybean}] \text{ (U/m}^3\text{)} = \frac{(\text{U/well}) \times \text{filter elution volume (ml)}}{\text{volume of air sampled (m}^3\text{)}}.$$

The minimum amount of allergen detectable with this assay was calculated as the percentage of the maximum inhibition from which the curve is no longer linear. This limit varied in each assay and is expressed as U/ml. In the assays carried out in this study, the detection limit oscillated between 300 and 1,860 U/ml, 1 U was indirectly estimated to contain approximately 1 ng of allergen [12]. These limits were used to calculate the detection limits for a mean sampled volume of 150 m³. These limits oscillated between 10 and 62 U.

Reproducibility

The reproducibility of the assay was assessed in two ways: (1) by comparing the slope of the standard curve of four consecutive assays. The percent inhibition data for each of the curves were plotted against log concentration. The regression line in each case was con-

structed from at least six points. Significant differences in the slopes of the lines were then tested by analysis of covariance (ANCOVA); and (2) by calculating the coefficient of variation (CV) within and between assays. The CV of the assay was calculated by determining the CV of the percent inhibition data for each point of several independent standard curves each from the same assay (intra-assay) and from a separate assay (inter-assay). The standard curves of the same assay were independently diluted.

Comparison between the RAST and ELISA Inhibition Methods

The values for 338 air samples collected in glass microfiber filters of automated air samplers were analyzed by the method described in this paper and compared with the RAST inhibition method described by Swanson et al. [13]. The two assays used the same elution method and the soybean-LMM extract previously described as antigen and reference preparation. Agreement between both methods was assessed after classifying the results as lower and higher than 165 U/m³, since this value was the highest value recorded in 22 days after the interventions [6].

Statistical Analysis

ANCOVA was used in the reproducibility study when comparing the slopes of the regression lines obtained for the standard curves. The significance of the test was then obtained from F tables. Correlation coefficients between soybean-LMM and HSP were subjected to the Spearman rank test. The 'difference plot' statistical procedure recommended by Bland and Altman [20] was used to compare the soybean concentration obtained by the ELISA inhibition and the RAST inhibition methods; after logit transformation of the data, the difference between the paired measurements was plotted against the mean of the measurements. The 95% confidence interval for the differences was used to assess the differences between both methods. The limits of this interval were given by the mean difference ± 1.96 SD. The kappa index and intraclass correlation coefficient were used to assess the agreement between the two methods.

Results

Validation of Allergen Extract

Protein Composition. Figure 1 shows the Coomassie brilliant blue stain of 5–17% SDS-PAGE gel of soybean-LMM extract. The extract demonstrated predominant bands <14.4-kD MW markers.

Specificity of the Reference Preparation. For the 32 values of specific IgE (A_{450 nm}) against soybean-LMM and HSP in asthmatic patients, the medians (ranges) were 0.187 (0.092–0.887) for soybean-LMM and 0.274 (0.109–1.068) for HSP. No significant differences were found between them (r = 0.939, p < 0.0001).

Variation in Sample Duplicates and Criteria for Result Acceptance

Owing to the variability of these methods, specific criteria for result acceptance were established. Figure 2 shows the variation in duplicates of 84 samples used to

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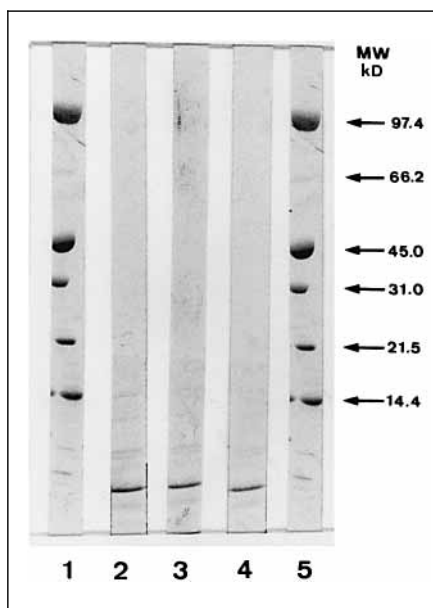
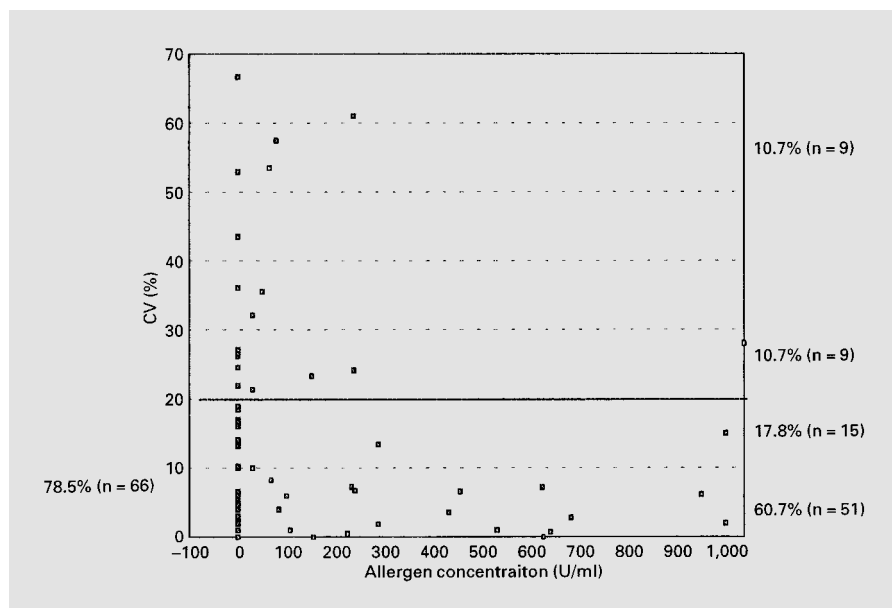


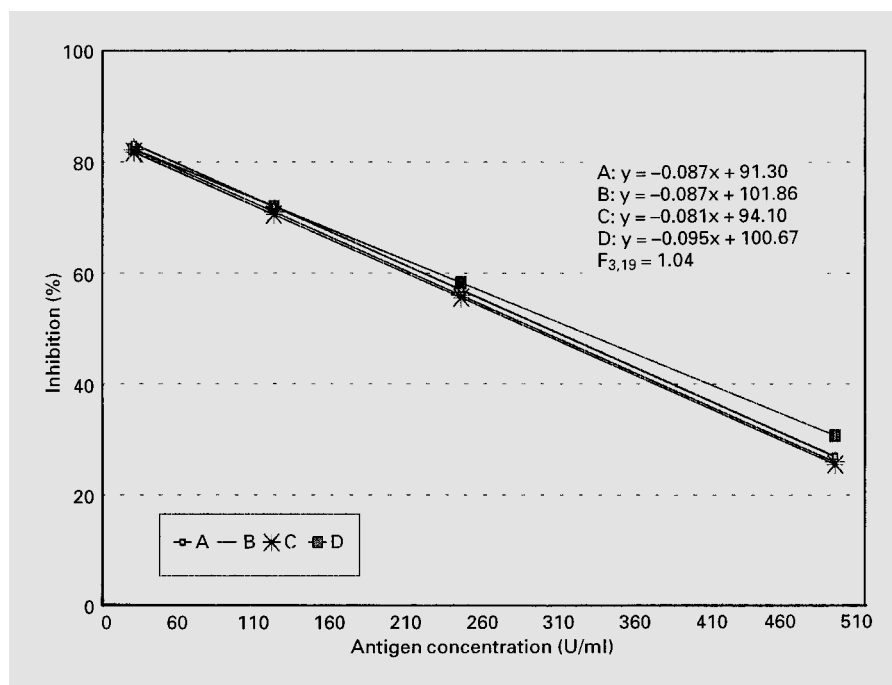
Fig. 1. Coomassie brilliant blue stain of 5–17% SDS-PAGE gel of soybean-LMM standard. Lanes 1 and 5: MW markers; lanes 2–4: soybean-LMM (20, 20 and 25 µg). The extract demonstrated predominant bands <14.4-kD MW markers.

Fig. 2. Variation in duplicates of 84 samples. We consider only values with a CV(%) of less than 20% as a criterion for acceptance of results. 78.5% of the samples were within this criterion.

Fig. 3. No significant difference was found between the slopes of the four standard curves (ANCOVA) performed consecutively. These results show that the assays are comparable.



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3

standardize the method. Only values with a CV(%) less than 20% were considered acceptable. 78.5% of the samples satisfied this criterion. All tested samples were repeated until this criterion was met.

Reproducibility

The regression lines derived from the standard curves of four consecutive assays are shown in figure 3. No sig-

nificant differences were found among the slopes of the four standard curves plotted consecutively. These results show that the assays are comparable. The CV between assays varied between 4 and 22% (for the assay range used in the reference standard) and was greater than the CV within assays, which ranged from 5 to 10%.

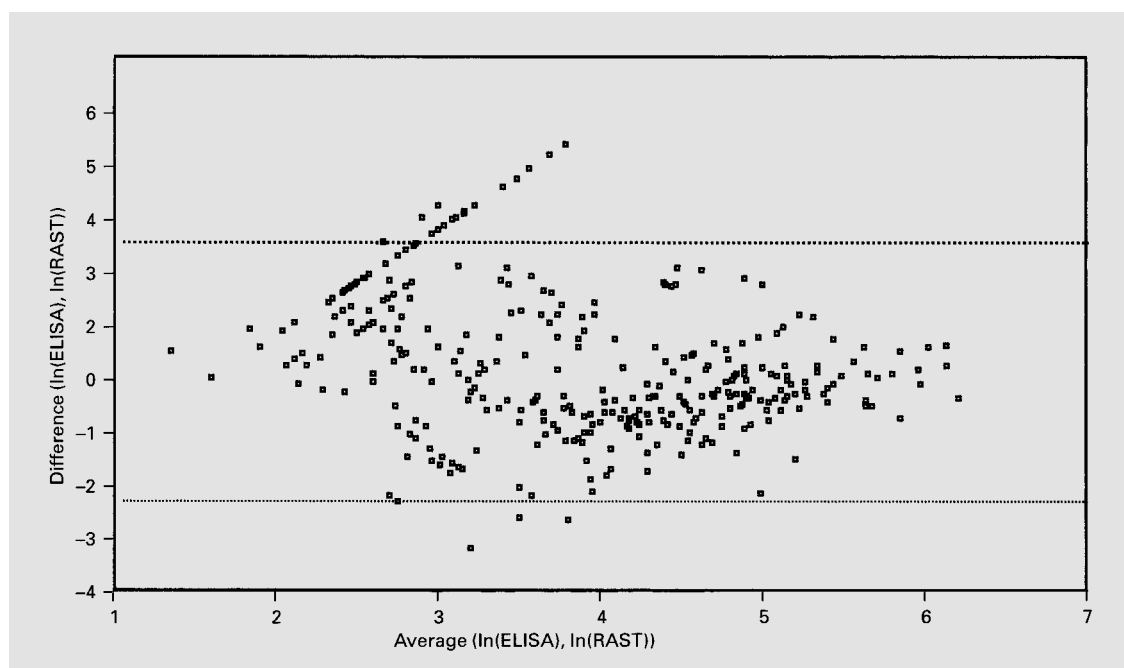


Fig. 4. log difference plot of 336 samples measured by the two immunoassays with limits of agreement (dashed lines).

Comparison between the RAST and ELISA Inhibition Methods

Median concentrations of soybean allergens were calculated as the distributions were not normal. The median (range) values of the 338 air samples were 50 U/m³ (0–636) for the ELISA inhibition method and 54 U/m³ (0–610) for the RAST inhibition method. Figure 4 shows the difference plot analysis of the concentrations (U/m³) of the 338 air filter eluates. The differences between logarithms were –2.24 and 2.6 in approximately 95% of the cases. The intraclass correlation coefficient was 0.456 ($p < 0.001$). After classifying the results of both methods as lower than or equal to 165 U/m³ and higher than 165 U/m³, the value of the kappa index was 0.46 ($p < 0.001$).

Discussion

Both the epidemiological studies and the studies on specific IgE to soybean carried out following the asthma epidemics in Barcelona attributed their cause to the unloading of soybean in the harbor [1, 3]. The environmental intervention in September 1987 with the installation of bag filters in a silo led to a decrease in airborne soybean concentrations and the subsequent disappear-

ance of the epidemics [6]. The 1,555 U/m³ level of soybean allergen detected during a determined asthma epidemic day [12, 21] was substantially higher than the highest value (165 U/m³) recorded in 22 unloading days after the interventions [6]. These data show that the measurement of soybean aeroallergen levels in harbors loading and unloading soybean is of increasing importance in controlling the efficiency of the environmental interventions in order to prevent new asthma outbreaks in these areas.

The present study reports the assessment of a new amplified ELISA inhibition method for the measurement of this airborne soybean allergen, with a biotin-streptavidin system which increases the sensitivity of this assay. Several techniques have been used to measure the allergens in the air [18, 22–24]. The direct ELISA or RIA methods are used for the measurement of allergens for which there are specific monoclonal antibodies and purified antigen for use as a reference standard. Rapid advances have been made in the last few years in allergen characterization and sequence determination using chemical and molecular biological approaches. However, in many cases neither specific monoclonal antibodies nor purified antigen are available. In these cases, the other alternatives are competitive immunoassays. The principle of these methods is the comparison of specific immu-

noglobulin binding by the soluble material extracted from the filters with a reference allergen source.

Competitive inhibition assays are considered to be semiquantitative methods [19], and accurate measurement requires assessment of the efficacy of these techniques. One of the limitations is the absence of standards, which are currently unavailable for quantifying soybean aeroallergen. The results of the present study refer to a reference preparation (soybean-LMM) with an assigned value of 10^6 U/ml. The soybean-LMM reference proved to be a suitable reference preparation for the measurement of soybean exposure, as shown by the results of the SDS-PAGE gel (fig. 1) and the correlation study of specific IgE to HSP. These studies demonstrated that this standard is composed of the LMM proteins responsible for the asthma epidemics and contains most of the soybean allergenic activity [2].

A further disadvantage of these methods is their poor reproducibility. In our study, the results of intra- and interassay CVs and the slope of the inhibition curves (fig. 3) show them to be comparable to those reported for similar immunoassays [18, 25]. However, in order to improve the quality of the method, it is necessary to establish a specific criterion for result acceptance. Only values with a CV(%) in duplicates less than 20% were considered as acceptable. This seems to be a valid criterion considering that these are semiquantitative methods and are similar to those adopted by other authors. Gordon et al. [25], in a study of measurement of airborne rat urinary allergens, allowed more than 30% variation in duplicates. On the other hand, another factor which decreases the variability of the method is the use of PBS/0.2% BSA/0.1% Tween 20 as elution buffer, as the addition of Tween 20 to the elution buffer is known to be the simplest, most sensitive and reproducible method for aeroallergen elution.

In the absence of a 'gold standard' for measuring soybean allergen in airborne dust samples, the present study compared the two methods currently available for the measurement of this aeroallergen. This study showed that results obtained by the two methods gave similar relative values but that individual concentrations showed differences. It is common practice to use correlation coefficients and regression lines when comparing two methods. This approach, however, does not give the measure of agreement between the methods. An alternative method is the use of difference plots [20, 26], which was that used in this study. The intraclass correlation coefficient was $r = 0.456$, $p < 0.001$, but once the results were classified as lower and higher than 165 U/m^3 , the kappa index of 0.46,

$p < 0.001$ may be taken as presenting fair to good agreement beyond chance [27].

Differences between these immunoassays could theoretically be due to the dust-sampling method and filter elution or to the immunoassays used [28, 29]. Given that the dust-sampling method and filter elution are the same for both assays, in this case differences could be due to the immunoassay used. Other comparative studies for the measurement of airborne rat allergen levels have shown that identical air samples would yield different results depending on the assay technology used, with the most important factors being the source and type of antibodies employed [30, 31]. In this study, the different sources of antibodies used could explain some of the differences obtained in the individual concentrations, as demonstrated by Swanson et al. [32], in a study of airborne mite allergens using monoclonal antibodies, rabbit polyclonals and human sera as detection antibodies.

This study shows that the ELISA inhibition assay described is a useful tool for the measurement of soybean aeroallergen. The practical significance of the results of this assay is that the allergen can be measured with a competitive ELISA method, since although monoclonal assays had high sensitivity, specificity and reproducibility, they were limited in that they do not detect other allergens present that may cause symptoms. This method is a marker of allergen load and, together with the measurement of meteorological atmospheric conditions, permits a control during the unloading of soybean in Barcelona to be established. This assay also informs on the concentration of this allergen in the atmosphere and facilitates the monitoring of the impact of environmental intervention controls used, e.g. unloading with a system of pumps that reduces the quantity of dust in the atmosphere, thereby avoiding excessive levels of soybean allergen.

In conclusion, the amplified ELISA inhibition method described appears to be an effective and reproducible method to be used for the measurement of soybean allergen in the environment where this bean is manipulated.

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References

- Antó JM, Sunyer J, Rodríguez-Roisin R, Suarez M, Vazquez L: Asthma epidemics associated with soybean dust relevant during harbor activities. *N Engl J Med* 1989;320:1097-1102.
- Rodrigo MJ, Morell F, Helm RM, Swanson M, Greife A, Antó JM, et al: Identification and partial characterization of the soybean-dust allergens involved in the Barcelona asthma epidemic. *J Allergy Clin Immunol* 1990;85:778-784.
- Sunyer J, Antó JM, Rodrigo MJ, Morell F: Epidemic asthma and soybean IgE antibodies. *Lancet* 1989;i:179-182.
- Gonzalez R, Varela J, Carreira J, Polo F: Soybean hydrophobic protein and soybean hull allergy. *Lancet* 1995;346:48-49.
- Odani S, Koide T, Ono T, Seto I, Tanaka T: Soybean hydrophobic protein: Isolation, partial characterization and the complete primary structure. *Eur J Biochem* 1987;162:485-491.
- Antó JM, Sunyer J, Reed CE, Sabria J, Martinez F, Morell F, et al: Preventing asthma epidemics due to soybean by dust-control measures. *N Engl J Med* 1993;329:1760-1763.
- White MC, Etzel RA, Olson DR, Goldstein IF: Reexamination of epidemic asthma in New Orleans, Louisiana, in relation to the presence of soy at the harbour. *Am J Epidemiol* 1997;145:1-7.
- Cocco G, Schiano M, Sacerdoti G: Functional characteristics in soybean asthma (abstract). *Am J Respir Crit Care Med* 1995;152:469.
- Navarro C, Marquez M, Hernando L, Galvan F, Zapatero L, Caravaca F: Epidemic asthma in Cartagena, Spain, and its association with soybean sensitivity. *Epidemiology* 1993;4:76-79.
- Garcia-Ortega P, Rovira E, Mora E: Epidemias de asma alérgica a semilla de soja en ciudades pequeñas. *Med Clin (Barc)* 1997;108:677.
- Ballester F, Soriano JB, Otero I, Rivera ML, Sunyer J, Merelles A, et al: Asthma visits to emergency rooms and soybean unloading in the harbours of Valencia and A Coruña, Spain. *Am J Epidemiol* 1999;149:315-322.
- Aceves M, Grimalt JO, Sunyer J, Antó JM, Reed CE: Identification of soybean dust as an epidemic asthma agent in urban areas by molecular marker and RAST analysis of aerosols. *J Allergy Clin Immunol* 1991;88:124-134.
- Swanson MC, Li J, Wentz-Murtha PE, Trudeau WL, Fernandez-Caldas E, Greife A, et al: Source of the aeroallergen of soybean dust: A low molecular mass from the soybean tela. *J Allergy Clin Immunol* 1991;87:783-788.
- Li JT, Swanson MC, Rando RJ, Wentz-Murtha P, Ovsyannikova IG, Morell F, et al: Soybean aeroallergen around the port of New Orleans: A potential cause of asthma. *Aerobiologia* 1996;12:173-176.
- Newman-Taylor A, Tee RD: Environmental and occupational asthma. Exposure assessment. *Chest* 1990;5:209S-211S.
- Carpenter AB: Enzyme-linked immunoassays; in Rose NR, Folds JD, Nakamura RM (eds): *Manual of Clinical Laboratory Immunology*, ed 5. Washington, D.C., American Society for Microbiology, 1997, pp 908-915.
- American Academy of Allergy, Asthma and Immunology (AAAAI). The use of standardized allergen extracts. *J Allergy Clin Immunol* 1997;99:583-586.
- 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;27:1314-1321.
- 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;54:135-140.
- Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;i:307-310.
- Soriano JB, Antó JM, Plasencia A, and the Barcelona Soybean-Asthma Group: Repeaters count: A sentinel method for asthma outbreaks. *Thorax* 1995;50:1101-1103.
- Luczynska CM, Arruda LK, Platts-Mills TAE, Miller JD, Lopez M, Chapman MD: A two-site monoclonal antibody ELISA for the quantitation of the major *Dermatophagoides* spp. allergens, *Der p* 1, *Der f* 1. *J Immunol Methods* 1989;118:227-235.
- Bollinger ME, Wood RA, Chen P, Eggleston PA: Measurement of cat allergen levels in the home by use of an amplified ELISA. *J Allergy Clin Immunol* 1998;101:124-125.
- Houba R, Van Run P, Doekes G, Heederik D, Spithoven J: Airborne levels of α -amylase allergens in bakeries. *J Allergy Clin Immunol* 1997;99:286-292.
- Gordon S, Tee RD, Nieuwenhuijsen MJ, Lowson D, Harris J, Newman-Taylor AJ: Measurement of airborne rat urinary allergen in an epidemiological study. *Clin Exp Allergy* 1994;24:1070-1077.
- Holls S: Analysis of method comparison studies. *JIFCC* 1997;9(1).
- The measure of interrater agreement; in Fleiss JL: *Statistical methods for rates and proportions*. New York, Wiley, 1981, pp 212-236.
- Vaughan NP, Chalmers CP, Botham RA: Field comparison of personal samplers for inhalable dust. *Ann Occup Hyg* 1990;34:553-573.
- Kenny LC, Aitken R, Chalmers C, Fabries JF, Gonzalez-Fernandez E, Kromhout H, et al: A collaborative European study of personal inhalable aerosol samplers performance. *Ann Occup Hyg* 1997;41:135-153.
- Hollander A, Gordon S, Renström A, Thissen J, Doekes G, Larsson PH, et al: Comparison of methods to assess airborne rat and mouse allergen levels. I. Analysis of air samples. *Allergy* 1999;54:142-149.
- Renström A, Gordon S, Hollander A, Spithoven J, Larsson PH, Venables KM, et al: Comparison of methods to assess airborne rat and mouse allergen levels. II. Factors influencing antigen detection. *Allergy* 1999;54:150-157.
- Swanson MC, Campbell AR, Klauck MJ, Reed CE: Correlation between levels of mite and cat allergens in settled and airborne dust. *J Allergy Clin Immunol* 1989;83:776-783.

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