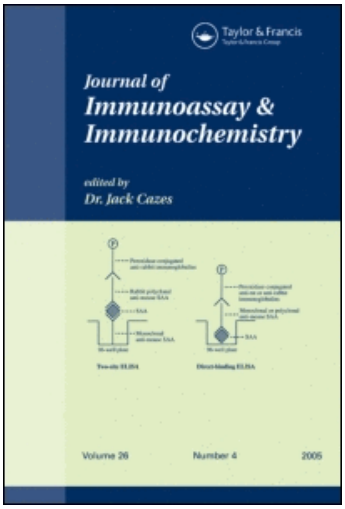

Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Two Elisa Methods for The Measurement of Airborne Swine Epithelial Antigens

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To cite this Article Virtanen, Tuomas I.(1990) 'Two Elisa Methods for The Measurement of Airborne Swine Epithelial Antigens', Journal of Immunoassay and Immunochemistry, 11: 1, 63 — 76
To link to this Article: DOI: 10.1080/01971529008053258
URL: http://dx.doi.org/10.1080/01971529008053258

JOURNAL OF IMMUNOASSAY, 11(1), 63-76 (1990)

TWO ELISA METHODS FOR THE MEASUREMENT OF AIRBORNE SWINE EPITHELIAL ANTIGENS

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ABSTRACT

A new double antibody sandwich ELISA for measuring swine epithelial antigens in swineries was compared to an adaption of our previously developed ELISA inhibition method. The sandwich ELISA is more sensitive than the inhibition ELISA, and its reproducibility is better. The quantitative results from swinery samples of the sandwich ELISA are about ten times higher, but correlate well (r_s 0.88, p=0.004) with the results of ELISA inhibition. The progression in magnitude of the results in relation to the sample dilution was examined.

(Key words: airborne allergen, agricultural environment, ELISA inhibition, capture ELISA, non-parallel ELISA curves)

INTRODUCTION

During the past few years immunochemical methods have become increasingly popular for measuring the levels of airborne allergens. They are the only way to detect antigens or allergens (i.e. allergenic

antigens) which cannot be quantitated by morphological or microbiological examination.

We have used the ELISA inhibition method to measure the levels of airborne bovine epithelial antigens in cowsheds (1, 2, 3). The rationale for measuring antigens instead of specific allergens is the need to avoid laborious allergen purifications in a situation where relevant components for measurement are not known. The aim of this study was to develop the double antibody sandwich ELISA for the measurement of swine epithelial antigens (SEA) in swineries and then to compare it to an adaption of our ELISA inhibition method in order to determine which of the methods should preferentially be used. Particular attention was paid to the phenomenon of the progression in magnitude of the results in relation to the sample dilution and to the quantitative differences of the results between ELISAs.

MATERIALS AND METHODS

Collection and Handling of Dust Samples

Samples of airborne particulate material were collected as described previously (2).

Filters were eluted in beakers with 5 ml of 0.01 M phosphate-buffered saline, pH 7.4, containing 0.5 % (V/V) nonionic detergent, Nonidet P40 (BDH Chemicals Ltd., UK) and 0.02 % (W/V) thiomersal (BDH Chemicals Ltd, England) for 2 h at room temperature with an agitation rate of 150 rpm. After centrifugation at

36,600 g for 45 min, the supernatant was collected and stored at -70 °C.

Antigen Preparation

For preparation of SEA, the powdered raw material of swine hair and skin scraping was purchased from Allergon AB (Ängelholm, Sweden). The antigen was made in Coca's solution in essentially the same manner as the previously described preparation of bovine epithelial antigen (3). The protein content of the SEA was 4.22 mg/ml (4).

Rabbit Antisera to SEA

Rabbits were immunized with SEA every two weeks for a 4 month period, each time receiving 2.0 mg of protein. The antigen solution was emulsified with Freund's incomplete adjuvant. Injections were given subcutaneously. Blood was drawn several times, and the antisera were stored at -70 °C.

Purification of Rabbit IgG

Rabbit IgG was purified as described by McKinney and Parkinson (5). The IgG preparation was stored at -70 °C. The purity of the preparation was examined by immunoelectrophoresis.

Biotinylation of Rabbit IgG

Biotinylation of rabbit IgG was carried out as described by Yolken et al. (6). The biotinylated IgG preparation was stored at -70 °C.

Determination of SEA Concentrations by ELISA Inhibition

The ELISA inhibition procedure for measuring SEA was based on a previously described method for the measurement of bovine epithelial antigen in cowsheds (3). Necessary titrations for optimal reagent concentrations were carried out. The following changes were made: Microelisa plates were coated with SEA at a concentration of 0.5 ug/ml of protein in a coating buffer containing thiomersal 0.02 % (W/V). After coating, the plates were incubated for 2 h at 37 °C with diluent containing thiomersal 0.02 % (W/V). Several sample dilutions were used. Rabbit anti-SEA antiserum was used at a dilution of 1:20,000 - 1:25,000 (depending on the coating batch of the plates). Biotinylated goat anti-rabbit IgG was incubated without agitation.

For determination of the amount of antigen in swinery samples, the method of point estimation at the level of 50 % inhibition of maximal absorbance achieved in diluent wells was used. The amount of antigen corresponding to the 50 % inhibition was calculated from the standard curve of absorbance against the log concentration of the standard antigen. The sample results were then obtained by multiplying by the appropriate dilution factors.

Determination of SEA Concentrations by Double Antibody Sandwich ELISA

For the double antibody sandwich ELISA, prior checkboard titrations of the reagents were carried

out. After determining the optimal incubation times and temperatures, the following procedure was adapted: A microelisa plate (Nunc-Immuno Plate I, Denmark) was coated for 2 h with rabbit anti-SEA-IgG at a concentration of 0.1 mg/ml of protein in the same coating buffer as in ELISA inhibition in volumes of 200 ul/well at 37 °C. Next, the plate was washed and incubated for 2 h at 37 °C with a diluent which contained casein (BDH Chemicals Ltd, England) 0.1 % (W/V) instead of gelatine. After incubation, the plate was stored at -70 °C. Before use, the plate was allowed to reach room temperature and was then washed. The standard antigen and sample dilutions in 200 ul volumes were added in duplicate, and the plate was incubated at 37 °C for 18 h. After washing and incubation with diluent for 1 h at 37 °C, biotinylated rabbit anti-SEA-IgG at a dilution of 1:100, 200 ul/well, was added. The plate was incubated for 2 h at 37 °C. After washing, Vectastain ABC Standard Kit reagents were used to develop the color reaction in a manner similar to that of ELISA inhibition (3).

For the determination of SEA in swinery samples, the standard curve of absorbance against the log concentration of the standard antigen was plotted. The amount of antigen in the samples was then calculated as a mean of 2 - 4 dilutions from the linear part of this curve.

RESULTS

ELISA Inhibition for SEA

Figure 1 shows the SEA standard curve with two samples tested at a series of two-fold dilutions. The

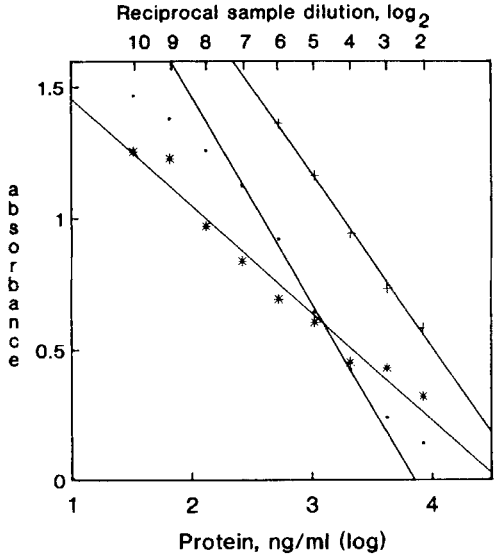

FIGURE 1 The linear parts of a SEA standard curve of ELISA inhibition (·) and of dilution curves for samples A (+) and B (*).

linear part of the SEA standard curve begins approximately at the level of 200 ng/ml indicating roughly the level of sensitivity. The dilution curves of the samples exhibit lower slopes than the standard curve. The results of the two samples calculated from different dilutions are presented in Table I. The progression in magnitude of the results is due to the non-parallelism of the standard and the sample dilution curves.

The mean intertest coefficient of variation calculated from 5 samples, three samples tested three times and two samples four times, was 22 % (range 15 - 35 %).

TABLE I

Results of Two Samples Showing the Progression in Magnitude of the Results in Relation to the Sample Dilution

Sample A		Sample B	
Dilution	ug/filter	Dilution	ug/filter
1/4	24	1/32	208
1/8	22	1/64	243
1/16	24	1/128	346
1/32	26	1/256	326
1/64	39	1/512	333

Double Antibody Sandwich ELISA for SEA

As shown in Fig. 2, the linear part of the standard curve begins approximately at a concentration level of 100 ng/ml. An unrelated protein preparation, swine feed antigen, did not interfere with the specific reaction (data not shown).

The standard curve and sample dilution curves of the sandwich ELISA are not parallel (Fig. 2). This non-parallelism is perhaps not as strong as in ELISA inhibition, but the use of the sandwich-type of ELISA does not eliminate the phenomenon. The mean intertest coefficient of variation calculated from 3 samples, individual samples tested three times, was 12.9 % (range 8.1 - 18.3 %).

Comparison of the Results of the Swinery Samples Obtained Using the Two ELISA Methods

The results for the two ELISAs are shown in the first and second columns of Table II. The results of

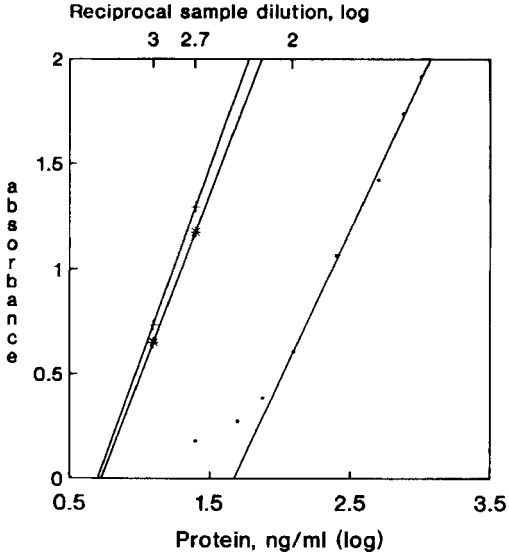

FIGURE 2 The linear parts of a SEA standard curve of a sandwich ELISA (·) and of dilution curves for samples C (+) and D (*).

TABLE II

Results of Swinery Samples
Using the Three Different ELISAs
ug/m³

Sample	ELISA inhibition	Sandwich ELISA	Casein ELISA inhibition
A	20.1	143.2	12.0
B	97.6	739.4	51.5
C	55.8	857.7	33.4
D	41.3	502.3	33.2
E	10.2	97.8	5.5
F	4.2	61.3	2.9
G	38.0	275.4	28.5
H	84.9	620.2	44.1

the sandwich ELISA are about ten times higher than the results of ELISA inhibition. They are also statistically significantly different ($p=0.012$; Wilcoxon signed-rank test). However, the results of the ELISA methods were found to correlate well (r_s 0.88, $p=0.004$; Spearman correlation analysis).

Because the only difference between the reagents and materials in these two ELISAs was the blocking agent (gelatin in the ELISA inhibition and casein in the sandwich ELISA), casein blocking (0.1 %, W/V) was tested in the ELISA inhibition. Apart from this change, the overall procedure was maintained except that the optimal dilution of antiserum was 1:12,000. Testing of the gelatin in the sandwich ELISA was impossible because of the previous observation that gelatin caused very high background absorbances in the sandwich ELISA. The results of swinery samples measured by casein-blocked ELISA inhibition are shown in the third column of Table II.

The results achieved by this casein-blocked variation of ELISA inhibition differ considerably from those of gelatin ELISA inhibition. Statistically, they are significantly lower ($p=0.012$; Wilcoxon signed-rank test) but correlate well (r_s 0.98, $p<0.001$; Spearman correlation analysis). This result of casein-blocked ELISA inhibition can be explained by the observed shift of the SEA standard curve along the abscissa toward lower SEA standard dilutions, concurrently increasing the sensitivity of the assay to the level of about 100 ng/ml, as determined by SEA standard curves (data not shown). Thus, when the sample dilutions used in both ELISA inhibitions were approximately the same, lower results were obtained with casein-blocked ELISA.

DISCUSSION

The purpose of this study was to examine whether the newly developed double antibody sandwich ELISA could offer any advantages over the ELISA inhibition in the measurement of swine-derived dust in swineries. In addition, the observed progression in magnitude of the sample results in relation to the sample dilution and the quantitative differences of the results between ELISAs were to be considered.

The sandwich ELISA is more sensitive than the gelatin ELISA inhibition. The linear part of the ELISA inhibition curve begins approximately at the level of 200 ng/ml and the corresponding part of the sandwich ELISA approximately at the level of 100 ng/ml. Interestingly, the use of casein as a blocking agent instead of gelatin in ELISA inhibition increased the sensitivity of ELISA inhibition to the level of the sandwich ELISA, as determined by standard curves.

Although the variation in sensitivity between gelatin ELISA inhibition and sandwich ELISA as determined by SEA standard curves was not great, the differences in swinery sample results were remarkable. The sandwich ELISA gave values which were about ten times higher than those for ELISA inhibition. The difference is even greater if a comparison is made between the sandwich ELISA and the casein-blocked ELISA inhibition (Table II). However, the results of the ELISAs correlate well.

The increased sensitivity of the sandwich ELISA was not a surprise in the light of previous data of other authors (7, 8). The surprising observation was,

however, the manner in which this increased sensitivity was manifest. It was seen as higher sample dilutions required in the sandwich ELISA than in the ELISA inhibition. For instance, Lockwood and Robertson (9) reported that although their ELISA for the measurement of an enterotoxin was nearly 10-fold more sensitive than their RIA, RIA produced higher results. The authors discussed the qualities of antisera. With respect to the present study, however, it is not a question of the quality of antisera since the same immune serum was used in both ELISAs. Rather, the phenomenon may be due to differences in the intrinsic dynamics of the ELISAs. A possible explanation for the higher results of the sandwich ELISA could lie in the efficient catching of SEA during the long incubation and its efficient presentation in the following step. It seems that when using pure antigen preparation (SEA standard) the reaction reaches its equilibrium more easily, and larger differences in sensitivity are not seen in the two ELISA types.

The progression in magnitude of the results in relation to the sample dilution was observed in both ELISA types. This is caused by non-parallelism between the standard antigen and sample dilution curves. In the analyses of allergenic extracts, non-parallelism between the standard antigen and sample dilution curves has been taken as an indication of partial antigenic differences between the standard antigen and the antigens present in the sample (10). Probably, the explanation for the phenomenon is competition between the antigen and related molecules for binding sites (11). In agricultural environments, probable competitive molecules are animal-derived and

they originate from other farm animals. It is also possible that the SEA standard antigen may differ from the natural swine antigens present in the dust, e.g., because of standard antigen preparation procedure or degradation of swine antigens in swinery environments. The phenomenon may also just reflect differences in relative epitope densities between the standard antigen and the antigens found in swineries. Consequently, it might be possible to reduce the phenomenon of non-parallelism by reducing competition in the ELISA reaction. This could be achieved by increasing the specificity of reactants.

The sandwich ELISA seems suitable for the measurement of SEA in swineries. In particular, its better sensitivity and reproducibility make it preferred over the ELISA inhibition. On the other hand, because of its general simplicity and the need for very few self-produced reagents, ELISA inhibition may be useful for preliminary measurements and purposes where demands are less stringent.

ACKNOWLEDGEMENTS

I thank Allergy Research Foundation (Finland) for supporting this study.

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