

RAST inhibition: a collaborative study on its suitability and reproducibility for the assay of cocksfoot pollen extracts

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Summary

The suitability and reproducibility of the inhibition mode of the radioallergosorbent test (RAST) as a method for the assay of allergen extracts was examined in a collaborative study involving seven laboratories. Participants assayed five coded extracts of cocksfoot grass (*Dactylis glomerata*) pollen, including three batches from one manufacturer, both by a common method and by their local methods of RAST inhibition. For the versions of RAST inhibition employed in this study the estimates of relative potencies of the extracts were in agreement. Further, when laboratories used their local methods, the inter-laboratory variation in potency estimates was similar to that found when laboratories used the common method. It was concluded that RAST inhibition has a useful role in the assessment of the potencies of allergen extracts, and of the consistency of serial batches of extracts from individual manufacturers.

Introduction

The inhibition mode of the radioallergosorbent test (RAST) was developed in the early 1970s [1–3] and has been usefully applied to the comparison of commercially available extracts for allergens such as ragweed, June grass, timothy and birch pollen. We have shown in a previous study [4] that the potency estimates obtained by different laboratories were in close agreement when a common method of RAST inhibition and common reagents were used. The aims of the present study were to investigate the suitability of RAST inhibition to compare the potencies of batches of cocksfoot pollen extract from two manufacturers; and to compare the reproducibility of potency estimates when local methods were used with that found when a common method was used. This paper describes the results and findings of that study.

The studies were carried out under the auspices of the Allergen Technical Working Group (ATWG). Cocksfoot grass (*Dactylis glomerata*) pollen was chosen as the

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allergen for the studies, since a British Standard for this allergen had recently been established.

Materials and methods

Cocksfoot (Dactylis glomerata) pollen extracts

P, R and T were different batches of cocksfoot pollen extracts from a single manufacturer. Q and S were prepared from a batch of cocksfoot pollen extract obtained from another manufacturer; extract Q was a 1:3 dilution of extract S. The undiluted extracts were expected to have roughly (within two-fold) the same activity as each other. The extracts were supplied to NIBSC in a freeze-dried form, and subsequently dissolved in sterile distilled water at the concentration of 4.5 mg dry weight per ml. These solutions were then distributed in 1 ml volumes into ampoules and freeze-dried.

Human anti-cocksfoot serum

The anti-cocksfoot serum, an NIBSC reagent coded 77/503, was prepared from a pool of human sera from individuals having high levels of specific serum IgE antibodies to cocksfoot pollen; it was distributed into 0.5 ml aliquots, freeze-dried and stored at -20°C . A pool of normal human sera (NHS) was distributed in 1 ml volumes and similarly freeze-dried.

Allergen discs

Paper discs (Schleicher & Schüll, D-3354 Dassel, W. Germany) were activated with cyanogen bromide after the method of Ceska *et al.* [5] and coupled with a cocksfoot pollen extract (supplied by Pharmacia) at 20 μg protein per disc.

Labelled anti-human IgE antibody

The anti-human IgE antibody radiolabelled with ^{125}I was from Phadebas RAST kits (Pharmacia, Uppsala, Sweden).

Participants

Workers in seven laboratories in five countries participated in this study; they are listed in the Appendix but the order in which they appear does not necessarily correspond with the numerical order of the code numbers.

Design of the study

The study was co-ordinated by NIBSC, who were responsible for coding and distributing the reagents. Participants were requested to perform three assays of the five coded cocksfoot pollen extracts by the study method, described below, and three assays by their local method of RAST inhibition. Sufficient ampoules of the coded extracts and labelled anti-IgE for six assays were supplied to the participants. Additionally, for the study method, laboratories were provided with Falcon microtitre plates, allergen discs, anti-cocksfoot serum, normal human serum and horse serum [6]. The raw data were to be returned to the NIBSC on the data forms provided.

Study method

This method was designed so that one assay could be performed on a single microtitre

plate: in each assay the coded extracts were tested at six, threefold serial dilutions from 1:3 to 1:729 in duplicate. Controls were included to estimate zero inhibition (human anti-cocksfoot serum without extract present) and non-specific background (NHS instead of anti-cocksfoot serum). All dilutions of the extracts, anti-cocksfoot serum and NHS were made in a horse serum diluent, a 1:10 dilution of horse serum (Gibco Europe Ltd, Paisley, Scotland) in PBS. Equal volumes (100 μ l) of each dilution of extract and the serum at a 1:20 dilution were incubated together in the microtitre plate for 30 min at 37°C, a disc was added to each well and the plates were held overnight at room temperature. The discs were then washed six times with buffer (PBS containing 1% Tween 20) with a 3-min incubation for each wash, 50 μ l of ^{125} I anti-human IgE were added to each disc, the plates held overnight at room temperature and then the discs were washed as before. The radioactivity bound to the discs was counted in a gamma counter for 120 sec; the counts obtained were recorded on the supplied data forms.

Local methods

Workers in six of the seven collaborating laboratories carried out RAST inhibition using their local methods and reagents; brief details of these and of the study method are given in Table 1. Five laboratories used activated paper discs, with Cocksfoot pollen on discs; one laboratory used Matrex particles as the solid phase.

Statistical analysis

Assay data were analysed by the parallel line method [7] relating the logit of the percentage inhibition of IgE antibody binding to log dose of allergen by weighted linear regression [8]. Analysis of variance was used to assess the significance of the deviations of the log dose-response relationships from linearity and from parallelism. The raw data were also plotted to aid the interpretation of the results. Potency ratios for the extracts expressed in terms of P were calculated as the antilogarithms of the horizontal differences between the fitted parallel lines of each of the extracts and extract P; in addition, the potency ratio for Q was calculated in terms of S.

Assays for which the deviations of the log dose-response relationship from parallelism or linearity were found to be significant ($P < 0.01$) were examined more closely. In most of these assays, examination of the slopes for the regression lines of extract S and its diluted form Q indicated that the apparent statistical invalidity was probably the result of underestimation of the residual error due to inadequate replication within the assays. Estimates for these assays were, therefore, considered to be statistically valid. Any assay for which the apparent invalidity did not appear to be due to underestimation of error was taken to be statistically invalid. However, estimates from all assays were included in the subsequent analyses.

Potency estimates were combined by taking the geometric mean of the individual estimates. The variability of the potency estimates was expressed as the geometric coefficient of variation [9] (GCV). Analysis of variance of log potencies was used to test whether or not the differences observed between the potency estimates of individual extracts could be attributed solely to the between-assay variation within laboratories.

Results

Workers in all except laboratory 2 performed assays by their local methods and those in all except laboratory 7 performed assays by the study method with the supplied reagents. Data were contributed from eighteen assays by the study method and from

Table 1. Details of reagents and incubation conditions used in the study method and local methods of RAST inhibition

Method and lab. code	Containers	Solid phase (SP)	Source of antigen on solid phase	Human anti-cockfoot serum (HS) (no. in pool)	Negative control	Source of ¹²⁵ I anti-IgE	Incubation times/temperatures		
							HS + AE	HS&AE + SP	SP + α -IgE
Study method	microtitre plates	CNBr PD	Pharmacia	77/503 (NK)	NHS	Pharmacia	30 min/37°C	ON/RT	ON/RT
Local methods									
Lab. 1	Plastic tubes	CNBr PD	lab's own	77/503 (NK)	NHS	lab's own	ON/RT	3 h/RT	ON/RT
Lab. 3	microtitre plates	CNBr PD	lab's own	lab's own (22)	NSA	Pharmacia	1h/RT	3 h/RT	ON/RT
Lab. 4	microtitre plates	CNBr PD	Pharmacia	77/503 (NK)	NHS	lab's own	1h/37°C	ON/RT	ON/RT
Lab. 5	plastic tubes	CNBr PD	lab's own (Allergon pollen)	lab's own (15)	BSA	lab's own	1h/RT + mixing	3 h/RT + mixing	ON/RT + mixing
Lab. 6	plastic tubes	Matrex PEL 102	lab's own	lab's own (35)	NHS	Pharmacia	none	ON/20°C	ON/20°C
Lab. 7	plastic tubes	CNBr PD	Phadbas RAST®	lab's own (NK)	HS + undiluted extract	Pharmacia	3h/23°C + mixing	3 hr/23°C + mixing	18 hr/23°C + mixing

SP = solid phase, HS = human serum, AE = allergen extract, CNBr PD = cyanogen bromide activated paper discs, NK = not known, ON = overnight, RT = room temperature, NHS = normal human serum, NSA = normal serum albumin, BSA = bovine serum albumin.

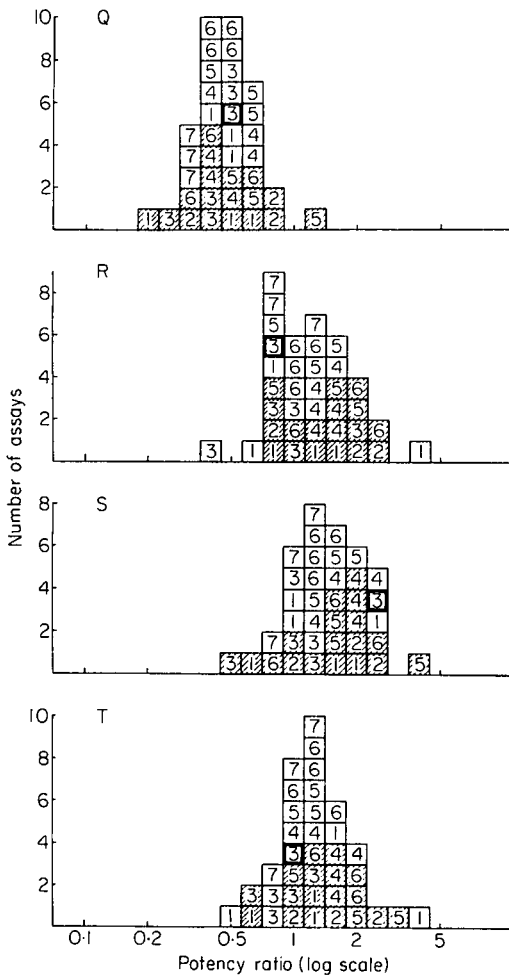


Fig. 1. Frequency distributions of potency estimates from individual assays in terms of extract P for extracts Q, R, S and T. Each box represents the result from one assay, the numbers in the boxes refer to the code numbers of the laboratories: ■ = study method; □ = local method; ▨ = statistically invalid estimate.

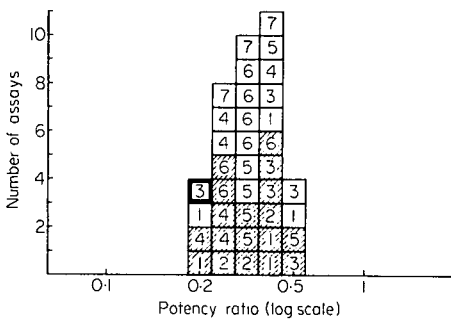


Fig. 2. Frequency distributions of potency estimates of extract Q in terms of S from individual assays. Each box represents the results from one assay, the numbers in the boxes refer to the code numbers of the laboratories: ■ = study method; □ = local method; ▨ = statistically invalid estimate.

Table 2. Geometric means (GM) and coefficients of variation (GCV) of the laboratory mean potency ratios for extracts Q, R, S and T relative to P and for extract Q relative to S

	No. of labs	Q		R		S		T		Q relative to S	
		GM	GCV	GM	GCV	GM	GCV	GM	GCV	GM	GCV
Study method	6	0.47	29%	1.38	19%	1.50	39%	1.40	31%	0.32	23%
Local methods	6	0.46	20%	1.12	19%	1.41	21%	1.17	22%	0.33	6%

nineteen assays by local methods.

All assays by the study method and all but one by the local methods were judged to be statistically valid; the exception was an assay from laboratory 3 which had significant deviations from parallelism. However, there was some evidence for underestimation of error within the assays by the local methods of two of the other laboratories (6 and 7).

The variation between the potency estimates for each extract was examined by analysis of variance. No significant differences were found between laboratories when either the study method or local methods were used. The frequency distributions of the potency estimates of extracts Q, R, S and T relative to P from individual assays are shown in Fig. 1 and that for Q in terms of S is shown in Fig. 2. The potency estimates of Q relative to S were generally close to the expected value of 0.33 (Fig. 2); all estimates were within a two-fold range of this value.

The geometric means of the potencies from replicate assays of the individual laboratories were calculated. The geometric mean of these means for the study method and the local methods were then calculated (Table 2). There was close agreement between the overall mean potencies for Q and S relative to P for the study and local methods. The mean potencies for extracts R and T from the study method were, however, slightly higher, although not significantly so, than those from the local methods. There was also some indication of differences between the potencies of the batches from the one manufacturer, i.e., P, R and T, with both the study and the local methods giving mean potencies for extracts R and T close to each other but somewhat higher than those for P. Nevertheless, the estimated potencies of these extracts differed by no more than 50%.

Table 2 also shows the inter-laboratory variation as measured by the GCV of the laboratory mean potencies. The GCVs for the local methods were small, and indeed were somewhat smaller than those for the study method. This was particularly the case with the potency ratio for Q in terms of S.

Discussion

One of the aims of this study was to assess the ability of RAST inhibition to compare potencies of allergen extracts. However, the validity of any comparison of extracts depends on whether they differ qualitatively, possibly owing to differences between their starting materials or manufacturing processes. If there were such differences between the extracts tested in this study—which is possible as the extracts were from two manufacturers—these differences might have manifested themselves in the following ways: (i) a lack of comparability of the dose-response relationships of the extracts within individual assays; and (ii) by disagreement between estimates of relative potency obtained when different assay procedures or reagents were used. No such

findings were obtained with the possible exception of a single assay (from laboratory 3) which gave some suggestion of a lack of comparability of the dose-response relationships of the extracts. Thus for the assay systems used in this study, the extracts appeared to behave similarly and RAST inhibition appeared to be a suitable method by which to assay them. Similar findings were obtained in a recent collaborative study involving timothy pollen extracts [10]. In contrast, in another collaborative study on *D. pteronyssinus* extracts [11], one extract was found to differ qualitatively from all the other extracts resulting in a high incidence of statistically invalid assays and disagreements between the laboratories in their estimation of potency estimates for this extract.

For the local methods the inter-laboratory variation in potency estimates was similar to that found in other studies involving pollens; for example in the timothy study mentioned above [10] the GCVs of the laboratory mean potencies ranged from 32 to 45%. Also, no significant differences were found in our study between laboratories in their estimation of potency. Further, in this present study, the use of the study method did not reduce the variation between laboratories. These findings could be attributed to a lack of difference between the local methods, but in fact the antisera, the antigen on the solid phase, the labelled anti-IgE and the incubation conditions varied considerably between methods. Antisera are considered important potential sources of differences between methods, yet in the study five different pools of sera, some from different countries, were employed. In addition, one laboratory (6) used a method involving inactivated Matrex particles which was very different from those of the other laboratories, yet its potency estimates fell within the ranges of estimates obtained by the other laboratories. A more likely explanation for the agreement between laboratories is that since similar materials were being compared, the differences in specificities of the different assay methods had little influence on the potency estimates.

In this study, three branches from one manufacturer were compared in six laboratories by their local methods of RAST inhibition and the potency estimates were found to be in agreement even though different methods and reagents were employed among the collaborating laboratories. It was therefore concluded that RAST inhibition has a useful role in the assessment of potencies of allergen extracts and the consistency of serial batches of extracts from individual manufacturers.

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Appendix:

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