

A method for quantitation of food biologic activity: Results with peach allergen extracts

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Objective: The aim of this study was to develop a quantitative skin test assay for measurement of the biologic activity of food allergen extracts, as well as to report the results obtained with a peach extract labeled in food biological units (FBUs).

Methods: We prepared a biologically quantified peach extract. The biologic activity was measured in FBUs by assigning 100 FBU/ml to the biologic activity of the extract that elicited a wheal area with a geometric mean equal to that produced by the prick-prick method by using the food itself in a population of 30 patients allergic to food. We evaluated 265 patients, including 70 patients allergic to peach and 195 control subjects (100 nonatopic subjects and 95 subjects allergic to pollen). The biologically quantified peach extract was used during the study and was compared with four commercial peach extracts, which were labeled in weight per volume.

Results: The sensitivity of the nonstandardized commercial peach extracts varied from 4.3% to 74%, with biologic activity being very low in all of them. The sensitivity of the biologically quantified peach extract was 100%, with a 100% concordance between the prick-prick and the skin prick test results.

Conclusion: These data demonstrate that if peach allergen preparations were biologically standardized in FBUs, the quality of these food extracts used for diagnosis would be improved. (*J Allergy Clin Immunol* 1998;102:275-80.)

Key words: Biologic activity, food, standardization, peach, skin test, food allergy

Physicians working with allergen extracts ought to know the quality and biologic activity of the materials they use to ensure safety and precision both for diagnosis and immunotherapy. In this context biologic standardization provides very useful information about the biologic response that can be expected when an extract is used for specific diagnosis and has become a quality

Abbreviation used

FBU: Food biological unit

guarantee of the allergen extract that physicians handle.¹ It is now possible to quantify the concentration of some individual allergens—at least the major allergens—in mass units, and this is highly desirable.²⁻⁵ However, even in this case, biologic units will also be needed because the biologic activity of allergen preparations will continue to be of interest.

Despite this, food allergen extracts continue to be labeled in protein nitrogen units or weight per volume values, which is far from adequate for the labeling of allergen extract potency.

The purpose of this study was to develop a quantitative skin test assay for measurement of the biologic activity of food allergen extracts, as well as to report the results obtained with a peach extract labeled in food biological units (FBUs).

METHODS

Study design

This study was carried out in two parts with several steps. We first defined a quantitative skin test assay for measurement of the biologic activity of food allergen extracts and afterwards prepared a peach extract with sufficient potency, which was labeled in FBUs. We next evaluated the diagnostic efficacy of the biologically quantified peach extract (100 FBU/ml) together with other commercial peach extracts and compared them with prick-prick test results (fresh food). This step is shown in the Results section as diagnostic evaluation in three patient groups (patients allergic to peach, control patients allergic to pollen, and nonatopic patients).

Patients

The patients allergic to peach were selected for the study by having typical symptoms of previous peach allergy reactions, a positive prick-prick test response to peach, and a positive peach challenge result.

Two hundred sixty-five patients took part in this study to evaluate skin test results obtained on use of the biologically quantitated peach extract (100 FBU/ml) in comparison with those derived from the prick-prick test and other commercial peach extracts. These 265 subjects were 70 patients allergic to peach and 195 patients used as a control group (95 patients allergic to pollen and 100 nonatopic patients). The control

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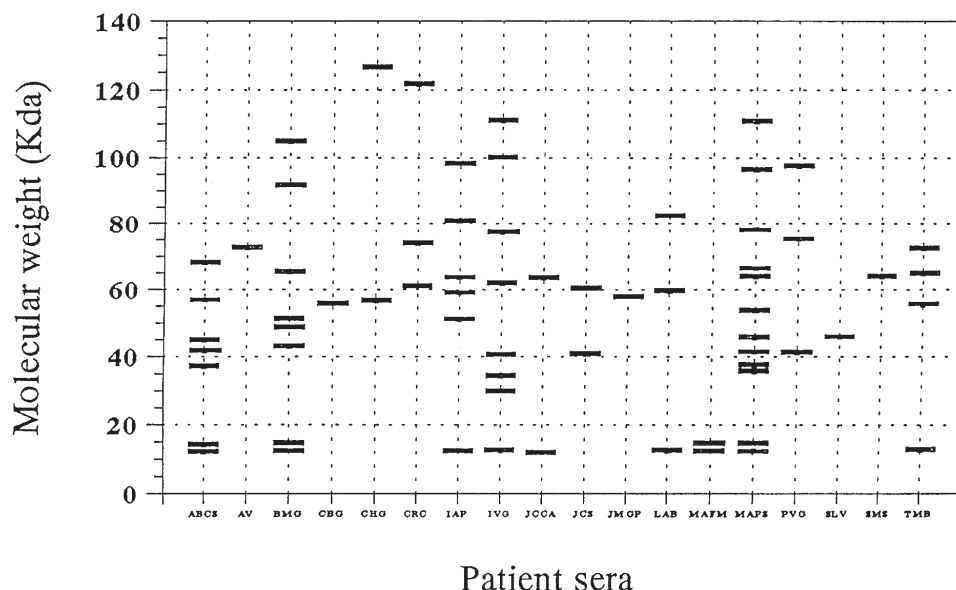


FIG. 1. Peach antigens detected in sera of patients with peach allergy by sodium dodecylsulfate-polyacrylamide gel electrophoresis immunoblotting.

patients allergic to pollen were selected by pollen allergy criteria and were also evaluated to determine whether they had peach allergy. The patients gave their written consent to participation in the study.

Peach extract preparation

Peaches purchased in a local market were the raw material used in the production of the peach peel extract. Peach peel (250 g) was homogenized in a blade homogenizer (Waring type; Braun, Kronberg, Germany) for 5 minutes at 20,000 rpm, followed by magnetic stirring (2000 rpm) at 4° to 8° C for 1 hour. The Björkstén solution⁶ was used as a solubilizing agent (10 mmol/L potassium phosphate buffer [pH 7.5] containing 2% suspended solid polyvinyl pyrrolidone, 2 mmol/L ethylenediamine tetraacetic acid disodium salt, and 10 mmol/L sodium diethyl dithiocarbamate). The extract was then clarified by centrifugation at 43,000 *g* for 30 minutes. The pellet was discarded, and the supernatant containing the solubilized proteins was dialyzed by diffusion in Visking tubing (Medicell, London, U.K.). The dialyzed extract was sterilized by filtration through a 0.22 μ m pore diameter membrane and freeze-dried. Average yields after this process were 4.7% (4.7 g of the lyophilized material for each 100 g of the peach peel). The peach extract was characterized by sodium dodecylsulfate-polyacrylamide gel electrophoresis immunoblotting (Fig. 1), according to the method previously described by Martinez et al.⁷ in which the protein content of peach extract was increased from 0.72 mg/ml to 2.62 mg/ml by using ion-exchange chromatography.

Skin tests

Skin tests were carried out by the skin prick test method and were performed according to The European Academy of Allergology and Clinical Immunology guidelines.¹ The ALK-Lancet needle (ALK, Denmark) was used during the study. A result was considered positive when a wheal 3 mm or greater in diameter was obtained. Skin tests with fresh peach peel were performed by a skin prick-prick test, according to the technique described by Dreborg and Foucard.⁸

Skin tests were also carried out in duplicate in 70 patients allergic to peach with the biologically quantified peach extract (100 FBU/ml), four commercial peach extracts (10 mg/ml peach peel [extract A]; peach 1:10 wt/vol [extract B], peach 1/10 [extract C],

and peach 5% wt/vol [extract D]), and the prick-prick test with fresh peach. Moreover, skin tests with the biologically quantitated peach extract and the prick-by-prick test were also carried out in the 195 control patients.

Peach challenge test

The oral challenge tests were carried out with fresh peach, according to the modified method described by Pastorello et al.⁹ Briefly, each dose was chewed and then swallowed. The peach doses were gradually increased until a positive response was obtained or the entire peach was eaten without symptoms. The in vivo tests with fresh peach were carried out from June to October, which is the peach season in Spain.

Method for quantitation of food biologic activity

The biologic activity of the peach extract was measured in FBUs, according to a new standardization method. One hundred FBUs was defined as the biologic activity of the food allergen extract eliciting a wheal area the geometric mean of which was equal to that produced by the prick-prick method by using the food itself in a population of 30 patients allergic to food.

Skin prick tests were carried out in quadruplicate with several concentrations of the glycerinated peach extract (0.72, 0.36, 0.072, and 0.014 mg/ml) and the fresh peach peel itself by means of a prick-prick test. Positive and negative controls were also performed (histamine and normal saline solution). The dose response curves for each patient and the relationship between allergen concentration and the wheal area were calculated by using the following model: $\text{Log } Y = k + m \text{ Log } X$, where Y signifies the geometric mean of the wheal area size, X signifies the concentration of allergen extract, constant k signifies the intercept with the y-axis, and m signifies the slope of the line. The logarithmic transformation of the skin response normalizes the distribution of wheal areas and homogenizes their variances over the response interval.

Once both the dose-response relationship (protein concentration-wheal area) and the geometric mean of the prick-prick test were calculated, the food biologic activity was assigned by applying the definition either through data interpolation or by means of the formula.

To apply this method, it was necessary for the patient to fulfill the following minimal criteria: (1) the patient must have food aller-

TABLE I. Characteristics of patients with peach allergy

No.	Age (yrs)	Sex	Pollen allergy	Pollen symptoms	Peach symptoms	Evolution time (yrs)
1	13	M	No	—	OAS	5
2	10	M	Yes	RC,A	OAS	6
3	9	M	Yes	RC,A	CU,SS	6
4	20	F	Yes	RC,A	OAS	1
5	26	F	Yes	RC,A	CU,OAS,SS	8
6	14	M	Yes	RC	CU,OAS	3
7	28	M	Yes	RC,A	CU,OAS,SS	19
8	15	M	Yes	RC,A	OAS	2
9	24	F	Yes	RC,A	CU,OAS	8
10	26	M	Yes	RC,A	OAS	3
11	25	F	Yes	RC,A	CU,SS	4
12	7	M	No	—	CU,OAS	3
13	34	M	Yes	RC,A	CU,OAS	10
14	19	M	Yes	RC	CU,OAS	14
15	25	M	Yes	RC,A	OAS	15
16	18	M	Yes	RC,A	OAS	3
17	18	F	Yes	RC	OAS	8
18	8	M	Yes	RC	CU,OAS	1
19	35	F	No	—	OAS	5
20	34	M	No	—	OAS,CU	17
21	24	F	Yes	RC,A	OAS	3
22	16	M	Yes	RC	CU,SS	8
23	13	F	Yes	RC	OAS	3
24	19	F	Yes	RC	CU,OAS	7
25	29	F	Yes	RC,A	OAS	8
26	30	M	Yes	RC,A	OAS	7
27	28	M	Yes	RC,A	CU,SS	20
28	10	F	No	—	CU,OAS	4
29	19	F	Yes	RC,A	OAS	3
30	16	F	Yes	RC,A	OAS	2

A, Asthma; CU, contact urticaria; OAS, oral allergy syndrome; RC, rhinitis/conjunctivitis; SS, systemic symptoms.

gy (typical symptoms, positive skin test response, and positive challenge test response); (2) a statistically significant correlation between the food extract and the prick-prick test with the food itself must be demonstrated; and (3) the patient must not have a positive response to fewer than three consecutive concentrations or have a correlation coefficient (*r*) of allergen dose-response relationship less than 0.85 or a slope (*m*) less than 0.1.

RESULTS

Determination of the biologic activity of the extract

The age, sex, type of symptoms, and pollen allergy symptoms of the 30 patients allergic to peach are given in Table I. The geometric mean of the wheal area elicited by each peach extract concentration, as well as those for the peach peel prick-prick test (fresh food) and histamine are shown in Table II.

There was a statistically significant correlation ($r = 0.70$, $p < 0.01$) between the wheal area elicited by the peach peel prick-prick test and that elicited by the skin prick test when the biologically quantified peach extract was used (Fig. 2).

Fig. 3 represents the dose-response relationship obtained from the peach extract, as well as its regression equation. One hundred FBUs per milliliter were assigned to the 0.20 mg/ml peach extract protein content, which produced the same wheal size (66.2 mm²) as the geometric mean of the wheal area obtained with the fresh food (prick-prick method). The biologic activity of the

TABLE II. Wheal area elicited by several concentrations of peach peel extract and fresh peach

Concentration (protein mg/ml)	Geometric mean (mm ²)	Confidence limit ($p < 0.05$)	Range (mm ²)
0.72	96.5	81-114	42-230
0.36	80.9	67-97	32-214
0.07	51.6	42-64	13-165
0.01	25.4	20-33	7-93
Fresh peach	66.2	55-80	32-216

four concentrations used was later calculated (Table III) on the basis of the new regression equation ($\text{Log } Y = 1.46 + 0.338 \text{ Log } X$, with *Y* being the wheal size and *X* being the biologic activity in FBU/ml). A peach extract protein content of 0.72 mg/ml (Bradford method) was found to correspond with a biologic activity of 350 FBU/ml.

This method might be complemented with the measurement of the other biologic units: histamine equivalent prick (HEP) or Brighton modified method (BU). The extract concentration that produced 1 HEP was 0.070 mg/ml, corresponding to 34 FBU/ml; the concentration that produced 100 BU was 0.295 mg/ml, corresponding to 145 FBU/ml.

Diagnostic evaluation

Results in patients allergic to peach. Seventy patients allergic to peach (34 men and 36 women) were evaluat-

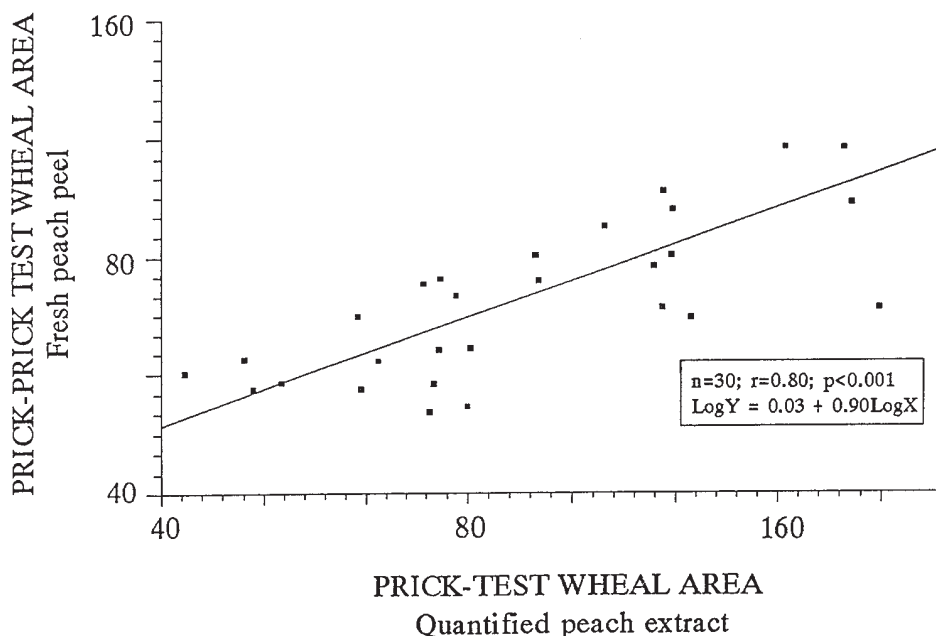


FIG. 2. Relationship between wheal area elicited by peach peel prick-prick test and skin prick test when biologically quantified peach extract was used (logarithmic scale).

TABLE III. Biologic activity of the several peach extract concentrations

Wheal size (mm ²)	Protein concentration (mg/ml)	Biologic activity (FBU/ml)
96.5	0.720	350
80.9	0.360	175
51.6	0.072	35
25.4	0.014	7
66.2	0.240	100

ed in this study group. The mean age was 20.3 ± 8.2 years, ranging from 4 to 43 years. Patient symptom types were oral allergy syndrome (86%), contact urticaria (61%), and systemic symptoms (26%).

The biologically quantified peach extract elicited a positive cutaneous response in the 70 patients allergic to peach (100%) who had reported typical peach allergy symptoms and had a positive peach challenge and positive peach peel prick-prick test response. Fig. 4 shows the differences in sensitivity obtained by using the various commercial peach extracts compared with those resulting from the prick-prick test and the extract quantified in FBUs. Three of these commercial peach extracts scarcely showed biologic activity, and the fourth did not detect peach allergy in 26% of the patients allergic to peach, with its biologic activity being very low in the remaining 74% (<10 FBU/ml).

To evaluate the specificity of the biologically quantified peach extract, we analyzed the results obtained in a nonatopic group without peach allergy and in an atopic group with pollen allergy.

Results in control patients allergic to pollen. Twenty-

five of the 95 patients allergic to pollen (26.3%) had positive prick-prick test responses. Only 12 of these 25 patients (48%) reported peach allergy symptoms and also had a positive peach provocation test response. Accordingly, the control patients allergic to pollen were divided into three groups: those without peach allergy (70 patients), those with peach allergy (12 patients), and those with asymptomatic hypersensitivity (13 patients).

The biologically quantified peach extract elicited a positive cutaneous response in the 12 patients allergic to peach (100%) and also in the 13 patients with asymptomatic hypersensitivity (100%), while no such response occurred in the patients not allergic to peach with a negative prick-prick test response (0%). Concordance between the prick-prick test and the skin prick test results was 100%.

Results in nonatopic patients. A negative result (0%) was observed in all of the nonatopic patients with no peach allergy and a negative peach prick-prick test response.

DISCUSSION

Skin testing is often used as a diagnostic tool for evaluating food allergy in spite of the fact that the oral challenge test is the gold standard.¹⁰ Standardized food extracts are not available, although many food allergens have been well characterized. The results of this study (Fig. 4), as well as those previously reported,¹¹⁻¹⁶ have shown that commercial food extracts vary widely in skin test potency, with some being of minimal potency. We found no relationship between the biologic activity and the labeled protein nitrogen unit or weight per volume values. The current situation for food allergen extracts is at least as bad as that for common aeroallergens before biologic standardization.¹⁷ Some investigators believe that food extract stan-

PEACH EXTRACT *Dose-response relationship*

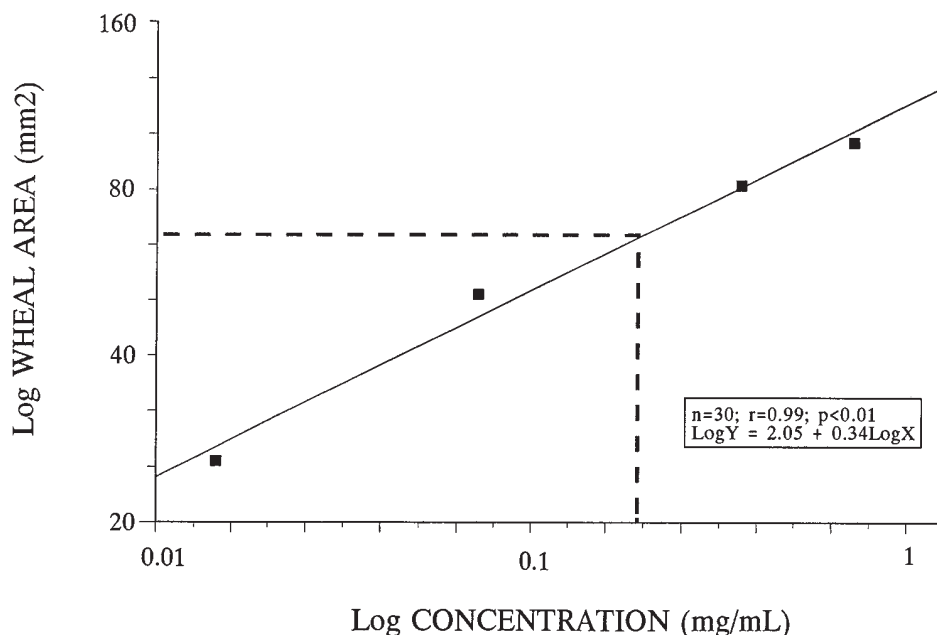


FIG. 3. Dose-response relationship obtained from peach extract (logarithmic scale). *Dashed line* represents peach allergen concentration producing same wheal area obtained with fresh food (prick-prick method).

standardization is not important, but it is our opinion that if food extracts are used, they must be better standardized, at least in potency. In this context we have designed a new method for measurement of food extract biologic activity.

Food extract standardization is not a new idea or concept; attention has been drawn for many years to the need for having better standardized food extracts.^{10,18,19} Patient biologic response to an extract depends on two sets of factors, the patient-dependent factors (e.g., age and symptom type) and the potency of the extract itself. There currently exist three biologic standardization methods (Aas et al.,²⁰ Brighton et al.,²¹ and Turkeltaub et al.²²); however, according to Backman et al.,²³ the biologic standardization of food-based allergenic preparations is not possible because patients do not show the same symptom types or age distribution, and their sensitization does not show the same time course.

In this study we have developed a quantitative skin test assay for measurement of the biologic activity of peach allergen extract, and we show the results obtained on use of extracts labeled in FBUs. The main contribution of this methodology is to bypass differences in biologic activity quantitation caused by differences attributable to patient sensitivity, such as symptom type, age distribution, and time course of sensitization, as reported by Backman et al.²³ With our method, the differences in wheal area size encountered on use of the various food extract concentrations, as well as use of the food itself, would be caused by the different biologic activity of each reagent because the responses to both reagents (the peach extract and the peach

itself) were elicited in the same patient. This is also upheld by the statistically significant correlation found between the skin test results elicited by the peach extract and by the food itself, in spite of differences in patient age, sex, sensitization time course, symptom type, and so on.

Validation procedures should be carried out so as to ascertain whether these theoretical assumptions are borne out. A statistically significant correlation between the test extract and the prick-prick test with the food itself must be demonstrated because if were it not, no precise estimation of patient sensitivity would be possible. Moreover, patients with a positive response to fewer than three consecutive concentrations, as well as those with a correlation coefficient less than 0.85 or a slope less than 0.1 must be excluded as too flat, or undefined regression lines might influence the final result in an unpredictable way.

The difference in diagnostic accuracy on the use of a biologically quantified peach extract and on the use of an extract labeled in weight/volume units is clearly illustrated in Fig. 4, in which the FBU-labeled extract elicited as many positive responses as the food itself in the prick-prick tests. The specificity was also good, as demonstrated in the nonatopic group. According to May²⁴ and Atkins et al.,²⁵ the positive peach skin test results obtained in the control group of patients allergic to pollen but without peach symptoms indicates asymptomatic hypersensitivities rather than nonspecific irritant reactions. This fact has also been reported in well-standardized common aeroallergens (e.g., pollens, mites, and cats) and constitutes a part of the medical art in the diagnosis of allergy.

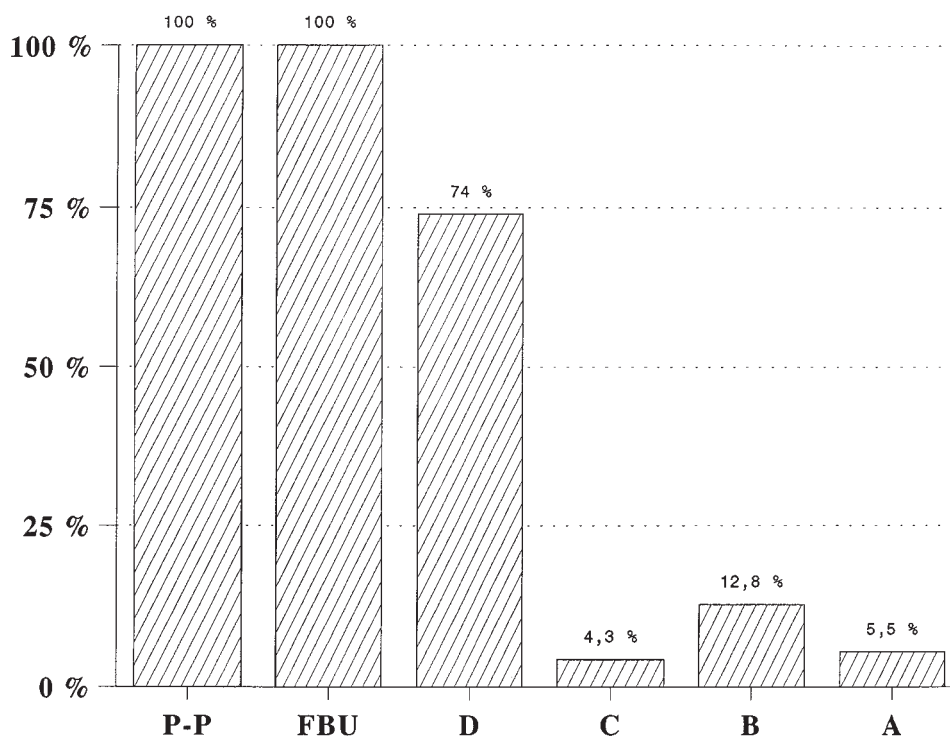


FIG. 4. Sensitivity obtained with various commercial peach extracts (A to D) compared with those resulting from prick-prick test and extract quantified in FBUs. *P-P*, Fresh peach peel; *FBU*, biologically standardized peach extract; *A*, CBF-LETI; *B*, Bayer Diagnostic; *C*, Stallergenes-Pasteur; and *D*, ALK-ABELLO.

Finally, it is our belief that if the biologic activity of food allergen preparations were labeled in FBUs, the quality of food allergen preparations used for diagnosis would be improved, and in consequence, the practicing clinician would have a more reliable choice criteria.

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