Journal of Clinical Laboratory Analysis 11:251–257 (1997)

Determination of IgE Antibodies to the Benzylpenicilloyl Determinant: A Comparison of the Sensitivity and Specificity of Three Radio Allergo Sorbent Test Methods

Juan J. Garcia,[1]* Miguel Blanca,[1] Felisa Moreno,[1] Jose M. Vega,[1] Cristobalina Mayorga,[1] Javier Fernandez,[2] Carlos Juarez,[3] and Antonino Romano,[4] and Enrique de Ramon[2]

[1]Allergy Unit, Research Unit for Allergic Diseases, Carlos Haya Hospital, Malaga, Spain
[2]Allergy Unit, Hospital Seguridad Social, Elche, Alicante, Spain
[3]Allergy Unit, Hospital Clinico, Granada, Spain
[3]Internal Medicine Department, Carlos Haya Hospital, Malaga, Spain
[4]Instituto de Medicina Interna e Geriatria, University Sacro Cuore, Rome, Italy

The quantitation of in vitro IgE antibodies to the benzylpenicilloyl determinant (BPO) is a useful tool for evaluating suspected penicillin allergic subjects. Although many different methods have been employed, few studies have compared their diagnostic specificity and sensitivity. In this study, the sensitivity and specificity of three different radio allergo sorbent test (RAST) methods for quantitating specific IgE antibodies to the BPO determinant were compared. Thirty positive control sera (serum samples from penicillin allergic subjects with a positive clinical history and a positive penicillin skin test) and 30 negative control sera (sera from subjects with no history of penicillin allergy and negative skin tests) were tested for BPO-specific IgE antibodies by RAST using three different conjugates coupled to the solid phase: benzylpenicillin conjugated to polylysine (BPO-PLL), benzylpenicillin conjugated to human serum albumin (BPO-HSA), and benzylpenicillin conjugated to an aminospacer (BPO-SP). Receiver operator control curves (ROC analysis) were carried out by determining different cut-off points between positive and negative values. Contingence tables were constructed and sensitivity, specificity, negative predictive values (PV–), and positive predictive values (PV+) were calculated. Pearson correlation coefficients (r) and intraclass correlation coefficients (ICC) were determined and the differences between methods were compared by χ^2 analysis. Analysis of the areas defined by the ROC curves showed statistical differences among the three methods. When cut-off points for optimal sensitivity and specificity were chosen, the BPO-HSA assay was less sensitive and less specific and had a lower PV– and PV+ than the BPO-PLL and BPO-SP assays. Assessment of r and ICC indicated that the correlation was very high, but the concordance between the PLL and SP methods was higher than between the PLL and HSA or SP and HSA methods. We conclude that for quantitating IgE antibodies by RAST to the BPO determinant, BPO-SP or BPO-PLL conjugates offer advantages in sensitivity and specificity compared with BPO-HSA. These results support and extend previous in vitro studies by our group and highlight the importance of the carrier for RAST assays. J. Clin. Lab. Anal. 11:251–257, 1997.
© 1997 Wiley-Liss, Inc.

Key words: benzylpenicilloyl; IgE antibodies; polylysine; human serum albumin; spacer

Abbreviations: IgE = immunoglobulin E; BP = benzylpenicillin; AX = amoxicillin; BPO = benzylpenicilloyl determinant; MDM = minor determinant mixture of BP; HSA = human serum albumin; PLL = poly-L-lysine; SP = aminospacer; BPO-PLL = benzylpenicilloyl-polylysine conjugate; BPO-HSA = benzylpenicilloyl-human serum albumin conjugate; BPO-SP = benzylpenicilloyl-aminospacer conjugate; RAST = radio allergo sorbent test; r = Pearson correlation coefficient; ICC = intraclass correlation coefficient; PV = predictive value; PBS = phosphate-buffered saline; ROC = receiver operator control.

Contract grant sponsor: <FIS>; Contract grant number <94/940>; Contract grant sponsor: <Junta de Andalucía>; Contract grant number <1994>; Contract grant sponsor: <CYCIT>; Contract grant number <BIO 92/0305>

*Correspondence to: Miguel Blanca, Allergy Unit, Research Unit for Allergic Diseases, Carlos Haya Hospital, Malaga, Spain.

Received 15 November 1996; accepted 22 November 1996.

© 1997 Wiley-Liss, Inc.

INTRODUCTION

In common with allergy caused by other low molecular weight compounds, penicillin allergy involves recognition of haptenic structures covalently bound to homologous and heterologous proteins. In the case of benzylpenicillin (BP), the major haptenic structure is the benzylpenicilloyl determinant (BPO) formed by conjugation of the carbonyl group of the penicillin betalactam ring with amino groups of the carrier protein (1–3). Other determinants (minor determinants) have been identified and investigated (4–7), but the BPO structure remains the major determinant involved in inducing immunoglobulin E (IgE)-mediated allergic reactions (4,8).

The quantitation of in vitro IgE antibodies to BPO has been a useful tool for evaluating allergic reactions to penicillins (9–11). Although initially this method used penicillin conjugated to human serum albumin (HSA), other carriers have been employed. These include red blood cells (12), polyacrylamide particles (13), transferrin (14), polylysine (7), epoxy-activated resins (15), an aminospacer (SP) (16), and direct conjugation of BP to a polystyrene solid phase (17). Both radioimmunoassay (7,9,12,15,16,18–20) and enzyme-linked immunoassay (11,14,17,21–23) methods have been used. A few comparative studies of in vitro methods using either antibodies from experimental animals (24) or human antibodies (12,16,25) have been reported, but no systematic attempts to determine the sensitivity and specificity of the assays have been carried out.

In this study, a radio allergo sorbent test (RAST) assay using three different solid phases was carried out. BP was coupled to three different carriers, poly-L-lysine (PLL), HSA, and an aminospacer (SP). The results were compared using 30 positive sera from subjects allergic to penicillins and 30 negative sera from subjects who had a good response after being given penicillin. Sensitivity, specificity, negative predictive values (PV–), and positive predictive values (PV+) were calculated and Pearson correlation (r) and intraclass correlation (ICC) coefficients obtained. Differences between methods were compared by χ^2 analysis.

MATERIALS AND METHODS

Positive Control Sera

Serum samples from 30 subjects who had suffered an immediate allergic reaction to a penicillin derivative and were skin test positive to the BPO determinant at the moment they were evaluated for the study were employed as positive controls. Mean subject age was 49 years, ranging from 19 to 68. There were 10 male and 20 female patients. In all sera where the RAST value was over 5% of the label uptake (see below), RAST inhibition studies were carried out as described (26) to confirm the specificity of the IgE antibodies to the BPO determinant. In brief, monomeric conjugates were made by conjugating BP, amoxicillin (AX), and AMP with butylamine as reported (27). RAST inhibition studies were not made in sera with a RAST value below 5% because, in our experience, no accurate results can be obtained.

Negative Control Sera

Negative control sera were obtained from 30 subjects with no history of allergy to penicillin and negative skin tests to BPO, a minor determinant mixture of BP (MDM), AX, and AMP. The subjects were age and sex matched to the positive control group and had similar total IgE levels.

Reagents for In Vitro Use

PLL, molecular weight 10 Kd, and HSA were obtained from Sigma Chemical Company. N-Boc-1,6-diamino-hexane-hydrochloric, the SP used in this study, was obtained from Fluka, Zaragoza, Spain. BP was obtained from Antibiotic S.A., Leon, Spain. Radiolabeled anti-human IgE was obtained from Pharmacia, Uppsala, Sweden.

Skin Test Procedure

The following reagents were used for skin testing: BPO-PLL (5×10^{-5} M, Allergopharma), MDM (2×10^{-2} M, Allergopharma), AX (20 mg/ml, Beecham), and AMP (20 mg/ml, Llorente). Skin tests were carried out as described (28).

Preparation of BPO RAST Discs

BPO-PLL, BPO-HSA, and BPO-SP RAST discs were prepared as indicated below.

BPO-PLL discs

PLL, 9 Kd, was coupled to cyanogen bromide-activated cellulose discs at a concentration of 10 mg/ml following the method previously described by Edwards et al. (7). Two hundred discs were treated with 40 mg of PLL in 4 ml of 0.1 M $NaHCO_3$. Discs were gently mixed for 3 hours at room temperature and then blocked in 4 ml of 50 mM ethanolamine in 0.1 M $NaHCO_3$ for 1 hour. This was followed by alternate washes in 0.1 M $NaHCO_3$ and 0.1 M acetic-acetate buffer pH 4. Discs were washed in 0.05 M carbonate-bicarbonate buffer, pH 10.2, and 4 mL of a solution of 50 mM of BP in carbonate-bicarbonate buffer, pH 10.2, was added and kept for 48 hours at 37°C. After this period, discs were washed in 0.1 M $NaHCO_3$ and finally in phosphate-buffered saline (PBS). These were kept in PBS at 4°C until use. In addition, 10 mg/ml of PLL was also coupled to discs in parallel to obtain the single PLL control discs.

BPO-HSA discs

Five milliliters of HSA at a concentration of 100 mg/ml was mixed with 5 ml of BP at a concentration of 200 mg/ml in 0.05 M bicarbonate-carbonate buffer, pH 10, for 48 hours

at 37°C. After this, the free hapten was removed from the conjugate in a Sephadex G-25 column. The conjugate was adjusted to a concentration of 50 mg/ml and coupled to the cyanogen bromide-activated cellulose discs as described above for BPO-PLL discs. The same amount of HSA alone (50 mg/ml) was also coupled in parallel to discs to obtain the single HSA control discs.

BPO-SP discs

These were obtained as described (29) following a modification of the method previously described by Daxun et al. (16). An aliphatic SP (N-Boc-1,6-diamino-hexane-hydrochloric) consisting of six carbons with one amino end free and the other blocked by CIH was dissolved in 0.5 M $NaHCO_3$, pH 9.5, at a concentration of 50 mg/ml. Cyanogen bromide-activated paper discs were added and shaken at room temperature for 24 hours. Then, the discs were washed in 0.5 M $NaHCO_3$, pH 8.4, and in a second step blocked in 500 mM ethanolamine in 0.1 M $NaHCO_3$ at room temperature for 2 hours. After washing, discs were treated with 4 M HCl for 2 hours. Then, discs were washed and treated with a solution 50 mM of BP in 0.05 M bicarbonate-carbonate buffer, pH 10.2, for 24 hours at room temperature. Finally, discs were washed and stored in PBS containing 0.05% sodium azide, pH 7.5, at 4°C until use. SP single control discs were also prepared in parallel.

RAST Procedure

RAST assays were carried out as described (12). These were carried out in duplicate using 50 µl of serum and 50 µl of radiolabeled anti-IgE. Results obtained with BPO-derivatized discs (BPO-PLL, BPO-HSA, and BPO-SP) were expressed as percentage of label uptake after subtracting the uptake of the respective carrier single disc (PLL, HSA, and SP). Optimal cut-off points between positive and negative were determined by receiver operator control (ROC) curves (30).

Statistical Studies

In order to compare the three assays tested in this study, the statistical analysis of the differences obtained between the areas defined under the ROC curves for each one of the methods was carried out as described (30). Assays were correlated by the r and ICC coefficients as described by Bartko (31). Sensitivity and specificity were compared by χ^2 analysis. In addition, the PV+ and PV– in the three assays were calculated.

The relationship between total IgE values and RAST values in the negative control group was compared by linear regression analysis.

Informed consent was obtained from all subjects participating in the study and this was approved by the Ethical Committee of the Hospital.

RESULTS

RAST values for the positive and negative control groups are presented in Tables 1 and 2, respectively. The mean values/SD in the negative control group for BPO-PLL, BPO-HSA, and BPO-SP were 0.10/0.22, 0.62/0.53, and 0.08/0.15, respectively. The statistical analysis of the areas defined by the ROC curves obtained for the three sorbents assessed in this study is presented in Table 3. Results showed significant differences when BPO-PLL and BPO-HSA were compared ($P < 0.005$), and between BPO-HSA and BPO-SP ($P < 0.05$). No significant differences existed when BPO-PLL and BPO-SP were compared ($P < 0.49$). The cut-off points that offered the best compromise between sensitivity and specificity for the three assays were 0.3 for BPO-PLL, 0.9 for BPO-HSA, and 0.5 for BPO-SP. According to these, contingence tables were obtained and presented in Figure 1. For BPO-PLL discs, 26 sera were positive, 27 sera were negative, 4 sera were false negative (sera 2,4,9, and 21), and 3 were false positive (sera 11,15, and 30). For BPO-HSA discs, 19 sera were positive, 25 were negative, 11 were false negative (sera 1,2,3,7,10,11, 14,17,21,22, and 30), and 5 sera were false positive (sera 4, 11,14,15, and 24). For BPO-SP discs, 26 sera were positive,

TABLE 1. RAST Values of the Positive Control Group Using BPO-PLL, BPO-HSA, and BPO-SP[a]

Subject	BPO-PLL	BPO-HSA	BPO-SP
1	3.2	0.1	2.5
2	0.1	0.5	0.6
3	0.6	0.2	0.5
4	0.1	1.1	0.2
5	3.5	1.3	4.2
6	12.9	1	12.4
7	5.1	0.8	4.9
8	12	2.2	9.3
9	0	1.2	0.2
10	0.7	0	0
11	1	0.6	1.1
12	20.1	3.9	4.9
13	0.3	1.2	2.3
14	3.1	0.7	2.2
15	4.4	2.6	4
16	5.2	2.4	4.6
17	3.4	0.6	2.6
18	21	8	18
19	1.3	2.2	0.6
20	3.1	2.5	2.3
21	0.2	0.3	0.1
22	2.7	0.5	1.8
23	17.6	3.1	15.6
24	1.4	0.9	1
25	0.7	1.5	1.1
26	3.6	1.6	2.7
27	2.7	1.4	2.1
28	4.1	1	4.3
29	17.5	5.3	16.9
30	2	0.6	2.6

[a]Results are expressed as percentage label uptake.

TABLE 2. RAST Values of the Negative Control Group Using BPO-PLL, BPO-HSA, and BPO-SP[a]

Subject	BPO-PLL	BPO-HSA	BPO-SP
1	0	0.6	0
2	0.1	0.5	0.1
3	0	0.2	0
4	0	1.2	0
5	0.1	0.6	0
6	0.1	0.5	0
7	0	0.7	0.1
8	0.1	0.6	0
9	0	0.8	0
10	0	0	0
11	0.7	0.9	0
12	0	0.7	0.2
13	0	0.4	0
14	0	1	0.1
15	0.8	3	0
16	0	0.5	0.2
17	0	0.2	0
18	0	0.4	0.1
19	0	0.3	0.1
20	0.2	0.6	0.1
21	0	0.8	0.1
22	0	0.4	0.1
23	0.1	0.6	0
24	0	1	0
25	0.1	0.4	0.4
26	0	0.1	0.1
27	0.1	0.5	0
28	0	0.5	0
29	0.1	0.6	0.1
30	0.7	0.1	0.7

[a]Results are expressed as percentage label uptake.

29 were negative, 4 sera were false negative (sera 4,9,10, and 21), and 1 serum sample was false positive (serum 30). Table 4 shows sensitivity, specificity, PV+, PV–, and the *P* value of the χ^2 analysis for the three assays. For the PLL and SP conjugates, PV+ and PV– approached or exceeded 90%, for the HSA conjugate these values were less than 80%. Linear regression analysis comparing the positive RAST for the three assays tested in the positive control group is presented in Figure 2. Pearson correlation coefficients (r) were 0.75 for BPO-PLL vs. BPO-HSA, 0.86 for BPO-PLL vs. BPO-SP, and 0.70 for BPO-HSA vs. BPO-SP. In the three methods, the significance had a *P* value <0.001. ICC for the three RAST systems was 0.86, but pairwise comparisons gave 0.70 for BPO-PLL vs. BPO-SP discs, 0.07 for BPO-HSA vs. BPO-PLL, and 0.09 for BPO-HSA vs. BPO-SP, showing the high concordance between BPO-PLL and BPO-SP discs.

TABLE 3. Comparison of the Areas Defined Under the ROC Curves for Each One of the Methods Assessed in This Study

	Area under the curve	Standard error	*P* value*
BPO-PLL (1)	0.94	0.03	0.01 (1 and 2)
BPO-HSA (2)	0.76	0.06	0.01 (2 and 3)
BPO-SP (3)	0.94	0.03	0.49 (1 and 3)

**P* was significant when BPO-PLL and BPO-SP were compared to BPO-HSA.

In 17 sera from the positive control group, RAST values were positive with all three methods. Comparative results are presented in Figure 3. It can be seen that for most sera, the RAST results with the BPO-PLL and BPO-SP conjugates were higher than for the BPO-HSA system, with results for sera 6, 8,12,18,23,28, and 29 being more than 50% greater.

DISCUSSION

The diagnosis of allergy to penicillin is made by a positive clinical history and the presence of specific IgE antibodies to BPO, usually detected with skin tests (32). Studies have shown that subjects who have a negative skin test, even if they have a positive history, can usually tolerate the administration of penicillin (8,33,34). In general, skin tests have proved to be more sensitive than RAST for the determination of IgE antibodies specific for the BPO determinant (33–34), although there have been cases where only RAST has been positive (6, 10 and personal experience).

Although technically more complex, RAST offers several advantages over skin tests. There is no risk of a systemic reaction, serum samples can be tested and retested at any time, the presence of IgE antibodies can be investigated following fatal reactions, and serum samples can be exchanged between

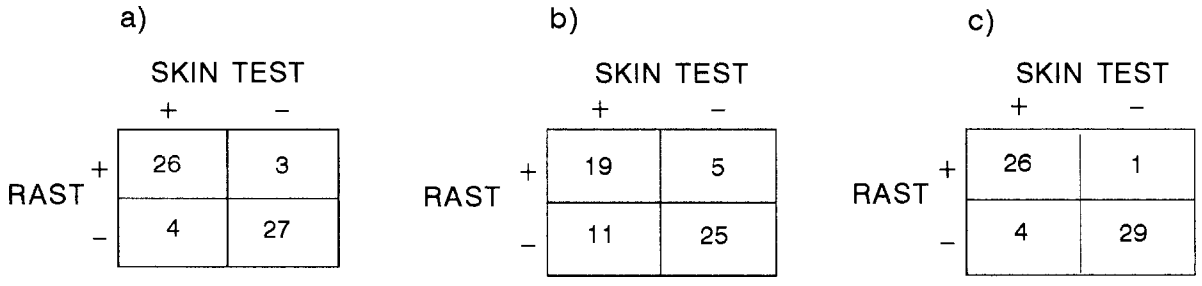

Fig. 1. Contingence tables with positive and negative results in both negative and positive control groups using BPO-PLL **(a),** BPO-HSA **(b),** and BPO-SP **(c)** RAST discs.

TABLE 4. Sensitivity, Specificity, PV+, PV–, and *P* Value of χ^2 Analysis for the Three Methods Assessed Using the Optimal Cut-Off Points Obtained by ROC Curves

	Sensitivity (%)	Specificity (%)	PV+ (%)	PV– (%)	*P*
BPO-PLL	86.66	90	89.65	87.09	<0.001
BPO-HSA	66.33	83.33	79.16	69.44	<0.001
BPO-SP	86.66	96.66	96.29	87.87	<0.001

investigators in order to validate results and carry out population studies. In addition, RAST inhibition has proved to be a useful tool for defining the specificity of IgE antibodies and studying the cross-reactivity with other betalactams (26,35). The assay that we describe is a classical RAST method where discs were activated with cyanogen bromide as reported elsewhere (6,7,8,10). In two of the assays, in a first step discs were conjugated to the spacer (PLL and SP) and in the BPO-HSA assay optimal conjugates of the hapten with the carrier were first made and in a second step these were coupled to the solid phase. In previous studies with BPO RAST systems (12), we have reported differences in sensitivity when using PLL and HSA carriers, and other studies have drawn attention to the importance of hapten density in relation to assay sensitivity (36,37). In comparing HSA and PLL, HSA has a much lower capacity for coupling penicillin molecules than PLL. Studies have also shown that not all the BPO haptens in BPO-HSA are accessible to antibodies (38). With regard to hapten density, similar results have also been observed in in vivo studies using PLL conjugates for skin testing (33,39,40). In particular, Levine and Zolov (33) found that a BPO-PLL conjugate was more effective than BPO-HSA or other BPO-protein conjugates for detecting IgE antibodies by skin testing.

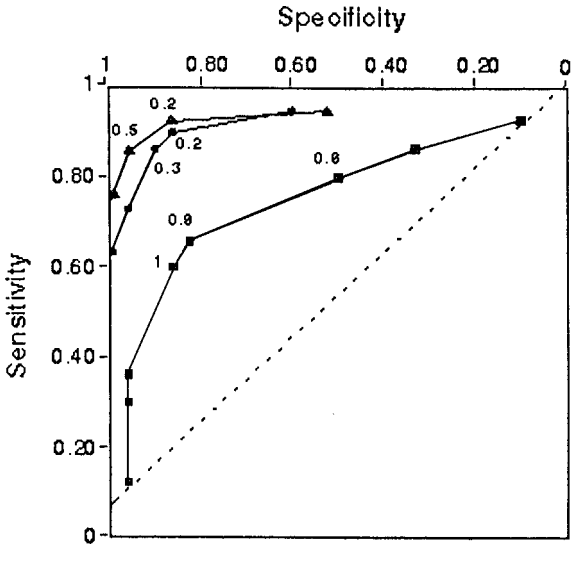

Fig. 2. Linear regression analysis comparing the three RAST methods tested in this study (BPO-PLL, BPO-HSA and BPO-SP).

In the present study, we chose to investigate three different hapten carriers (PLL, HSA, and SP) on a RAST paper disc. While PLL and HSA have been widely used, the use of an aminospacer as a carrier for determining IgE-specific antibodies has only recently been reported by Daxun et al. (16) who suggested it led to an increase in assay sensitivity. This assay has also been studied by our group for side chain-specific antibodies to AX and we have shown that there are also differences (29). No evidence has been reported so far about sensitivity and specificity studies with the three different RAST assays reported in our system in a well-defined group of subjects with an immediate allergic reaction to penicillin, a skin test positive to the BPO, and IgE antibodies specific to the BPO determinant. Our results indicate that the three RAST systems used are highly correlated (high r). However, the ICC was higher between the PLL and SP methods (0.70) than either of these with the HSA method (0.07 and 0.09, respectively), indicating a major concordance between the PLL and SP methods. We also found that sensitivity and specificity were higher in the PLL and SP methods than in the HSA method. Considering the results obtained, the best method was BPO-SP, closely followed by BPO-PLL. While sensitivity and PV– were very similar between these two systems, the SP system was superior in terms of specificity and PV+. Interestingly, although BPO-SP was the most sensitive RAST assay, there were instances where the sera were positive to only one of the assays. These occurred in one instance with the BPO-PLL, in two cases with the BPO-HSA, and in one case with the BPO-SP. At the moment, we see no explanation for these findings since we do not know what the final structure and configuration of the BPO hapten is in any of the systems used. In any case, it suggests that if the RAST is negative with one of the assays, an alternative system can be used and positive results may be found. The carrier producing most false positive results was BPO-HSA with five sera. This was due to the very high concentration of carrier used for conjugating to the solid phase (50 mg/ml). In our experience, this false positive value can be reduced, but if concentration is reduced there is a corresponding important decrease in the RAST values of positive cases and a decrease in sensitivity.

Summarizing, we conclude that for quantitating IgE antibodies to penicillin, the use of solid phases with BPO-SP or BPO-PLL conjugates offers an increase in sensitivity and specificity over the use of BPO-HSA conjugates. These results confirm and extend previous data reported by our group (12,29) and other authors (7,16).

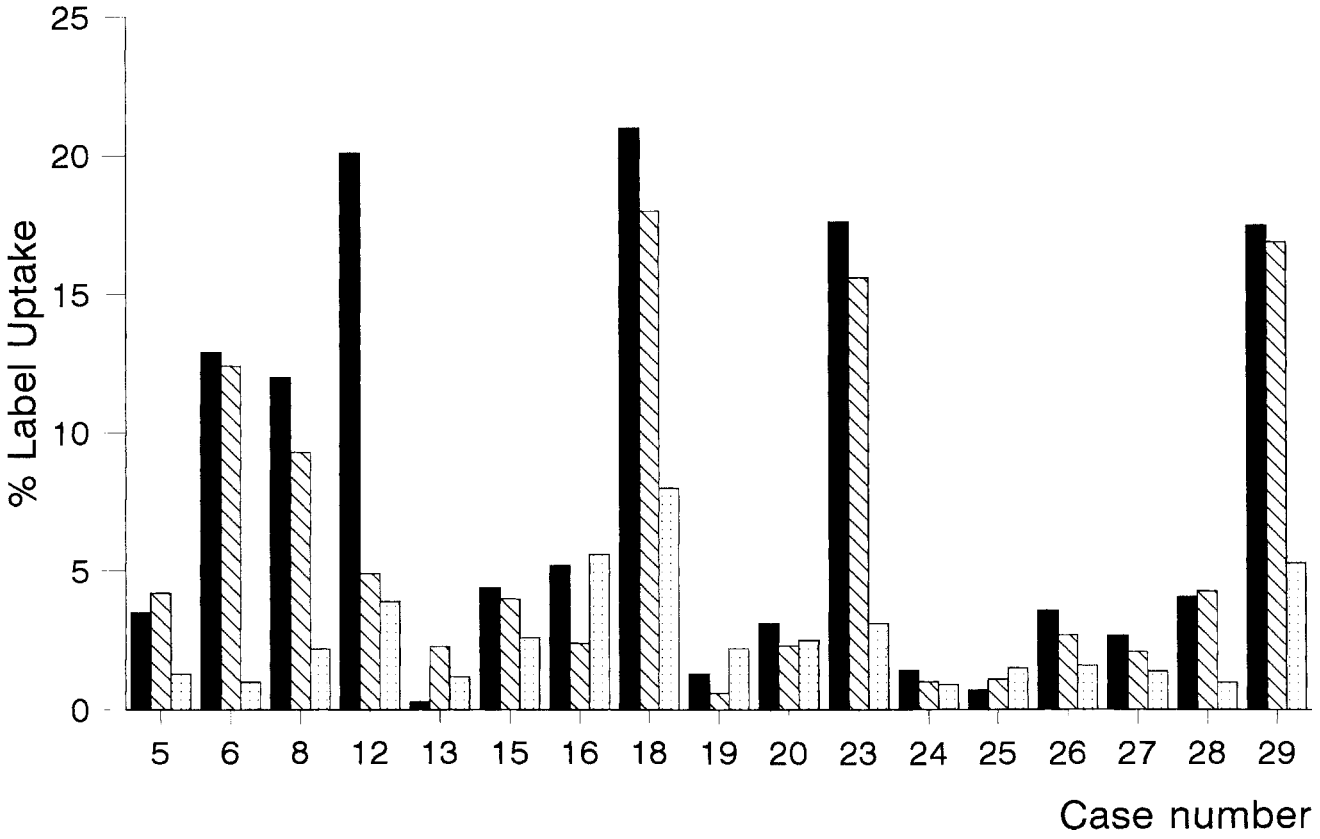

Fig. 3. Comparison of the RAST values obtained in the seventeen sera from the positive control group which were positive in all three assay systems. ■, BPO-PLL; ▨, BPO-SP; ⬚, BPO-HSA.

REFERENCES

1. Batchelor FR, Dewdney JM, Gazzard D: Penicillin allergy: The formation of the penicilloyl determinant. *Nature* 6:362–364, 1965.
2. Levine BB: Immunochemical involved in penicillin hypersensitivity in experimental animals and in human beings. *Fed Proc* 24:45–50, 1965.
3. De Weck AL, Blum G: Recent clinical and immunochemical aspects of penicillin allergy. *Int Arch Allergy Appl Immunol* 27:221–229, 1965.
4. Parker CW, Shapiro J, Kern J, Eisen HN: Hypersensitivity to penicillenic acid derivatives in human beings with penicillin allergy. *J Exp Med* 115:821–828, 1962.
5. Dewdney JM: Immunology of the antibiotics. In *Antigens 5.* M Sela, ed. Academic Press, New York, 1977, p 73–245.
6. Kraft D, Berglund A, Rumpold H, Roth A, Ebner H: Radioallergosorbent test with conjugates specific for "minor" haptenic determinants in the diagnosis of IgE-mediated penicillin allergy in man. *Clin Allergy* 11:579–587, 1981.
7. Edwards RG, Spackman DA, Dewdney JM: Development and use of three new radioallergosorbent tests in the diagnosis of penicillin allergy. *Int Arch Allergy Appl Immunol* 68:352–357, 1982.
8. Adkinson NF, Thompson WL, Maddrey WC, Lichtenstein LM: Routine use of penicillin skin testing on an inpatient service. *N Engl J Med* 285:22–24, 1971.
9. Juhlin L, Wide L: Detection of penicillin allergy by the radioallergosorbent test. *Lancet* 2:261, 1979.
10. Jarisch R, Roth A, Boltz A, Sandor I: Diagnosis of penicillin allergy by means of Phadebas RAST penicilloyl G and V and skin tests. *Clin Allergy* 11:155–160, 1981.
11. Christie G, Coleman W, Newby S, Mcdiarmaid-Gordon A, Hampson JP, Breckenridge AM, Park BK: A survey of the prevalence of penicillin-specific IgG, IgM and IgE antibodies detected by ELISA and defined by hapten inhibition, in patients with suspected penicillin allergy and in healthy volunteers. *Br J Clin Pharmacol* 25:381–386, 1988.
12. Kraft D, Wilson DV, Dervey ME: Penicillin allergy studies by modified red-cell-linked antigen-antiglobulin reaction. *Int Arch Allergy Appl Immunol* 52:248–256, 1976.
13. Ohno Y, Stahmann M: A new detection method for penicillin antibodies and hypersensitivity in humans using penicillin coupled to polyacrylamide particles. *Immunochemistry* 9:1087–1093, 1972.
14. de Haan P, Kalsbeek GL: Induction of benzylpenicilloyl specific antibodies, including IgE, by long-term administration of benzylpenicillin. *Clin Allergy* 13:563–569, 1983.
15. Harle DG, Baldo BA: Drugs as allergens: An immunoassay for detecting IgE antibodies to cephalosporins. *Int Arch Allergy Appl Immunol* 92:439–444, 1990.
16. Daxun Z, Adriansson J, Martin A, Malmberg E, Ahlatedt S: Diagnostic capabilities of penicillin-based structures as tools in the in vitro diagnosis of penicillin allergy. *J Invest Allergol Clin Immunol* 2:240–246, 1992.
17. Klein GF, Stanzl U, Fritsch PO, Varga JM: Enzyme-linked immunosorbent assay for detection of antibodies to the unmodified beta-lactam ring. *Allergy* 48:151–157, 1993.
18. Wide L, Juhlin L: Detection of penicillin allergy of the immediate type by radioimmunoassay of reagins (IgE) to penicilloyl conjugates. *Clin Allergy* 1:171–175, 1971.
19. Urena V, Delgado RG, Daroca, Lahoz C: Polystyrene tube radio-

immunoabsorbent assay for IgE anti-penicillin antibodies. *Lancet* 1:1210–1211, 1977.
20. Blanca M, Mayorga C, Pérez E, Suau R, Juárez C, Vega JM, Carmona MJ, Perez-Estrada M, García J: Determination of IgE antibodies to the benzyl penicilloyl determinant. A comparison between poly-L-lysine and human serum albumin as carriers. *J Immunol Methods* 153:99–105, 1992.
21. de Haan P, Kalsbeeck GL: The humoral immune response to some benzylpenicillin preparations. *Allergy* 38:319–324, 1983.
22. Isetta AM, Minghetti A, Radojkovic J, Vasile C, Celada F: Affinity and specificity of penicillin-antibody interaction determined by an enzyme (*E. coli* β-galactosidase) immunoassay. *Eur J Immunol* 6:737–742, 1976.
23. Botello A, Moneo I, Cuevas M, Alvarez E: Detección de anticuerpos antipenicilina: correlación entre la técnica de RAST y el enzimoinmunoanálisis a doble paso. *Allergol Immunopathol* (Suppl VII):297, 1980.
24. Mamas S, Dray F: Viroimmunoenzymoassay for detection of antipenicilloyl antibodies and penicilloyl residues: Comparison of results obtained by radio-, viro- and enzymoimmunoassay. *Ann Immunol Inst Pasteur* 30:407–418, 1979.
25. Caneva E, Di Genaro P, Farina F, Orlandi M, Rindone B: Synthesis and characterization of a penicillin-poly(L-lysine) which recognizes human IgE anti-penicillin antibodies. *Bioconjugate Chem* 4:309–314, 1993.
26. Moreno F, Blanca M, Mayorga C, Terrados S, Moya MC, Pérez E, Suau R: Studies of the specificities of IgE antibodies found in sera from subjects with allergic reactions to penicillins. *Int Arch Allergy Appl Immunol* 108:74–81, 1995.
27. Mayorga C, Obispo T, Jimeno L, Blanca M, Moscoso J, Carreira J, Garcia JJ, Juárez C: Epitope mapping of beta lactams antibiotics with the use of monoclonal antibodies. *Toxicology* 97:225–234, 1995.
28. Blanca M, Vega JM, García J, Carmona MJ, Terrados S, Avila MJ, Miranda A, Juárez C: Allergy to amoxicillin with good tolerance to other penicillins. Study of the incidence in patients allergic to beta lactams. *Clin Exp Allergy* 20:475–481, 1991.
29. Blanca M, Moreno F, Mayorga C, García J, Fernández M, Pérez E, Juárez C, Suau R: The nature of the carrier in the RAST assay influences the capacity for detecting IgE antibodies to penicillins. *J Clin Immunoassay* 17:1–5, 1994.
30. Sackett DL: Basic research. Clinical research. Clinical epidemiology and general internal medicine. *J Gen Intern Med* 2:40–47, 1987.
31. Bartko: The intraclass correlation coefficient as a measure of reliability. *Psychol Rep* 19:3–11, 1966.
32. Weiss ME, Adkinson NF: Immediate hypersensitivity reactions to penicillin and related antibiotics. *Clin Allergy* 18:515–540, 1988.
33. Levine BB, Zolov DM: Prediction of penicillin allergy by immunological tests. *J Allergy* 43:231–244, 1969.
34. Gadde J, Spence M, Wheeler B, Adkinson F: Clinical experience with penicillin skin testing in a large inner-city STD clinic. *JAMA* 20:2456–2463, 1993.
35. Blanca M, Fernández J, Miranda A, Terrados S, Torres MJ, Vega JM, Avila MJ, Pérez E, García J, Suau R: Cross-reactivities between penicillin and cephalosporins. Clinical and Immunological Studies. *J Allergy Clin Immunol* 83:381–385, 1989.
36. Lew AM: The effect of epitope density and antibody affinity on the ELISA as analysed by monoclonal antibodies. *J Immunol Methods* 72:171–176, 1984.
37. Lee D, Dewdney JM, Edwards RG: The influence of hapten density on the assay of penicilloylated proteins in fluids. *J Immunol Methods* 84:235–243, 1985.
38. Lafaye P, Lapresle C: Fixation of penicilloyl groups to albumin and appearance of anti-penicilloyl antibodies in penicillin-treated patients. *J Clin Invest* 82:7–12, 1988.
39. Ressler C, Mendelson LM: Skin test for diagnosis of penicillin allergy-current status. *Ann Allergy* 59:167–170, 1987.
40. Parker CW, Thiel JA: Studies in human penicillin allergy: A comparison of various penicilloyl-polylysines. *J Lab Clin Med* 3:482–491, 1963.