Serum Prostatic Acid Phosphatase (PAP): Monoclonal Enzyme-Linked Immunoassay Compared to Polyclonal Radioimmunoassay

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ABSTRACT

A new enzyme linked immunosorbent assay (ELISA) kit which utilizes a monoclonal antibody against prostatic acid phosphatase (PAP) was compared with current radioimmunoassay (RIA) methodology which uses a polyclonal antibody. Both assays are double antibody immunoassays with the major difference being the method of antibody preparation. A study of intrarun precision using control material showed an average within run coefficient of variation (CV) percent as 7.0 percent and 6.9 percent for ELISA and RIA, respectively, while between run CV percent averaged 11.1 percent and 11.5 percent, respectively. Thus, precision results compare similarly between the two assays. The specificity showed significantly different results. Patterns of correlation between the two methods indicate differing specificities of the primary antibodies. The values for ELISA were greater than RIA for control sera and patient samples when values fell outside the reference range; however, RIA values exceeded ELISA values with patient samples which fell within the reference ranges as provided by each manufacturer.

Therefore, there exists a question of specificity of antibody employed in each of the two assays. The PAP antigen is prepared from two different sources for each kit. The ELISA manufacturer prepares antigen from seminal fluid and RIA manufacturer prepares antigen from normal human prostate. The question of specificity may be influenced by: (1) source of antigen used in immunizing animals and (2) monoclonal versus polyclonal means of producing antibody.

Introduction

The association of increased serum levels of prostate-specific acid phosphatase, PAP, (EC 3.1.3.2) and adenocarcinoma of the prostate has been known for quite some time. Methods exist for determination of PAP by either enzymatic activity or by molecular mass. The lack of specificity using tartrate inhibition and using the various substrates, such as p-nitrophenyl phosphate,
1-napthyl phosphate, and thymolphthalein monophosphate, to differentiate PAP from other acid phosphatases, is well documented.\textsuperscript{3,13,15} Owing to the lability of enzyme activity, the \textit{in vitro} enzymatic activity may not reflect \textit{in vivo} serum PAP concentration; however, with immunochemical methods, antigenic sites are preserved and thus may closer approximate serum PAP levels \textit{in vivo}.\textsuperscript{9} These immunochemical methods measure molecular mass by means of immunoprecipitation or solid phase immunobosorption. Immunological methods such as radioimmunoassay (RIA)\textsuperscript{9,16} and enzyme linked immunosorbent assay (ELISA)\textsuperscript{11} measure mass; while immunoenzymatic assay (EIA)\textsuperscript{11} and fluorescent immunoassay (FIA)\textsuperscript{14} separate PAP from serum by immunochemical means and then measure enzymatic activity by absorbance and fluorescence of product, respectively. The upper limit of the reference range associated with these RIA and ELISA lies from 2.0 to 7.15 ng per ml.\textsuperscript{4,11} All of these immunoassays depend heavily on specificity of antibody production, and this may explain the variation in reference values. The purpose of this study is to present a comparison of a new monoclonal ELISA kit with an established RIA methodology which employs a polyclonal antibody for PAP.

\section*{Materials and Methods}

\textbf{Enzyme-linked Immunosorbent Assay (ELISA)}

The Abbott “PAP-EIA” kit (Lot #41-418HT) is a solid phase double antibody enzyme immunoassay based on the “sandwich” (ELISA) principle. Beads coated with mouse (monoclonal) anti-PAP are incubated with standards, controls, and serum samples. The PAP remains bound to the solid phase while the unbound material is aspirated as the beads are washed. A second mouse (monoclonal) anti-PAP antibody, directed against a different antigenic site and conjugated to horseradish peroxidase (EC 1.11.1.7) serves as an enzyme “tag”. The PAP protein is thus “sandwiched” between two antibodies exposing the peroxidase marker on the second antibody. After washing, the beads are reacted with ortho-phenylene-diamine (ODP) in the presence of $\text{H}_2\text{O}_2$ to produce oxidized ODP which is read spectrophotometrically at 492 nm. The peroxidase activity is related to the concentration of the PAP which has been immunologically isolated.

The assay is performed as follows: To a well of the reaction tray are added in sequence: 100 $\mu$l of standard, control, or serum; 100 $\mu$l of the protein stabilized specimen dilution buffer; and, an antibody coated bead. The reaction tray is covered and incubated for one hour at 37°C. Using the Abbott ‘Pentawash system’*, the beads are washed twice with 4 to 5 ml of deionized water and excess fluid aspirated. To each well are added 200 $\mu$l of anti-PAP: peroxidase conjugate. The tray is covered and incubated again for one hour at 37°C. The excess fluid is aspirated and the beads are transferred to appropriate (12 x 75 mm) tubes. The OPD-substrate solution is prepared by diluting one OPD tablet (12.8 mg o-phenylenediamine • $2\text{HCl}$) per 5 ml of OPD diluent (citrate-phosphate buffer containing 0.02 percent $\text{H}_2\text{O}_2$). The OPD-substrate solution (300 $\mu$l) is added to each tube, and they are then incubated at room temperature (15 to 30°C) for 30 minutes. The enzymatic reaction is terminated by the addition of one ml of 1 N sulfuric acid to each tube and absorbance determined at 492 nm with the Quantum 1 photometer.* The manufacturer's upper limit of detection is 30 ng per ml. It has been stated that

\* Abbott Laboratories, North Chicago, IL 60064.
other acid phosphatase isoenzymes show less than 0.1 percent cross-reactivity in the assay system and lipemic, icteric, and hemolyzed specimens do not interfere with the assay.17

Radioimmunoassay (RIA)

The Clinical Assays “Gammadab” 125I-PAP RIA kit† (lot #0108 and 0109) utilizes a competitive binding assay followed by immunoprecipitation. Binding of both labeled and unlabeled antigen is accomplished with a polyclonal goat anti-PAP serum and the precipitating antisera is an equine anti-goat serum. For technical details of the assay consult the manufacturer’s package insert.18

Samples

Patient samples for this study were those routinely received in the laboratory for PAP assay. Both ELISA and RIA determinations were performed on each sample on the same day. If delay was experienced, samples were stored at −20°C.

Control material was obtained from Ortho Diagnostics.‡ Levels A, B, and C came from lots 016X01, 017X01, and 018X01, respectively. The PAP used in control material is prepared from normal prostate. With samples requiring dilution, it was determined that the respective kit sample diluents were not interchangeable; therefore, dilutions were made using the appropriate specimen dilution buffer to optimize conditions for each assay.

Statistical Analysis

A three-way analysis of variance (ANOVA) was performed to determine statistical significance for within-run and between-run studies (table I). Student’s T-test was employed to evaluate statistical significance for dilution experiments (table II). Statistical significance was obtained if p < 0.05.

Least squares linear regression was employed to determine the best fit lines. Non-standard abbreviations used in figure 1 and figure 2: S = slope, I = intercept, R = correlation coefficient.

Results

The standard curve for the ELISA method demonstrated linearity to 10 ng per ml with the slope decreasing as concentration approached 30 ng per ml. The comparison of mean values and precision for control material of the two methods are shown in table I. Intrarun CV percent averaged 7.0 percent and 6.9 percent for ELISA and RIA, respectively while the interrun CV percent for these two assays averaged 11.1 percent and 11.5 percent for ELISA and RIA, respectively. The values for serum PAP in the control materials were significantly greater with the monoclonal ELISA method than in the polyclonal RIA method (table I). This was observed in the within run study as well as an overall between run study in which the controls were interspersed with patient samples. Comparison of results for patient samples by the two methods is illustrated in figure 1. Most noticeable is the slope of 0.61 which indicates the ELISA values are greater than RIA in patient samples; however, as values approach 0, the RIA values tend to be greater than ELISA as shown by an intercept of 1.35. This variance in values obtained by the two methods in patient samples as values approach 0 was not evident in a comparison of control samples (figure 2), since the intercept was close to 0 while still maintaining a slope less than 1. This was probably due to: (1) the majority of control samples greater than one ng per ml; and
Comparison of Accuracy and Precision of Prostatic Acid Phosphatase Assays by Radioimmunoassay and Enzyme Linked Immunosorbent Assay

TABLE I

<table>
<thead>
<tr>
<th>Analysis A</th>
<th>Analysis B</th>
<th>Analysis C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RIA</strong></td>
<td><strong>ELISA</strong></td>
<td><strong>RIA</strong></td>
</tr>
<tr>
<td><strong>Within Run</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>2.56 ± 0.10</td>
<td>3.14 ± 0.35</td>
</tr>
<tr>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
</tr>
<tr>
<td>Day 2</td>
<td>2.89 ± 0.10</td>
<td>3.22 ± 0.23</td>
</tr>
<tr>
<td>n = 10</td>
<td>n = 8</td>
<td>n = 10</td>
</tr>
<tr>
<td>Day 3</td>
<td>2.57 ± 0.31</td>
<td>3.33 ± 0.13</td>
</tr>
<tr>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
</tr>
<tr>
<td><strong>Between Run</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.76 ± 0.36</td>
<td>3.36 ± 0.39</td>
</tr>
<tr>
<td>n = 46</td>
<td>n = 73</td>
<td>n = 47</td>
</tr>
</tbody>
</table>

Ortho Tri Level (A: Lot # 016X01; B: Lot # 017X01; C: Lot # 018X01).

Values expressed as ng per ml: mean ± SD.

RIA method is significantly different than ELISA method at p < 0.01 by a three-way analysis of variance (ANOVA).

(2) the Ortho control material was spiked with PAP obtained from human prostate.

Since the intercepts vary quite drastically, there appears to be a problem with sensitivity as well as specificity. The methods are both highly correlated as evidenced by similar slopes with both patient data (figure 1) and control data (figure 2); however, the intercepts are quite different. One possible explanation may be the sensitivity of each assay and the source of antigen (human prostate for the RIA and seminal fluid for the ELISA) may influence the values, particularly at lower patient values with an intercept of 1.35 (figure 1). This observation of an unusual intercept is not evident in control sera (spiked with PAP obtained from normal human prostate). The prostate is thought to contain three isoenzymes of PAP and seminal fluid only one of these three. Thus, it appears that the monoclonal ELISA and polyclonal RIA recognize the same antigen at values close to reference range for control material (figure 2) while there exists another antigen which is detected in patient sera by the polyclonal RIA as evidenced by an intercept of 1.35 ng per ml (figure 1).

Dilutions of the lowest level control were made (table II). The ELISA serum PAP value was smaller than the RIA value when the concentration was less than one ng per ml; however, ELISA values of the diluted control material followed the same pattern evident in the patient samples, with ELISA values exceeding RIA values as serum PAP levels increased. These data support the hypothesis that increased sensitivity and specificity are obtained when using a monoclonal antibody. If a plot of dilution versus amount from the data in table II is examined, correlation coefficients of 0.999 and 0.993 are obtained for ELISA and RIA, respectively. This plot indicates that both assays are independently linear, with the relative accuracy statis-

TABLE II

<table>
<thead>
<tr>
<th>A*</th>
<th>ELISA</th>
<th>RIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:9</td>
<td>0.31± 0.10 (n = 15)§</td>
<td>0.45 ± 0.13 (n = 17)</td>
</tr>
<tr>
<td>1:3</td>
<td>0.84 ± 0.08 (n = 16)</td>
<td>0.89 ± 0.13 (n = 18)</td>
</tr>
<tr>
<td>1:1</td>
<td>1.84 ± 0.16 (n = 15)§</td>
<td>1.49 ± 0.19 (n = 18)</td>
</tr>
<tr>
<td>3:1</td>
<td>2.92 ± 0.18 (n = 16)§</td>
<td>2.16 ± 0.13 (n = 19)</td>
</tr>
<tr>
<td>None</td>
<td>3.76 ± 0.18 (n = 16)§</td>
<td>2.51 ± 0.19 (n = 18)</td>
</tr>
</tbody>
</table>

* Dilution of Ortho Tri Level A (Lot # 016X01)
† Values expressed as ng per ml: mean ± SD.
§ p < 0.05
¶ p < 0.001
Figure 1. Comparison of polyclonal RIA versus monoclonal ELISA in patent sera (line drawn according to linear regression equation). Values expressed as ng/ml. Nonstandard Abbreviations: R = correlation coefficient, S = slope, I = intercept.

Statistically significant in all cases except the 1:3 dilution (table II).

Discussion

If PAP is to serve as the marker to determine (a) the extent of adenocarcinoma of the prostate, and, (b) the efficacy of therapy, then the clinical value of serum PAP determination lies with the ability to discern acid phosphatase specific for prostate from other acid phosphatases, particularly those of erythrocyte, leucocyte, and platelets. Currently, PAP is not used as a diagnostic tool: rectal and histological examinations are primary to diagnosis. It is used to assess the extent of metastases once the diagnosis of prostate cancer is made histologically. If the tumor is sufficiently anaplastic and therefore poorly differentiated or if the tumor is intracapsular, it will be difficult to detect increases in serum PAP. However, the method of choice, with well defined reference limits, is one in which early increases are observed. The method should be free of error owing to lack of specificity of antibody and/or to cross reactivity with other acid phosphatases. Since detectable levels of serum PAP are found in total prostatectomy patients and in women, there is convincing evidence that the polyclonal PAP assays are not specific for prostatic acid phosphatase.

The manufacturer of the monoclonal ELISA kit utilized in this study claims serum levels of less than two ng per ml in 99 percent of healthy male subjects, 89 percent of patients with benign prostatic hypertrophy (BPH), and 93 percent of patients with other benign diseases. The manufacturer's published data state that in the case of prostatic carcinoma, 71 percent of stage A, 59 percent of stage B, 45 percent of stage C, and 28 percent of stage D fell within reference range of 0 to 2 ng per ml. These values indicate that the use of a monoclonal antibody cannot detect all carcinomas. The values compare favorably with the study of Griffiths et al., although only 11 percent had an elevated PAP in BPH while Griffiths observed 20 percent incidence of increased serum PAP in BPH. The upper limits of the reference range are 2.0 ng per ml and 3.2 ng per ml for the ELISA and RIA, respectively. Our work with patient sera confirms these differences when patient values fell within the reference range.

The occurrence of higher values with the ELISA than the RIA is evident as serum PAP values increase within and outside the reference range (figure 1). A
possible source of these differences is the source of antigen used in preparation of antibody. The manufacturer of the ELISA kit prepares PAP from seminal fluid, while the RIA kit manufacturer prepares PAP from normal human prostate. These different sources of antigen may explain the different intercepts with the control material (spiked with PAP from normal human prostate) and patient sera. As discussed by Ablin et al., human prostatic tissue is thought to contain three prostate-specific isoenzymes and two of these three are found in tissue while the third occurred in both tissue and prostatic fluid. Therefore, the antibodies employed in the two assays may be directed against different antigens. This could lead to method-dependent differences in serum PAP assays by mass measurement, leading to significant differences in patient management. One must also consider the source of antigen utilized as a spike in quality control material since the antibodies utilized in immunoassays may not be specifically prepared against that antigen source.

The findings of this study indicate that specificity of PAP immunoassays may be influenced by: (1) source of antigen used in immunizing animals and (2) monoclonal versus polyclonal means of producing antibody.

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References