Novel bispecific immunoprobe for rapid and sensitive detection of prostate-specific antigen

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Prostate-specific antigen (PSA) is one of the most useful tumor markers for the screening and follow-up of prostate cancer. Bispecific monoclonal antibodies (bsM Abs) are unique immunopropbes that incorporate two different binding sites in the same antibody molecule. This antibody design can bring important advantages in the development of new immunoassays. We have developed a new hybrid hybridoma that secretes bsM Ab anti-PSA × anti-horseradish peroxidase. This bsM Ab has shown rapid kinetics and an excellent detection limit in a sandwich single-step assay with a total incubation time of 15 min and a 5-min substrate development. This assay in a manual format has a detection limit of 0.028 μg/L. Comparison with the Hybritech Tandem-E® PSA assay yielded a regression equation with slope = 0.433 [95% confidence interval (CI) = 0.415–0.451], intercept = 0.88 (CI = 0.45–1.31), and $S_{\text{obs}} = 1.83 \mu g/L (r = 0.98)$. This new immunoprobe can be used to develop a new generation of assays for clinical laboratories and can be adapted to screening devices for physicians' offices and even home diagnostics.

INDEXING TERMS: bispecific antibodies • tumor markers • screening • hybrid hybridoma • quadroma

Prostate-specific antigen (PSA), an important tumor-associated marker [1, 2], is a 34-kDa glycoprotein produced by the prostatic epithelium.1 PSA is a major protein in seminal plasma and is involved in the liquefaction of semen after ejaculation [3]. It is classified as a serine protease of the kallikrein family and is normally found in low concentrations (≤40 μg/L) in male blood plasma [3, 4]. Most of the naturally occurring PSA in serum remains inactive by association with α1-antichymotrypsin (ACT) and α2-macroglobulin [3, 5–7]. PSA is not a neoantigen or cancer-specific antigen, but rather is expressed by normal, benign hyperplastic, and neoplastic prostatic cells as well other nonprostatic tissues [4]. However, the total (free plus complexed PSA) can be increased in conjunction with prostate cancer, benign prostatic hyperplasia, and after surgical trauma to the prostate. More recently, assays have emerged that measure free, total, and complexed PSA forms, but the clinical significance and utility of the measurement of these different forms are still under investigation [5, 6, 8].

Currently, there are many different assays for the measurement of PSA [7, 9–15]. All of them involve monoclonal or polyclonal antibodies labeled with an enzymatic, fluorometric, or radioactive marker. Here we report the development of a novel bispecific monoclonal antibody (bsM Ab) that could be used for enzyme-based measurement of PSA, with some potential advantages over current methods of detection.

BsM Abs are uniquely engineered antibodies bearing two different binding sites (paratopes) in a single antibody molecule, in contrast with the monospecific antibodies that possess two congruous paratopes [16, 17]. This bifunctional design allowed us to develop a bsM Ab with one site capable of binding PSA and the other an enzymatic marker (e.g., peroxidase). This immunoprobe with intrinsic enzyme marker binding capability can be used directly as a tracer in immunoassays [17–19] approaching the theoretical limit of the specific activity, with every bsM Ab molecule uniformly bound to the enzyme marker.

Materials and Methods

CELL LINES
B80.3, a mouse hybridoma secreting an IgG1 anti-PSA M Ab, was kindly provided by Biomira, Edmonton, AB, Canada. YP4 is a rat hybridoma producing an IgG2a anti-horseradish peroxidase (HRPO) M Ab, obtained cour-
tesy of C. Milstein, MRC Laboratory of Molecular Biology, Cambridge, UK [17]. The YP4 cell line had been previously selected for resistance to azaguanine and ouabain (drYP4) [18]. LNCap, a prostate adenocarcinoma cell line from ATCC, Rockville, MD, was used to provide a convenient source of soluble PSA. All the cell lines were maintained in standard media: RPMI-1640 supplemented with 2 mmol/L l-glutamine, 50,000 units/L penicillin, 50 mg/L streptomycin, and 100 mL/L fetal bovine serum (Gibco BRL, Gaithersburg, MD).

**HYBRID HYBRIDOMA GENERATION**

The fusion protocol used to generate the quadroma or hybrid hybridoma was similar to a previously described method with some modifications [17,18]. Approximately 2.5 x 10^7 drYP4 cells were fused with 2.4 x 10^7 B80.1 cells by using a 500 mL/L polyethylene glycol (PEG) solution (Sigma, St. Louis, MO) for 7 min. The cells were washed to remove all PEG and the pellet resuspended in 100 mL of standard medium supplemented with 100 mL/L growth factor (IGEN, Rockville, MD), oxaaloeetic acid, sodium pyruvate, bovine insulin (Sigma), 0.75 mmol/L ouabain, 0.1 mmol/L sodium hypoxanthine, 0.4 mmol/L aminopterin, and 16 mmol/L thymidine (Gibco BRL). The cells were plated at a final concentration of 1.5 x 10^5 per well and incubated at 37 °C with 5% CO2. Screening of the hybrid hybridoma fusion supernatants was performed after 10 to 16 days of culture with a sandwich assay as described below. Fifteen of the stronger positive clones were selected, expanded, and frozen. The best clone was recloned twice by limiting-dilution method.

**BSMAB SANDWICH ASSAY FOR PSA**

Purified B87.1 MAb, kindly provided by Biomira, was used to coat ELISA plates (Nunc, Naperville, IL). This MAb is a mouse IgG1 anti-PSA antibody that recognizes a different epitope on the PSA and can be used in a heterosandwich assay along with monospecific 125I-labeled B80.3 for the measurement of PSA (M. Krantz and M.R. Suress, manuscript in preparation). After coating (1 μg/well of B87.1 overnight at 4 °C) and blocking with 30 g/L bovine serum albumin (BSA) in PBS for 2 h at 37 °C, the plates were washed and 100 μL of the PSA containing LNCap supernatant incubated for 2 h at room temperature. The plates were again washed and 75 μL of supernatant from each well containing quadroma clones were added. At the same time, 25 μL (100 mg/L) of peroxidase in PBS were also added to each well. The mixture was incubated for 1 h at 37 °C. After washing, 2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS; Kirkegaard & Perry Labs., Gaithersburg, MD) substrate was added and the absorbance measured at 405 nm. Affinity chromatography with a mixture of B80 and B87 MAbs bound to a CNBr-Sepharose preactivated matrix (Sigma) was used to obtain purified free PSA as described previously [20].

**PURIFICATION OF BSMABS**

**ANION EXCHANGE.** Bulk cultures (1-2 L) of the selected hybrid hybridoma (P57.3R2.21) cultures were prepared [17]. Approximately 1.5 L of supernatant was centrifuged to clarify and remove cells, and solid ammonium sulfate was gradually added with stirring to achieve 50% salt saturation. The stirring was continued overnight at 4 °C, and centrifuged for 30 min at 3600 g to collect the pellet. The precipitated immunoglobulins were dissolved in 20 mL of 10 mmol/L sodium phosphate (P2) buffer and dialyzed exhaustively with 200 volumes of 10 mmol/L P1 buffer. The crude immunoglobulin sample was loaded onto a 50-mL bed volume DE52 column equilibrated with 100 bed volumes of 10 mmol/L P1 buffer. The column was washed with 20 mL P1 buffer. The absorbance at 280 nm was monitored continuously. The washing was continued until all the unbound material had been removed and the absorbance reading was again at baseline. To elute the immunoglobulins, a linear ionic gradient was set up with 200 mL of 10 mmol/L P1 and 200 mL of 100 mmol/L P1. The column flow rate was 1.5 mL/min, and 50 fractions (7 mL each) were collected. The fractions were tested with the bsmAb assay described previously for screening of the quadroma supernatants. The fractions with highest bispecific activity were pooled and the purity was determined by a reducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) method with the Phast® gel system (Pharmacia, Uppsala, Sweden).

**Affinity purification.** HRPO (RZ 3.0, Sigma) was covalently linked to a CNBr preactivated Sepharose by using published protocols [21], at 10 mg of peroxidase per mL of gel. Unbound peroxidase was removed by washing with PBS. Supernatant from the hybrid hybridoma (500 mL) was repeatedly loaded onto this affinity column by using a closed-loop system overnight at 4 °C, with a speed of ~1 mL/min. The column was then washed with 10 mmol/L phosphate buffer, pH 6.8. The antibodies bound to the column were eluted with 100 mmol/L glycine, pH 2.8. The fractions (2 mL) were neutralized with 50 μL of 1 mol/L P, pH 8.0. Each fraction was assayed for bsmAb activity and pooled together on the basis of its activity.

**Results**

B80.3 and B87.1 are two high-affinity mouse MAbs developed by immunizing human seminal plasma PSA and using standard hybridoma techniques. These two MAbs belong to the IgG1 subclass and have an affinity constant $K_d$ of $2 \times 10^{-10}$ mol/L each. The two MAbs form an excellent sandwich pair. These two murine MAbs were used to develop an RIA, and, in limited testing, the optimized assay showed a good correlation ($r = 0.975$) with the Hybritech Tandem R kit with 154 prostate cancer, 28 benign prostatic disease, and 12 healthy donors (Krantz and Suresh, in preparation). The above two...
monospecific MAbs were the starting point for this work on bsMAbs.

QUADROMA DEVELOPMENT
The protocol involving PEG and double drug selection was very effective in generating anti-PSA × anti-HRPO hybrid hybridomas. From the original four microtiter plates, >90% of the wells contained clones. The initial screening to detect bsMAB was performed with a two-step bsMAB sandwich assay described in Materials and Methods. The results showed that almost 100% of these wells contained bsMAB-secreting clones. Some of the positive wells gave an absorbance of >3.0 in 15 min after addition of substrate. From these wells, 25 primary clones were selected on the basis of their growth characteristics and bispecific activity. Three of these were recloned by limiting dilution, and the best of these reclones was chosen and recloned again to ensure monoclonality of the quadromas and to develop one subclone (P57.3R2.21).

PSA PURIFICATION
The affinity column was loaded with ~1.2 L of the LNCap cell line with 0.990 mg/L PSA. The final yield after elution was 85% (1.012 mg). The purity by SDS-PAGE was ~95%, and only free PSA was present. We calibrated this standard with a pure PSA sample from Scripps Labs. (La Jolla, CA). All work on the optimization of the bsMAB immunoassay was performed with this affinity-purified PSA.

INITIAL IMMUNOASSAY KINETICS
With the supernatant from one of the most positive clones (P57.3), a preliminary study was done to develop a bsMAB PSA sandwich assay, schematically shown in Fig. 1. The antigen, two antibodies, and the enzyme form a tetrameric complex to generate the ELISA signal. In this assay the second step was done varying the incubation time from 30 s to 3 min, and the absorbance was measured 2 and 5 min after the addition of the ABTS substrate (Fig. 2a). The results show that even with a crude supernatant that contains competing monospecific antibodies that could potentially decrease assay sensitivity, the second step was almost complete at 60 s of incubation. The high absorbance at 405 nm was not significantly different when compared with the 3-min incubation period. This assay was performed with nonpurified LNCap supernatant containing close to 1 mg/L PSA. The high colorimetric yield and fast second-step kinetics achieved with a crude

![Figure 1](https://example.com/fig1.png)

Fig. 1. Schematic representation of the tetrameric complex formed in the bsMAB sandwich immunoassay to detect PSA.

![Figure 2](https://example.com/fig2.png)

Fig. 2. (A) Initial bsMAB assay kinetics for the second step of a forward sandwich assay; (B) initial single-step sandwich assay kinetics.

(A) The plate was coated with the catcher MAb (B87.1), blocked with 30 g/L BSA for 3 h, and incubated overnight with ~1 mg/L PSA for the first step of the sandwich assay to reach equilibrium. BSA was used as a negative control. After a wash step, the plate was incubated with the supernatant of the hybrid hybridoma plus 30 mg/L peroxidase. The incubation time was varied from 30 to 180 s. The absorbance at 405 nm was read after 2 and 5 min. (B) Crude quadroma supernatant was used in an initial single-step assay. The incubation times varied from 1 to 10 min.
cell supernatant from the bsMAB (anti-PSA/anti-peroxidase) in a two-step forward sandwich immunoassay was also seen in a single-step assay (Fig. 2B). Here all the components were incubated together for 1 to 10 min and the reaction kinetics monitored. These preliminary results showed promise for pursuing further work on the purification of bsMAB and optimization of the assay.

**bsMAB Purification**

*Ammonium sulfate precipitation.* We first prepared purified bsMAB by using an ammonium sulfate precipitation method. With this procedure, we could remove some of the protein contaminants of the culture supernatant and also concentrate the bsMAB sample. Approximately 1.5 L of tissue culture supernatant was precipitated with ammonium sulfate and the immunoglobulins resuspended in PBS. The final concentration factor after dialysis was 1:20. This concentrated sample of antibody was used in a PSA immunoassay; the dilution curve is shown in Fig. 3.

A bell-shaped curve was obtained as a function of the crude bsMAB concentration. This unique dilution could be explained by the fact that the presence of anti-PSA monospecific MAbs secreted by the quadroma could compete with the bsMAB, decreasing the signal in the more concentrated samples. At low dilutions of the concentrated sample (1:10 and 1:50), a strong competition between the monospecific anti-PSA and the bsMAB could ensue, in favor of the former. This competition decreases upon further dilution and the signal increases significantly. Higher dilution (1:1000) results in decreased signal because of low bsMAB mass.

**Anion exchange.** The ammonium sulfate-precipitated antibody was loaded on a DE52 ion-exchange column and the antibodies were eluted with a phosphate gradient from 10 to 100 mmol/L. The fractions representing the highest bispecific activity were pooled (data omitted). SDS-PAGE showed that the pooled fraction A was 65% pure immunoglobulins.

**Affinity chromatography.** Affinity-purified bsMAbs were obtained as a single eluted peak by using a 3-mL HRPO-CNBr-Sepharose column (data not shown). The affinity eluate is expected to contain both the bsMAB and the monospecific anti-peroxidase. Monospecific anti-PSA MAB is washed off as the unbound fraction. The electrophoretic analysis of this purification showed that some HRPO was leaching from the column during the purification step.

**Development of a Single-Step Two-Site Immunoassay**

The essential requirements of a sandwich enzyme assay are a matched pair of antibodies with high combined affinity and specificity, a solid-phase support for one antibody, an analyte preparation to be used as a calibrator, and an enzyme marker that remains highly detectable when linked to the antibody and does not reduce antibody affinity. The big advantage of bsMAB as tracer (Fig. 1) is the fact that the antibody bears an intrinsic binding site for the enzyme. This avoids the need for chemical conjugation, which could potentially (in some less-optimized conjugation methods) lead to loss of activity of the antibody or the enzyme, aggregation, and formation of undesirable complexes. Here we explored the optimization of the bsMAB performance as tracer in a single-step immunoassay. We chose a single-step format because of its convenience, even though there was a possibility of a hook effect at extreme high concentrations of PSA.

One of the factors in the optimization of immunoassays was the concentration of the bsMAB. In this evaluation, HRPO was always used in excess (30 mg/L). A standard dilution curve of the purified antibody was used to determine the optimal dilution to be used in the single-step assay. The direct PSA binding assay showed (data omitted) that the pooled bsMAB in a 1:10 dilution had the highest binding. None of the purified samples presented the characteristic bell-shaped curve that may indicate the successful removal of the monospecific anti-PSA. On the basis of these results, we proceeded with the experiments by using the pooled bsMAB from the DE52 purification diluted 1:10 in all subsequent single-step assay optimizations.

The above pool, diluted 1:10, was preincubated with excess HRPO (30 mg/L) and used in a set of experiments involving four points in the low range of PSA (0, 4, 10, and 25 μg/L). The incubation time was varied from 10 min up to 2 h. In all these assays the solid-phase MAb B87.1 was coated at 1 μg/well in 100 μL and the additional binding
sites on polystyrene blocked with 30 g/L BSA in PBS. There was no significant increase in the signal after 15 min of incubation time (data not shown). Hence, all subsequent experiments were performed with a 15-min incubation time.

DETECTION LIMIT
The lower limit of detection, defined as the unit value of antigen above the zero value signal plus 2 SD, was determined with 12 replicates. Note that we used the less sensitive ABTS substrate; the plate was read after 5 min and various times up to 1 h. The detection limit was expressed in relation to the substrate incubation time (data not shown). The detection limit of most commercial assays, with incubation times from 1 to 3 h, are between 0.3 and 0.05 μg/L (Table 1). Those limits could be reached with our 15-min assay even when using a less sensitive peroxidase such as ABTS, which required longer color development time. When we compared the detection limit with tetramethylbenzidine (TMB; Kirkegaard & Perry Labs.) as a peroxidase substrate under the same conditions as before, with 1 mol/L phosphoric acid added to stop the colorimetric reaction after 5 min, the detection limit of the assay was highly improved at 28 ng/L (Table 1).

PSA/ACT COMPLEXES (EQUIMOLAR ASSAY)
PSA has intrinsic proteolytic activity, and most of the naturally occurring PSA in serum remains inactive by association with ACT. The clinical utility of assay depends on the capacity of detection of the total PSA, which represents free PSA plus ACT-complexed antigen [3, 5]. The assay is defined as equimolar if it is equally reactive to ACT-bound PSA and free forms of PSA. We evaluated the effect of added ACT in the assay and the capacity of the assay to detect total PSA. Purified PSA was incubated with increasing molar amounts of ACT for 24 h [6]. The complex formation was also confirmed by SDS-PAGE. ACT-PSA was used as calibrator to compare with free PSA. ACT complexation had no significant influence on the recovery of PSA (data not shown). This indicates that this assay is also an equimolar assay, being equally reactive to free PSA as well PSA-ACT. The above results were confirmed also by using a commercially available (Scripps Labs.) pure PSA-ACT complex. In a directed comparison, the Hybritech Tandem® PSA calibrators were compared with the pure PSA-ACT calibrators for relative recoveries. The two recovery results were superimposable (data omitted).

METHODS COMPARISON
To further evaluate our new bsMAb-based PSA assay, we tested 138 samples obtained from the Tumor Marker Laboratory, W.W. Cross Cancer Institute, Edmonton, AB (courtesy of S. Popemma and Sheila Stelmaschuck) against the results obtained with the samples previously analyzed in an automated Hybritech enzyme immunoassay for PSA.

The results (Fig. 4A and B) show an excellent overall correlation: \( r = 0.98 \), slope = 0.433 [95% confidence interval (CI) = 0.415–0.451], intercept = 0.88 (CI = 0.45–1.31), and \( S_y/x = 1.83 \) μg/L. In the lower range from 0 to 10 μg/L, \( r \) was 0.95, slope 0.584 (CI = 0.539–0.629), intercept \( -0.33 \) (CI = \(-0.57--0.09\)), and \( S_y/x = 0.45 \) μg/L. The calibrators prepared by us were \(~50\%\) lower in assay values than the calibrators provided in the Hybritech kit; hence the decreased slope. No hook effect was detected up to 100 μg/L PSA.

The intraassay CV was 27% with PSA values of 0.05–

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<th>Table 1. Comparison of assay sensitivity of various PSA assays.</th>
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<td>DPC IRMA-Count®, radiometric</td>
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<td>DELFIA PSA® [7], fluorometric</td>
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<td>Serono SR1 enzyme immunoassay (EIA) [11], automated</td>
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<td>Khosravi et al. [12], EIA</td>
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<td>Vihko et al. [14], fluorometric</td>
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<td>Yu and Diamandis [15], fluorometric</td>
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PSA is one of the most used serum tumor markers because of its importance in the screening and follow-up of patients with prostate cancer. As we increase and automate the number of tests performed every day in clinical laboratories all over the world, development of assays with faster kinetics and higher sensitivity is needed, while preserving the technological simplicity of the assay. The focus of our efforts was to develop a rapid assay using a new generation of immunoprobes with the highest possible specific activity. We developed a bsMAb incorporating in one arm of the molecule an anti-PSA paratope and in the other an anti-peroxidase binding site. The specific activity could be further improved by using polyperoxidase homopolymers with 10 to 80 monomers cross-linked into one large molecule recently introduced by Research Diagnostics (Flanders, NJ).

Although there are new approaches for the development of bifunctional molecules [22–25], we opted for the generation of hybrid hybridomas as a convenient source of bsMAbs. Previously we had developed a double-resistant anti-peroxidase hybridoma (drYP4) that facilitated the development of the new anti-CA125 × anti-HRPO hybrid hybridoma [18]. In this example, CA125 was representative of a huge tumor antigen with a molecular mass in excess of 200 kDa. Despite this enormous size, we developed an ELISA based on bsMAbs [18]. Here, we chose PSA as an example of a small-molecular-mass antigen and two well-established anti-PSA clones (B80.3 and B87.1) to develop a rapid ELISA. The standard PEG fusion protocol generated bsMAb clones with high frequency. The PSA used in all our experiments was produced by the LNCap cell line, which produced close to 1 mg of PSA per liter of supernatant. These supernatants were used directly for all initial screening assays and also as the source of antigen by affinity purification.

Our initial evaluation of the bsMAb probe showed very high colorimetric yield. With 1 mg/L PSA, the absorbance was ~2.000 after only 180 s of incubation. These extremely fast second-step sandwich assay kinetics were our initial starting point for further development and optimization of a new PSA assay. Ammonium sulfate fractionation was used to remove some of the protein contaminants and also concentrate our antibody preparation. The serial dilution of this preparation demonstrated an interesting observation, namely a bell-shaped curve. At high concentrations (low dilution), the signal was low, likely due to a strong competition between the monospecific anti-PSA and bsMAb. As the dilution factor increased, the signal increased up to a point where the mass of bsMAb became limiting and the signal diminished. This enhanced signal due to dilution was also seen in primary quadroma cultures, and this phenomenon could result in an apparent decrease in the number of positive clones identified in hybrid hybridoma fusions if screening is not performed at various dilutions of culture supernatants.

Two methods for purification of the bsMAb were attempted: anion exchange and affinity purification. The affinity purification with HRPO coupled to preactivated CNBr-Sepharose was designed to produce an eluate containing the bsMAb and the monospecific anti-peroxidase.
but not free monospecific anti-PSA. The presence of monospecific anti-HRPO would not interfere in the optimization of the assay because saturating amounts of HRPO could be added, preventing any competition for signal quenching. The limitation of this method was the lower yield and also the possible loss of antibody activity due to the harsh conditions of elution. The HRPO column also showed slow leakage of coupled HRPO, which could contribute to the low yields. The anion-exchange column was effective and produced good yields even though the purity was only 60% by SDS-PAGE. The different fractions of purified material, when used in a serial dilution assay, did not present the bell-shaped curve, which indicated that bsMAb was relatively free of the monospecific B80 (anti-PSA) species.

Different assay conditions were optimized, resulting in the development of a 15-min single-step assay. This demonstrated that the bsMAb could be used in the generation of fast assays. Although the detection limit was comparable with most commercially available assays with longer incubation times, this 1-h substrate incubation would remove some of the advantages of fast kinetics of the assay. For this reason we used TMB as the substrate to reach a sensitivity of 0.028 μg/L in only 5 min of substrate development. The Hybritech Tandem-E PSA assay is a single-step EIA with 2 h of incubation and 30 min of substrate development, with a detection limit of 0.1 μg/L. It appears from Table 1 that our bsMAb assay has good sensitivity, even with a short incubation time. More recently, an ultrasensitive time-resolved fluorometric assay for PSA was reported, involving alkaline phosphatase plus diflusinal phosphate as the signal-generating system [26]. This assay has a detection limit of 0.3 to 1 ng/L in a 90-min assay. It would be interesting to compare if (a) our current peroxidase-based bsMAb assay would exhibit similar ultrasensitivity with luminescent substrate, or (b) our new anti-PSA bsMAb with alkaline phosphatase binding arm (unpublished results) can achieve those remarkable sensitivities.

Bispecific MAbS have intrinsic binding to any two predetermined antigens, and, in fact, these molecules can be considered macromolecular cross-linkers [16–18]. This uniformly reproducible 1:1 binding with the signal-generating arm has several advantages in immunohistochemistry and immunoassays [27]. Although conjugation chemistry is better refined now than earlier methods, cross-linking of two macromolecules cannot be controlled to form 1:1 heteroconjugates. Some MAbs molecules are either not labeled at all or have more than one signal molecule that could present size and steric problems due to random linking. In the bsMAbs, with excess HRPO, we expect that every molecule is uniformly bound without sterically interfering with the anti-PSA arm (Kreutz and Suresh, unpublished data). This could explain the high specific activity and fast kinetics. In addition, bsMAbs provide unique advantages in product development, with simplified production and greater assay consistency from lot to lot.

A portion of the naturally occurring PSA in serum remains enzymatically inactive by association with ACT. This fact should be considered in the development of assays capable of measuring the total PSA [free PSA + PSA-ACT (equimolar assay)]. Externally added ACT in increasing concentrations did not interfere in the recovery of PSA, which implied that the B87/P57 sandwich assay is an equimolar assay. Further, the recoveries were essentially similar when either pure PSA-ACT (Scripps Labs.) or Hybritech Tandem PSA calibrators were used.

Finally, we compared the bsMAb assay with the Hybritech Tandem EIA, also an equimolar assay. The $S_{\text{PSA}}$ from 0 to 100 ng was excellent (1.83 μg/L), and even in low range (0 to 10 ng), $S_{\text{PSA}}$ was 0.45 μg/L. No hook effect was observed up to 100 μg/L PSA, and with our exceptionally clean backgrounds, the tracer mass could be increased if necessary, to decrease potential hook effects. Also, the values obtained in our assay were approximately half those obtained by the Hybritech assay, which indicated a need to improve the preparation or conservation of our PSA calibrators. We have also recently used this bsMAb to map the potential epitope on the PSA molecule by synthetic peptide scanning techniques [20].

BsMAbs are powerful immunoprobes that could be used as ultimate enzymoimmunotracers, presenting a high uniform specific activity, wherein every bsMAb molecule is labeled uniformly with an HRPO molecule. We developed a bsMAb anti-PSA/anti-HRPO-secreting quadroma and optimized a manual sandwich single-step assay that can be performed in 20 min (incubation plus substrate development), preserving a high analytical sensitivity. This probe could also be used in the next generation of automated immunoassays as well as in rapid screening formats for physicians’ offices or home diagnostics.

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