Immunofluorometric assay for sensitive and specific measurement of human prostatic glandular kallikrein (hK2) in serum

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Prostate-specific antigen (PSA) and human prostatic glandular kallikrein (hK2) have 79% identity with the primary structure. When we used recombinant hK2 protein, only 7 of 23 monoclonal anti-PSA IgGs (monoclonal antibodies, Mabs) cross-reacted with hK2, which enabled us to design a novel immunofluorometric MAb-MAb assay for the specific detection of hK2. In the first incubation, an excess of MAb 2H11, which does not cross-react with hK2, is added to prevent both free and complexed PSA from reacting in subsequent immunoreactions. In the second incubation, biotinylated MAb H50, which cross-reacts with hK2 by an epitope overlapping with MAb 2H11, served to bind only hK2 to the microtitration wells coated with streptavidin. In the third step, Eu-labeled MAb H117, which cross-reacts with hK2, detected the immobilized hK2. The hK2 assay was calibrated with recombinant hK2. The detection limit of the assay was 0.1 µg/L, and the cross-reactivity with recombinant PSA was ≤0.7%. The concentration of hK2 was measured in serum samples from 334 males with total PSA concentrations ranging from 1 to 3400 µg/L. Most of the samples (57%) had hK2 concentrations below the detection limit. The proportions of hK2 relative to total PSA were 0–2% in 79%, 2–5% in 14%, 5–10% in 4%, and >10% in 3% of the samples. Gel filtration of 10 serum samples with increased hK2 concentrations showed a single peak of hK2 immunoreactivity with an apparent molecular size of ~30 kDa, corresponding to that of recombinant hK2 and free PSA.

INDEXING TERMS: prostate-specific antigen • monoclonal antibodies • prostate cancer • protease inhibitors • complexes

Human glandular kallikrein (hK2) and prostate-specific antigen (PSA or hK3) are closely related serine proteases belonging to the subgroup of glandular kallikreins [1]. The mature forms of PSA and hK2 comprise 237 amino acid residues that share 79% sequence identity [2]. The primary structure of hK2 suggests it is a trypsin-like protease [3], in contrast to PSA, which shows chymotrypsin-like substrate specificity [4]. PSA has been purified and characterized from seminal fluid and prostate tissue [5]. Thousand-fold lower levels of PSA immunoreactivity have been detected recently in breast cancer cytosols [6], in the milk of lactating women [7], and in amniotic fluid [8]. The major physiological function of PSA is thought to be the fragmentation of the gel-forming proteins in semen [9]. PSA has also been suggested to degrade insulin-like growth factor binding protein-3 [10]. The hK2 mRNA amounts are estimated to be 10–50% of that of PSA mRNA in prostatic tissue [11–13]. Androgens induce the expression of the genes encoding hK2 and PSA [14]. Despite the abundance of hK2 transcripts in the prostate, the purification, characterization, and concentration of the hK2 protein in body tissues and fluids has not been reported. Therefore, the physiological function of hK2 remains to be investigated.

The active single-chain form of PSA forms stable covalent complexes with several extracellular protease inhibitors: α₁-macroglobulin (AMG), α₁-antichymotrypsin (ACT), and protein C inhibitor (PCI). However, the immunodetection of PSA is sterically shielded by the complex formation with AMG, and the serum concentrations of these PSA-AMG complexes can

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These prostatic concentrations potential molecular Free therefore

Thereof the PSA-CT complex (PSA-C) is the major molecular form, whereas a minor proportion occurs in a free noncomplexed form (PSA-F) despite the 10^{-2} to 10^{-1} molar excess of ACT and AMG in blood [17, 18].

Increased serum concentrations of PSA (>4 mg/L) may result from various disease conditions of the prostate, especially in cancer of the prostate (CaP) [19]. Serum PSA measurements are widely used to monitor CaP [19–24], but the diagnostic potential of PSA measurements is limited by the fact that PSA concentrations are also increased in many subjects with benign prostatic hyperplasia (BPH) [25–27]. Recent studies have shown that the proportion of free PSA to PSA-ACT complexes in serum is significantly higher in BPH than in CaP [17, 28], which may prove to be clinically useful in improving the diagnostic specificity of the analysis of total PSA.

Recently, we reported the recombinant production of hK2 and PSA with the use of both eukaryotic and prokaryotic expression systems [29; Eerola et al., ms. submitted for publication]. Characterization of the immunological cross-reactivity of 23 monoclonal antibodies (MAbs) generated against PSA was performed to construct the epitope maps of PSA and hK2. These data have now been used to design an immunofluorometric assay for the specific measurement of hK2 in human serum.

**Materials and Methods**

PURIFIED PROTEINS, REAGENTS, AND INSTRUMENTATION

Superose 12 HR 10/30 prepacked FPLC-column, NAP-5, and NAP-10 gel filtration columns were purchased from Pharmacia (Uppsala, Sweden). Microtitration wells coated with streptavidin, anti-PSA Mab 2E9 and anti-PSA MAb H117, DELFIA® PSA Assay, DELFIA Eu-labeling kit, DELFIA 1234 Plate Fluorometer, DELFIA Buffer, DELFIA Wash Solution, and DELFIA Enhancement Solution for the immunofluorometric assays were from Wallac Oy (Turku, Finland). Biotin amidoacprolate N-hydroxysuccinimide ester solution and heparin were from Sigma (Deisenhofen, Germany). Purified PSA from seminal plasma (containing ~85% of catalytically active single chain form and ~15% of the inactive two-chain form), purified ACT, and PSA-ACT complexes were generated, purified, and stored as reported previously [30, 31]. Additional purified PSA-ACT [32] used in the cross-reactivity study was obtained as a gift from T.A. Stamey (Stanford, CA).

**MABS**

The reactivity of 23 anti-PSA MAbs with hK2 and PSA has been described previously [29]. Nine of these MAbs were used in immunoassays described here (Fig. 1). MAbs 2E9 and 2H11 were specific for free and complexed forms of PSA (PSA-T), whereas H50, H117, 3C1, 4H5, 5F11, and F5 also recognized recombinant hK2. MAb 5A10 recognized only PSA-F. MAb 241 was generated against ACT and used as an Eu-labeled tracer in the assay protocol for PSA-C [33].

**SAMPLE MATERIAL**

Serum samples from 334 males on which no clinical documentation was available were obtained from the Department of Clinical Chemistry, University Hospital, Malmö, Sweden. The total PSA concentrations ranged from 1 to 3400 μg/L. The procedures followed were in accordance with the Helsinki Declaration of 1975.

**PRODUCTION OF RECOMBINANT PSA AND HK2**

The recombinant production of PSA and hK2 with use of Semliki Forest virus (SFV)-infected BHK-21 cells and Escherichia coli has been recently reported and shown to result in the expression of 30-kDa proteins according to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blots [29; Eerola et al., ms. submitted for publication]. Cell culture supernatants of SFV cultures and periplasmic fractions of E. coli were used for binding studies, affinity measurements, cross-reaction estimations, and as calibrators in the hK2 assay.

**EU LABELING AND BIOTINYLATION OF MABS**

Tracer MAbs were labeled with 2–6 Eu/IgG according to the instructions in the DELFIA Eu-labeling kit. Biotinylation of MAb H50 was performed as reported previously [31]. The efficiency of the biotinylation was monitored with two subsequent incubations: In the first incubation, 200 μL of biotinylated MAb H50 (bH50), 250 μg/L, was added to streptavidin-coated microtitration wells and incubated for 1 h. After the first incubation, 100 μL of reaction solution was transferred to anti-mouse IgG-coated microtitration wells and incubated for 1 h, parallel to 100 μL of the 250 μg/L MAb H50 solution used as calibrator. The bound MAb was determined by using Eu-labeled anti-mouse IgG as the tracer antibody. The biotinyl-
tion procedure was judged to be adequate if >95% of hH50 was bound to streptavidin.

IMMUNOFLUOROMETRIC ASSAY PROCEDURES

**hK2 assay protocol.** The assay designed for the specific detection of hK2 (2H11+bH50/H117) included three incubation steps (Fig. 2): (a) Duplicates of calibrator or unknown samples in 25 μL together with 100 μL of DELFIA buffer containing an excess (2000 ng/well) of MAb 2H11, which does not cross-react with hK2, were incubated at room temperature for 1 h in streptavidin-coated microtitration wells; (b) bH50 (100 ng/well) in 50 μL of DELFIA buffer was added to the reaction mixture and incubated for 1 h; (c) after a wash step, the Eu-labeled tracer MAb H117 (100 ng/well), also cross-reacting with hK2, was added in 200 μL of DELFIA buffer to detect the immobilized hK2. Another washing step was followed by the addition of 200 μL/well of enhancement solution. The fluorescence was measured for 1 s in a plate fluorometer. In other hK2 assay constructs we investigated, the tracer MAb H117 was replaced with either Eu-labeled MAb 3C1, 4H5, 5F11, or F5.

**PSA assay protocols.** Four different in-house two-site PSA assays were run according to protocols similar to the commercial DELFIA PSA-T kit (2E9/2H11). These assays included the following MAb for capture and detection: PSA-T + hK2 (H117/H50), PSA-T (2E9/H117), PSA-F (5A10/H117), and PSA-C (H117/241). The protocol for the PSA-C assay differed from the others in three ways to minimize the nonspecific adsorbance of ACT to the microtitration wells, as reported earlier [31]: (a) the DELFIA buffer contained 25 000 IU/L of heparin, (b) the buffer was added to the well before the sample, and (c) the tracer MAb 241 was incubated overnight at 4 °C.

**Calibration.** Free PSA calibrators, purified from seminal plasma [31], were used in the assays for PSA-T + hK2, PSA-T, and PSA-F. As reported in Results, the proportion of hK2 relative to PSA was <0.3% in this preparation. The PSA-C assay included purified PSA-ACT complex calibrators formed in vitro [31], and the hK2 assay included recombinant hK2 calibrators produced in *E. coli*. The assay for PSA-T + hK2 (H117/H50) was used to assign the values for the PSA-ACT and recombinant hK2 calibrators, as it includes MAbS with similar affinity constants for recombinant hK2, recombinant PSA, purified free PSA, and purified PSA-ACT complexes [29]. Preparations were diluted in TSA buffer [50 mmol/L Tris, 150 mmol/L NaCl, 0.5 g/L sodium azide, and 75 g/L bovine serum albumin (BSA)] (pH 7.8).

**GEL FILTRATION**

A Superose 12 HR column (1 × 30 cm) attached to the Smart® purification system (Pharmacia) was equilibrated with TSA buffer (pH 7.2). The flow rate was maintained at 0.2 mL/min, and fractions of 0.25 mL/tube were collected. The elution volumes of carbonic anhydrase (30 kDa), ovalbumin (43 kDa), BSA (67 kDa), and lactate dehydrogenase (140 kDa) were used as molecular mass markers.

**Results**

**EPITOPE MAP OF PSA AND hK2**

An epitope map of the nine MAbS used in this study, based on previously reported data [29], is shown in Fig. 1. One MAb (5A10) was specific for PSA-F, whereas two MAbS (2E9 and 2H11) recognized PSA both in free and complexed form. Five MAbS (H50, H117, 3C1, 4H5, and 5F11) recognized both PSA-T and hK2 with similar affinities, whereas one MAb (F5) had an ~100-fold lower affinity for recombinant hK2 compared with PSA [Eerola et al., ms. submitted for publication].
VALIDATION OF THE HK2 ASSAY
The hK2 assay principle and the three-step incubation assay protocol as described in Materials and Methods is illustrated in Fig. 2.

Concentration of blocking MAb 2H11 and hH50. To minimize the cross-reaction of PSA in the hK2 assay, increasing amounts of MAb 2H11 (0–3200 ng/well) were added and analyzed in the presence of recombinant PSA, recombinant hK2, purified PSA, purified PSA-ACT (each 50 µg/L), patient serum 1 (hK2/PSA-T = 5%), and patient serum 2 (hK2/PSA-T = 0%). Efficient blocking of PSA-T by MAb 2H11 is achieved at concentrations >1000 ng/well.

hK2 calibrator. Serial 1:3 dilutions of the recombinant hK2 calibrator to the calibration diluent were used in the hK2 assay (Fig. 4). These calibration preparations showed good stability when stored at −20 °C or 4 °C for 5 weeks compared with the reference calibrators stored at −70 °C. The recovery of hK2 was 97.8% (SD 2.8%) at −20 °C and 99.1% (SD 3.1%) at 4 °C.

Cross-reactivity. The cross-reactivity with purified PSA, PSA-ACT, and recombinant PSA was 0.7% of PSA equivalents (Fig. 5). Purified PSA and recombinant PSA were diluted in calibration diluent because of complex formation with AMG in female serum, whereas purified PSA-ACT retained its immunoreactivity in female serum. Samples with total PSA concentration >250 µg/L were diluted in calibration diluent to yield a total PSA concentration below this limit. We corrected the results of all hK2 measurements by subtracting the cross-reacting PSA signal (0.7% of the PSA concentration in the sample) from the measured hK2 concentration.

Precision, analytical recovery, and detection limit. Between-assay precision was studied by analyzing 16 serum samples with increased hK2 concentrations in three subsequent assays. The mean CVs in three groups categorized according to the hK2 concentration in the samples were 13.9% (hK2 0.4–1.0 µg/L, n = 7), 9.2% (hK2 2.5–9.2 µg/L, n = 5), and 5.6% (hK2 11.4–59.2 µg/L, n = 4). Within-assay precision was measured in 34 replicates with sera from healthy women with four concentrations of recombinant hK2. The CVs were 12.5% (hK2 0.8 µg/L), 8.2% (hK2 2.2 µg/L), 3.4% (hK2 5.3 µg/L), and 4.2% (hK2 16.4 µg/L). Analytical recovery was studied with high-hK2-concentration serum samples (n = 3) diluted in
female serum and calibration diluent (1:4 dilutions). The recovery of hK2 was 91.4% (SD 2.3%) in female serum and 103.5% (SD 5.9%) in diluent as measured by the hK2 assay. The recovery was 108.5% (SD 7.2%) when samples were diluted in female serum or calibration diluent containing a constant amount of purified PSA (100 μg/L). The lower limit of detection, determined by 2 SD of the calibration diluent or female serum multiplied by the slope of the calibration curve, was 0.10 μg/L.

**ANALYSIS OF SERUM SAMPLES**

The hK2 concentrations were measured in serum samples from males (n = 334) with PSA-T concentrations ranging from 1 to 3400 μg/L. The PSA-T + hK2 concentrations were measured by the H117/H50 assay, from which we calculated the PSA-T concentrations by subtracting the measured hK2 concentration by the 2H11 + hH50/H117 assay, both assays involving the same MAb-MAb sandwich pair (H50 and H117). Table 1 shows the median values of hK2 concentrations in four groups categorized according to the PSA-T concentrations. The hK2 concentration was below the detection limit of the assay (<0.1 μg/L) in 190 of the 334 samples (57%). The proportion of hK2 relative to PSA-T was 0–2% in 79%, 2–5% in 14%, 5–10% in 4%, and >10% in 3% of the samples (Table 2). The scattergram in Fig. 6 shows the distribution of hK2 values ≥0.1 μg/L (n = 144) in relation to PSA-T. The incidence of samples with the hK2/PSA-T ratio >5% was not dependent on the PSA-T concentration. The DELFIA PSA-T assay was also used to measure the PSA-T concentration in the 334 serum samples. The correlation coefficient (r) between DELFIA PSA-T (2E9/2H11) and PSA-T + hK2 (H117/H30) assays was 1.00 (P < 0.0001, slope = 1.43) with all samples, and 0.97 (P < 0.0001, slope = 1.21) with samples in the PSA-T range 1–100 μg/L (n = 309).

Nineteen hK2 samples containing 2–43% of hK2/PSA-T were further evaluated with assays for PSA-T (2E9/H117), PSA-F (5A10/H117), and PSA-C (H117/2C1). The correlation coefficients of the hK2 concentration vs PSA-F, PSA-T, and PSA-C concentrations were 0.94 (P < 0.0001, slope = 0.275), 0.80 (P < 0.0001, slope = 0.26), and 0.78 (P < 0.0001, slope = 0.27), respectively. The results obtained by replacing the Eu-labeled tracer MAb H117 in the hK2 assay (2H11 + hH50/H117) with either Eu-labeled tracer MAbs 3C1, 4H5, 5F11, or F5 correlated well with the results of the original hK2 assay. When the 19 serum samples with increased hK2 were measured, the correlation coefficients with the tracer MAbs 3C1, 4H5, or 5F11 compared with the original hK2 assay were excellent (r = 1.00, P < 0.0001, slopes = 1.38–1.55). When the Eu-labeled tracer MAB H117 was replaced with Eu-labeled MAB F5, which has a 100-fold lower affinity with hK2, the slope of the regression line dropped substantially, whereas the correlation coefficient remained high (r = 0.98, P < 0.0001, slope = 0.36). The affinity constants of the hK2-detecting MAbs H117 and H50 were also determined with two serum samples containing high concentrations of hK2. Similar affinity binding constants were obtained for recombinant hK2 and the hK2 signal detected in the two serum samples.

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<th>Table 1. hK2 concentrations and hK2/PSA-T ratios in serum samples (n = 334).</th>
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<td><strong>hK2, μg/L (95% conf. limits)</strong></td>
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<td><strong>PSA-T range (μg/L)</strong></td>
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<th>Table 2. Frequency of high hK2/PSA-T ratios in serum samples (n = 334).</th>
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<td><strong>hK2/PSA-T ratio, %</strong></td>
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Fig. 6. Scattergram of serum samples with detectable hK2 immunoreactivity (hK2 ≥0.1 μg/L and PSA-T ≥1.0 μg/L).

The two lines represent the 5% (solid) and 10% (dashed) proportions of hK2/PSA-T. The correlation coefficients in PSA-T ranges 1–3400, 1–100, 1–20, and 1–10 μg/L were 0.84, 0.48, 0.32, and 0.14, respectively (P < 0.05).
GEL FILTRATION OF SERUM SAMPLES AND RECOMBINANT hK2

Seven serum samples with ratios of hK2:PSA-T >5%, three samples with hK2:PSA-T between 1% and 5%, and one sample with hK2:PSA-T <1% were analyzed by gel filtration. The fractions were monitored with five assays specific for different molecular forms of PSA and hK2: (a) PSA-T + hK2, (b) PSA-T, (c) PSA-F, (d) PSA-C, and (e) hK2. Fractions obtained by gel filtration of recombinant hK2 produced in \textit{E. coli} are shown in Fig. 7A. The major peak of the recombinant protein eluted at a position corresponding to \textasciitilde 30 kDa. In a sample with only 0.9% hK2/PSA-T (Fig. 7B), the concentrations measured with the hK2 assay in the gel filtration fractions are close to zero, whereas PSA-T + hK2, PSA-T, and PSA-F assays at the 30-kDa position gave very similar results. The analysis of a high-hK2 sample (hK2:PSA-T = 8.5%) in Fig. 7C shows that the sum of hK2 and PSA-F (or PSA-T) concentrations measured in the fractions at the 30-kDa position correspond closely to the concentration measured by the PSA-T + hK2 assay. All serum samples analyzed by gel filtration showed a single peak of hK2 immunoreactivity eluting at a position corresponding to \textasciitilde 30 kDa, whereas no hK2 complex peaks of higher molecular mass were detected.

Discussion

We demonstrate here a MAb-based sandwich assay for the specific and sensitive detection of hK2 in the presence of PSA. Because there are no specific anti-hK2 MAbs available currently, we used an indirect assay design made evident from cross-reactivity studies with recombinant hK2 and epitope mapping of 23 anti-PSA MAbs [29]. With the use of overlapping epitopes of MAb 2H11 (not cross-reactive with hK2) and MAb H50 (cross-reactive with hK2), we could distinguish between PSA and hK2. The concentration of hK2 in the presence of excess PSA can be determined by using a protocol with an excess of MAb 2H11, through which PSA was prevented from being measured by the sandwich pair of MAbs H50 and H117. Both MAbs H50 and H117 recognize PSA-F, PSA-C, and recombinant hK2 with similar affinities. The blocking procedure was highly selective for PSA, as neither the recombinant hK2 nor the hK2 immunoreactivity found in serum samples was affected by the excess of MAb 2H11.

Because no reference preparations of hK2 from biological sources are currently available, we have used recombinant hK2 as calibrator in the hK2 assay. A buffer matrix was used as a calibration diluent because the addition of recombinant hK2 to serum could not be quantitatively recovered, presumably because of complex formation with AMG in a similar way as reported with PSA [18]. The calibrators were calibrated with the PSA-T + hK2 assay (H117/H50), as both the H117 and H50 MAbs recognize purified PSA from seminal fluid, recombinant PSA, and recombinant hK2 with similar affinities.

The identity of the hK2 immunoreactivity in serum samples and recombinant hK2 measured by the hK2 immunoassay was shown in two ways: (a) The affinity constants of H50 and H117 were similar for the hK2 detected in serum and for the recombinant hK2, and (b) when comparing 19 serum samples with high concentrations of hK2, the replacement of the hK2 assay tracer MAb H117 with MAbs 3C1, 4H5, or 5F11 correlated closely with the original hK2 assay method (r = 1.00). Replacement of MAb H117 with MAb F5 resulted in equally good correlation (r = 0.98) but with a slope of only 0.36,
presumably due to the ~100-fold lower affinity of this MAb for the recombinant hK2 as compared with PSA.

Low concentrations of hK2 as compared with those of PSA were measured by the developed hK2 assay in 334 serum samples. Of these, 57% had hK2 concentrations below the detection limit of the assay. The frequency of samples with hK2 concentrations >10% of the PSA concentration was 3%. This may seem surprising, as the measured concentrations of hK2 mRNA in the prostate have been determined to be between 10% and 50% of that of PSA mRNA [10–12]. The purified PSA from seminal plasma that was used to calibrate the PSA-T + hK2, PSA-T, and PSA-F assays contained <0.1% hK2. Consequently, this preparation was considered adequate for calibrating these assays. Similarly low concentrations of hK2 (<1%) were measured when we analyzed the commercial DELFIA PSA calibrators, which consist of dilutions of seminal plasma.

According to the gel filtration studies performed, a single peak of hK2 immunoreactivity eluted with a molecular mass (~30 kDa) corresponding to that of recombinant hK2 and free PSA purified from seminal plasma. No evidence of high-molecular-mass forms of hK2 was detected in the analysis of 10 serum samples containing measurable concentrations of hK2 immunoreactivity. On the basis of the specificity deduced from the amino acid sequence of hK2, it is conceivable that the mature form of hK2 forms complexes with protease inhibitors in serum such as AMG, PCI, and α1-antitrypsin. However, hK2-AMG, like PSA-AMG complexes, would not be expected to be immunodetectable because of the steric shielding of AMG. Complex formation between hK2 and ACT is less likely to occur than between PSA and ACT mainly because of the suggested trypsin-like substrate specificity of hK2. However, the possibility remains that the epitopes recognized by MAb H50 and H117 used here in the sandwich assay of hK2, in contrast to the situation in the PSA-ACT complex, are not readily exposed in the serpin complexes that hK2 may form. Therefore, the possible presence of hK2 serpin complexes in the circulation remains to be further investigated.

As a corollary to our finding of the generally low proportion of hK2 relative to PSA in serum samples, we expect that there would be a close correlation between assays specific for PSA and the assays determining PSA + hK2. This suggestion is supported by the results of our analysis of 334 serum samples with the assays of PSA-T (2E9/2H11) and PSA-T + hK2 (H117/H50), which showed a highly significant linear correlation (r = 1.00 with all samples, and r = 0.97 with samples in the PSA-T range 1–100 µg/L). In comparison, PSA-T (x-axis) and hK2 (y-axis) assays showed lower correlation coefficients |r| = 0.84 (slope = 0.026) with all samples and |r| = 0.48 (slope = 0.025) with samples in the PSA-T range 1–100 µg/L). Very similar results were obtained with the 19 serum samples with increased hK2 immunoreactivities. When PSA-T or PSA-ACT assays (x-axis) were compared with the hK2 assay (y-axis), the correlation coefficients were between 0.78 and 0.80, with slopes of 0.026–0.027. A better correlation was obtained with PSA-F (x-axis) and hK2 (y-axis) assays |r| = 0.94, slope = 0.275.

Knowledge of cross-reaction of hK2 in existing PSA assays may explain some of the discrepancies observed between different PSA assays. With the availability of recombinant hK2, this issue can be addressed, although final verification with hK2 purified from biological sources will be needed. Discrepancies have mainly been noted between PSA assays involving either monoclonal–monoclonal or monoclonal–polyclonal assay designs. Some of these discrepancies are undoubtedly a consequence of different assays detecting PSA-F and PSA-ACT to varying extents. This equimolarity issue has been extensively described and debated in the literature [15, 34, 35]. In addition, some differences that have been described [36, 37] cannot be exclusively attributed to the differences in equimolar recognition of free and complexed PSA. These differences have mainly been seen in the monitoring of relapses of prostate cancers treated hormonally or by radical prostatectomy. Conceivably, differences in detecting PSA, or both PSA and hK2, may explain some of these discrepancies. The ACS™ PSA assay from Ciba Corning involves a monoclonal–polyclonal assay design with MAb F5 used as a capture antibody together with a polyclonal antibody used for detection [37]. Our results on the cross-reactivity of MAb F5 with hK2 reported here and elsewhere [Eerola et al., ms. submitted for publication] suggest that this PSA assay will codetermine hK2 to some degree. The double monoclonal assay from Hybritech (Tandem–R PSA), according to our unpublished results, utilizes an antibody with no detectable cross-reactivity with hK2, thus rendering this two-site assay specific for PSA-T. The described assay for hK2 will be valuable in investigating the reported discrepancies between commercial PSA immunoassays.

Recently, production of sheep antipeptide antisera to the 41–56-peptide region of hK2 has been reported and used in a competitive immunoassay [38]; however, the detection limit of this competitive assay is not sufficient for measuring the very low concentrations in serum samples reported in the present study.

In conclusion, we have developed an indirect sandwich assay that is based on M Abs raised against purified PSA. This assay measures hK2 specifically and with a high sensitivity in biological fluids. On the basis of the assay of 334 clinically undefined serum samples, the proportion of hK2 relative to PSA is generally low. The possible clinical significance of occasionally increased concentrations of hK2 remains to be determined. The different assays with well-characterized reactivities against PSA, hK2, and PSA + hK2 will be valuable tools upon validation of the clinical significance of the analysis of these two closely related kallikreins of mainly prostatic origin. They will also be helpful in resolving reported discrepancies in comparing results from differently designed commercial PSA assays.

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