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Evaluation of a rapid qualitative prostate specific antigen assay, the One Step PSA™ test

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Abstract

Recently, highly sensitive prostate specific antigen (PSA) kits have been developed and reported to be useful for the early identification of a chemical relapse. However, if the measurement time was short and the cost low, such an assay kit should be sufficient for cancer screening when dealing with a large number of samples. The One Step PSA test uses an immunochromatographic method to qualitatively, not quantitatively, judge a positive or negative result. We confirmed the sensitivity of the kit using purified PSA. Serum specimens from 147 men with or without prostate diseases were tested using the kit. PSA concentration of each serum specimen was independently measured by a quantitative ACS-PSA2 EIA kit (Chiron, cut-off: 2.1 ng/ml).The sensitivity of this kit was determined to be 4 ng/ml. All 33 samples with a value of greater than 4 ng/ml were clearly positive. Of the 94 samples with values less than 4 ng/ml, nine were judged as positive. The remaining 85 cases were judged as completely negative. These results indicate that the sensitivity of the One Step PSA test is 100% and the specificity is 90.4%.Tests using this kit can be easily performed at outpatient clinics or elsewhere. This kit is useful for initial cancer screening, because results can be obtained within 15 min and at a cost lower than that of ordinary PSA kits.

Keywords: Prostate specific antigen; Prostate cancer; Cancer screening; Qualitative method; Immunochromatography

1. Introduction

Recently, the incidence rate of prostate cancer has increased remarkably in Japan. In 1992 it was estimated to be 15.7 per 100 000 men, and is increasing exponentially. Accordingly, it is anticipated that deaths from prostate cancer will rise from the 5399 in 1995 to 13 494 in 2015. The importance of early diagnosis through screening and early treatment has gained acceptance, although the actual clinical significance of prostate cancer screening is still inconclusive. The serum prostate specific antigen (PSA) value, transrectal ultrasonography (TRUS) and digital rectal examination (DRE) have been used for screening. The sensitivity and positive predictive value (PPV) of PSA are now the highest among these three methods [1–7].

Determinations of PSA values now play an essential role in screening and the management of prostate cancer [8–11]. Recently, an ultra-sensitive PSA kit has been developed for the measurement of the PSA value and may be useful for early detection of relapse. However, the necessity of such an ultra-sensitive PSA kit for initial diagnosis is not well established. It would seem that a qualitative method by which only

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positive or negative results are judged is sufficient for mass screening whereby large numbers of specimens must be processed as rapidly and as inexpensively as possible. Actually, such qualitative methods have become widely used in various fields [12–15]. The most characteristic merits of these qualitative kits are that they require no instrumentation, results can be obtained quickly and the cost is low.

In this communication, we would like to report the first preliminary clinical evaluation of One Step PSA test strips for rapid detection in serum specimens with abnormally high PSA values.

2. Materials and methods

2.1. Development of One Step PSA test strip

The One Step PSA test was originally developed in our laboratory. This is a qualitative strip type assay kit, approximately 5×60 mm in size, that uses an immunochromatographic method to judge a positive or negative result visually, but not to measure the PSA value. The immunochromatography employs a sandwich assay system using two mouse monoclonal antibodies (PSA140 and PSA103) which were generated against purified PSA (Scripps Laboratories, San Diego, CA) in our laboratory. PSA140 is coated at the test band zone on the membrane to capture the PSA–antibody complex. PSA103 is conjugated with colloidal gold and contained in the absorbent area, so that the immunoreaction can finally be visualized at the test band zone. Mouse monoclonal antibodies used here can recognize both the free and complex type of PSA (total PSA). In the absence of PSA, no line will appear in the test zone. When PSA exists in samples, PSA bound with PSA103 antibody-dye conjugate migrates up from the absorbent area at the end of the strip and is then captured by solid-phase by another antibody PSA140. Finally, sandwiched PSA molecules fixed on the test band line can be visualized. Positive reaction forms a pink-rose band. On the control band area, unbound dye-conjugate is captured by anti-mouse antibody immobilized there to form a pink-rose color band regardless of the test sample composition. The clearly visible control band serves as an indicator of the assays validity. The intensity of this visualized band is equivalent to that of PSA 4 ng/ml. The sensitivity of the One Step PSA test kit was determined using purified PSA (Eiken Chemical Co., Tokyo, Japan) which was serially diluted from 10 to 1 ng/ml. When 120 μl of serum specimen is dropped onto the end of the strip, the liquid migrates upward along the membrane through capillary action. If the test band is equal to or darker than the control band after 15 min, the result indicates that the PSA level is at or above the cut-off point. If only the control band appears, it means that the PSA level of the sample is below the cut-off point. If the control band does not appear, the test result would be considered to be invalid. The results were confirmed by duplication.

2.2. Patients and sera samples

One hundred and forty-seven samples, including 37 male patients with prostate cancer (PC), (among archival frozen sera that had been stored at -80°C) who were examined for suspicion of benign prostate hyperplasia (BPH) or PC at our hospital between March, 1991 and May, 1999 were used. The diagnoses of cancer were pathologically confirmed using biopsy and/or surgical specimens. For comparative purposes, PSA values of the same 147 serum specimens were also determined quantitatively by the ACS-PSA2 EIA kit (Chiron, East Walpole, MA). The data from this kit was shown to significantly correlate ($R = 0.960$) with those from the Tandem-R PSA kit which is regarded as a synonym of equimolar type kits [16].

3. Results

We judged the results of the test as negative (−), positive (+) and strongly positive (+ +). If the test band did not appear within 15 min of application of the sample, it was judged as (−). When the test band was equal to or darker than the control band, the result was judged as (+). A markedly darker test band was designated as (+ +). A control band was used to validate the test results of all samples.

3.1. Sensitivity of the One Step PSA test

When a purified PSA standard was used for the assay, the test band did not appear at concentrations of 0 or 1 ng/ml, but did appear at 2 ng/ml or more. The

degree of coloration of the test band was equal to that of the control band at 4 ng/ml. Sensitivity of this One Step PSA test was finally determined to be 4 ng/ml (Fig. 1).

3.2. Evaluation of specimens by the One Step PSA test

Among the 147 specimens, 85 samples were (−), 42 were (+) and 20 were (+ +). Of the 85 samples that were (−), all 36 samples shown to have PSA concentrations less than 1 ng/ml by the ACS-PSA2 kit showed a completely negative result. However, one sample with a concentration of 1.9 ng/ml was estimated as positive. Between values of 2–3 ng/ml, seven samples were judged as (−) and six were judged as (+). Of samples with values between 3–4 ng/ml, three were judged as (−) and two were (+). This means that a total of nine samples (6.1%) were misjudged. In 53 samples with a value more than 4 ng/ml, all were correctly classified as (+) or (+ +) (Fig. 2, Table). These results indicate that the sensitivity of One Step PSA test is 100% and the specificity is 90.4%.

To provide further information, 34 of the 62 positive cases were diagnosed as having prostate cancer by prostate needle biopsy.

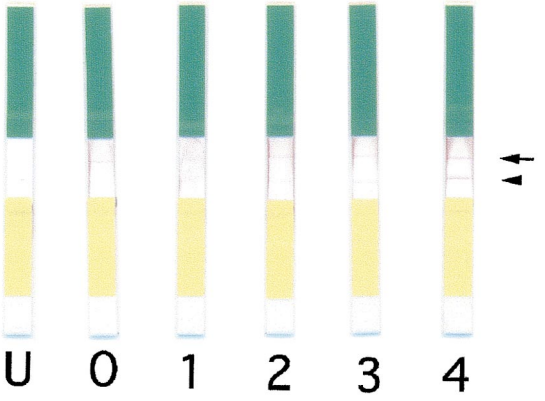

Fig. 1. Sensitivity of the One Step PSA test using purified PSA as a standard. If the test band is equal to or darker than the control band, the result indicates that the PSA level is at or above the cut-off point of 4 ng/ml. The arrow and the arrow head indicate the control band and the test band, respectively. The figures mean the purified PSA concentration (ng/ml) U. unused, The analytical sensitivity of the kit was concluded to be 4 ng/ml.

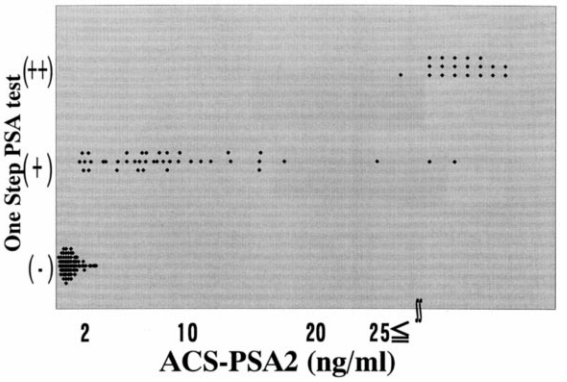

Fig. 2. Comparison of results measured by the One Step PSA test and ACS-PSA2 kit.

4. Discussion

The incidence rate of prostate cancer has increased remarkably. Since radical therapeutic methods are limited even now, early detection and treatment of prostate cancer is necessary. Screening for prostate cancer may be useful for early initiation of treatment, although its value is still inconclusive [1–2]. In Japan, various systems are used in screening for early detection of prostate cancer, with some facilities using all three available modalities (DRE, TRUS and PSA) and some using only one or two. Imai et al. reported that among the three modalities serum PSA measurements had the highest sensitivity (80.4%) and the greatest PPV (45.1%) [3]. In the US, Catalona et al. [4] conducted a prospective clinical trial of 6630 male volunteers aged 50 years or older who underwent PSA determination (cut-off point: 4 ng/ml) or DRE. They reported that the cancer detection rate was 3.2% for DRE, 4.6% for PSA and 5.8% for the two methods combined, and that the PPV was 32% for PSA and 21% for DRE [4]. In Europe, Reitbergen et al. [5] evaluated PSA, DRE and TRUS for prostate cancer screening and reported that PSA is the strongest predictor of early prostate cancers. These results indicate that the measurement of the PSA value has been established as a very effective method to detect prostate cancer.

Measurement of PSA values by a quantitative method requires special equipment and costs approximately 3000–4000 Japanese yen (25–35 $US) per assay. On the other hand, the One Step PSA test can

be priced much lower, because it utilizes a simple method. For mass screening of abnormal PSA values, a simpler, more rapid and cost effective method would be preferable. The One Step PSA test needs no special equipment, because this is a qualitative detection method that utilizes the principle of immunochromatography. Recently, the importance of equimolarity has been emphasized for PSA measurement. The monoclonal antibodies used here can recognize both free and complex types of PSA (total PSA). Therefore, this kit may be eligible to equally capture both free and complex PSA molecules in sera, though it is very difficult to precisely demonstrate the equimolarity of qualitative kit the One Step PSA test. In this study, we found that the test band became positive 15 min after applying sera if the PSA concentration in the sample was above 4 ng/ml (Fig. 1). Of 53 serum specimens with a PSA concentration greater than 4 ng/ml, all were judged as positive by the One Step PSA test. In those specimens with positive test results, the judgment was consistently easy. This high sensitivity should be noted here. Unfortunately, nine samples with values less than 4 ng/ml showed false positive results (Fig. 2, Table 1). This incorrect interpretation may have been caused by the mental pressure that 'no cancer must be missed' although, to our regret, the true reason is not known.

Recently, to improve the diagnosis rate of cancer and to decide upon the indications for prostate biopsy, some investigators have suggested the utility of PSA density (PSAD) [17], PSA velocity (PSAV) [18], age-specific PSA reference [19]and free/total PSA [20] etc. Because the One Step PSA is a qualitative detection kit, these cannot be calculated. However, a merit of this method is that it requires no special equipment except for a centrifuge to separate serum from blood. This kit is useful for initial screening of prostate cancer as an abnormal PSA value can be detected handily and rapidly if serum separation is performed. Currently, we are developing another type of kit that uses whole blood, thus enabling testing immediately after blood is drawn. We will test more samples in the near future to further examine the suitability of such kits for mass screening.

Table 1
All of the 53 samples with value shown to be grater than 4 ng/ml by the ACS-PSA2 kit were clearly positive on the test strips. Of the 94 samples with values less than 4 ng/ml, nine were judged as positive and 85 as negative

		One Step PSA test			
		−	+	++	Total
ACS-PSA2 (ng/ml)					
0 ≦	<1	36	0	0	36
1 ≦	<2	39	1	0.	40
2 ≦	<3	7	6	0	13
3 ≦	<4	3	2	0	5
4 ≦	<5	0	3	0	3
5 ≦	<10	0	18	0	18
10 ≦	<20	0	9	0	9
20 ≦		0	3	20	23
Total		85	42	20	147

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