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ORIGINAL RESEARCH

A Comparative Study of Prostate Specific Antigen (PSA) Point-of-Care-Testing (POCT) Techniques

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Abstract

Background: Prostate Specific Antigen (PSA) testing is widely used to diagnose and monitor clinical progress in patients with prostate cancer. The availability of various new Point-of-Care-Testing (POCT) equipment for PSA demands that the performance characteristics of these equipment be assessed before introducing them into clinical use to ensure accuracy and reliability.
Objectives: To compare the i-CHROMA® automated immunofluorescence serum total PSA assay with the Accubind® Enzyme Linked Immuno-Sorbent Assay (ELISA) as POCT among patients with suspected prostate cancer.
Methods: The study was conducted at the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Nigeria. Blood samples drawn from 20 consecutively selected patients were analysed for PSA using the i-CHROMA® immunofluorescence kit once and with the Accubind® ELISA protocol twice.
Results: The mean PSA using Accubind® ELISA was 12.9ng/ml, while it was 14.5ng/ml with the i-CHROMA® immunofluorescence assay. The bias between the two methods was 1.6ng/ml. The two methods had a good correlation: Passing Bablok regression equation was y = 1.264604x – 0.0300469, and the Spearman correlation coefficient between the two measurements was high (r = 0.956; Confidence Interval 0.889 - 0.983; p<0.0001). Agreement between the two methods was statistically satisfactory as the mean values of the samples fell within the 95% Confidence Interval of the differences on the Bland Altman plot.
Conclusion: The i-CHROMA® POCT assay showed good correlation and agreement with the well-known ELISA method. Therefore, the method is recommended for use in monitoring PSA in patients with prostatic cancer.

Keywords: Benign Prostatic Hyperplasia, i-CHROMA®, ELISA, Point of care testing, Prostate cancer, Prostate Specific Antigen.

Introduction

Analytical methods may be compared to assess the performance of laboratory techniques relative to another or to validate a new technique using an established and previously validated method as a reference. Accubind® enzyme-linked immunosorbent assay (ELISA) (Monobind Inc, 100 North Pointe Drive, Lake Forest, CA 92630, USA) is established in the assay of prostate-specific antigen (PSA). In contrast, the i-CHROMA® automated immunofluorescence assay (Boditech Med Incorporated, Gang-won-

do 200-883 Republic of Korea) is a relatively uncommonly used method.

The i-CHROMA® automated machine was introduced into the point of care testing (POCT) laboratory at the Department of Chemical Pathology, Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC). The Accubind® ELISA for PSA is the established PSA assay method used in the laboratory. It is known for its reliability and accuracy, consistent with the clinical state of patients with prostatic illness. However, prolonged turnaround time due to the necessity to pool samples before the assay and the time span of the actual procedure are major challenges with the ELISA method.

PSA is a protease belonging to the human kallikrein family of proteases. [1] It is also referred to as human kallikrein 3. [2] It is a 28.4 kilodalton protein consisting of a single-chain glycoprotein of 237 amino acid residues, four carbohydrate side chains, and multiple disulfide bonds. PSA is produced by the epithelial cells of the prostate gland and serves to liquefy the seminal coagulum, thus enhancing sperm motility. PSA exists in two primary forms in the serum: free and complexed. The majority exists as complexed PSA (cPSA) bound to either alpha2 - macroglobulin (AMG) or alpha1-antichymotrypsin (ACT). A lesser quantity exists as free PSA (fPSA). Most immunoassays measure both free and ACT-complexed PSA as the total PSA, although the AMG-complexed PSA is difficult to measure as the AMG molecule masks the binding epitope. [1,2] The insignificant amount of this AMG complex renders it unlikely to be of physiologic relevance. Subsequent references to PSA in this article refer to the total PSA.

Prostate cancer is the most common cancer among Nigerian males. [3] PSA is a valuable biomarker in prostatic cancer screening, diagnosis, and monitoring. However, it is a non-specific marker of prostate cancer, with considerable overlap between benign prostatic hyperplasia (BPH) and prostatic cancer, particularly at PSA levels of 4 – 10ng/ml. [1] This non-specificity limits PSA as a screening/diagnostic tool for prostate cancer. Its most widespread application, currently, is, therefore, in monitoring the disease and its treatment. Some of the measures taken to increase the sensitivity and specificity of PSA for prostate cancer detection include digital rectal examination, age-adjusted reference intervals, PSA velocity, PSA density, and fPSA index. [4]

Different methods of PSA assays have been reported to produce incongruent results, possibly owing to the differing antibodies employed or the assay technique. The first United States Food and Drug Agency (FDA) approved test for detecting and monitoring PSA was the Hybritech® Tandem-R immunoradiometric assay utilising purified human PSA. The World Health Organization (WHO), in an effort to improve assay standardisation, developed the PSA international standard (code: 90/670)using recombinant DNA techniques in 1999. [5] Commercially available assay methods are calibrated to either of the techniques mentioned above. The WHO-calibrated techniques yield about 20% lower PSA values than the Hybritech® calibrated technique. [6] Many commercial assay kits, including the POCT techniques, often do not refer to either of these primary calibration standards in their procedure manuals; this could hinder the interpretation of the results, especially when different assays may have been used to monitor the PSA in the same patient. POCT PSA assay techniques have also been observed to exhibit varying degrees of agreement with traditional laboratory methods. [7–12] Therefore, this study aimed to compare the i-CHROMA® assay technique with the Accubind® ELISA assay technique by assessing for analytical bias and other parameters before introducing the former into clinical laboratory practice. This may mitigate the current concerns about the agreement between the

ELISA method and the new commercially available techniques.

Methods

Following ethical approval by the Health Research Ethics Committee of the OAUTHC, Ile-Ife (ERC/2019/11/07), venous whole blood samples were drawn consecutively from 20 patients with prostate cancer selected via quota sampling at the POCT laboratory of the hospital between January and March 2020. Serum was separated from the blood samples immediately after clot retraction. PSA was assayed on the i-CHROMA® machine immediately after the serum was separated; the remaining serum was kept in a -20°c freezer for the ELISA analysis within two weeks of collection. ELISA analysis was carried out twice on each sample; the two batches were run on separate days.

The Accubind® PSA ELISA is an immunoenzymometric sandwich assay using high affinity and specific antibodies. Samples (25µl) were added to a streptavidin-coated microwell plate with immobilised "capture" antibodies specific for PSA. Biotinylated detection antibodies recognising a different epitope on the PSA molecule and linked with an enzyme were added. A substrate for the enzyme linked to the detection antibody was added. The addition of acid stopped the reaction. The colour developed by the reaction was proportional to the antigen concentration in the sample and read as absorbance with the microplate reader. The PSA ELISA had a limit of detection of 0.04ng/ml, and the assay was linear up to 50ng/ml. All concentrations initially exceeding this value were diluted sufficiently, the assay was repeated, and the final results were obtained by multiplying with a factor corresponding to the original dilution factor.

The manufacturer compared the Accubind® PSA ELISA (x) with an unnamed reference method (y) to give the linear regression equation: y = 0.9226x + 0.3500, with correlation coefficient r = 0.95, mean PSA of the technique was 5.04ng/ml, while the mean for the reference method was 4.92.
Intra-assay precision on three levels of PSA concentration (ng/ml) - 24 runs each: Level 1: mean: 0.90, coefficient of variation (cv%): 4.8; Level 2: mean: 3.99, cv: 5.8; Level 3: mean: 18.25, cv:5.4.
Inter-assay precision on three levels of PSA concentration (ng/ml) - 20 runs each: Level 1: mean: 0.92, cv: 5.5; Level 2: mean: 3.58, cv: 5.5; Level 3: mean: 18.39, cv: 4.4.

The i-CHROMA® automated PSA assay is a fluorescence sandwich immunoassay. A serum sample (75µl) was added to a detection buffer containing a biotinylated antibody to PSA conjugated to a fluorescent compound. The mixture was incubated at room temperature for 15 minutes. The mixture was then added to a test cartridge containing streptavidin bound antibody to PSA on a nitrocellulose matrix. The cartridge was inserted into the i-CHROMA® machine, where the fluorescence was read and displayed as the concentration of PSA in the sample.
The working range of the assay was: 0.1 - 100ng/mL.
Validation studies by the manufacturer included estimating PSA concentration in 100 serum samples with i-CHROMA® PSA and an automated Abbott AxSYM system (Abbott Laboratories, USA). The Abbott AxSYM PSA assay was calibrated using the WHO standard 90/670 as a reference. [13]
Linear regression and coefficient of correlation between the two tests yielded the regression equation y = 1.0283x – 0.0973 and r = 0.992, respectively. The intra-assay precision was calculated by one evaluator, who tested three different concentrations of control material ten

times, each with three different lots of i-CHROMA® PSA.
Control PSA (ng/mL) - Level 1: 0.5: mean: 0.50, cv (%): 7.0; Level 2: 4.0: mean: 4.06, cv: 6.5; Level 3: 25.0: mean: 25.44, cv: 5.3. The inter-assay precision was confirmed by three different evaluators with three different lots, testing three times each of three different concentrations of PSA control material. Level 1: 0.5: mean: 0.50, cv: 6.8; Level 2: 4.0: mean: 4.1, cv: 6.0; Level 3: 25.0: mean 25.9, cv: 4.9.

Statistical analysis
Data analysis was carried out using MedCalc statistical software version 19.2.6. (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2020). Frequency distributions were assessed for normality using the Shapiro-Wilk test, and a non-parametric assessment of differences between groups' median values was performed using the Wilcoxon signed-rank test. The F statistic was determined to assess the variance ratio between the groups.

A linear relationship between the methods was assessed using the Passing and Bablok regression curve. Outlier values were excluded from the differences between the assays to establish normality; agreement between the methods was subsequently examined with the Bland-Altman difference curve. Statistical significance was set at p less than 0.05 at a 95% confidence interval. For acceptability of difference, limits of agreement were set *apriori* at 95% confidence limits of the difference plot.

Results

Analysis of results for all 20 samples (Table I)
The mean PSA (ng/ml) for Accubind® ELISA was 12.9, while the mean PSA for the i-CHROMA® immunofluorescence assay was 14.5. The estimated bias between the two methods was 1.6ng/ml. Frequency distribution for ELISA PSA and i-CHROMA® PSA did not satisfy the Shapiro-Wilk test for normality: W = 0.4937, p < 0.0001, W = 0.5565, p < 0.0001, respectively. Wilcoxon signed-rank test also revealed a zero median for groups' differences: Z = -1.850, p = 0.064.
Comparison of variances for the two groups yielded an F statistic of: 1.0463, p = 0.927.

The Passing and Bablok regression curve (Figure 1) showed a good correlation between the two methods, yielding the regression equation: y = 1.264604x – 0.0300469. From the above regression equation, at medical decision limits of 4.0 and 10.0ng/ml, the i-CHROMA® assay would yield PSA results of 5.0 and 12.6ng/ml. The slope of the regression line was 1.27. The confidence interval was 1.03 - 1.47; these suggest a proportional systematic error of 27%. The intercept, an estimate of the constant proportional error between the methods, was -0.03 with a confidence interval: of -0.66 - 0.61. The confidence interval of the intercept included 0; hence was regarded as statistically insignificant.

The Spearman correlation coefficient r was 0.956, with a confidence interval: of 0.889 - 0.983 (p<0.0001).
Outlier values were excluded to establish the normality of differences, and the Bland-Altman difference plot was carried out with the remaining result pairs. The samples' mean values fell within the 95% confidence interval on the difference plot (Figure 2).

Table I: Serum Prostatic Specific Antigen levels

Serial number	*ELISA PSA (1) ng/ml*	*ELISA PSA (2) ng/ml*	*Average ELISA PSA ng/ml*	*i-CHROMA® PSA ng/ml*
1	4.9	5.6	5.3	6.8
2	7.7	7.1	7.4	5.5.
3	7.9	7.4	7.7	3.4
4	8.4	7.1	7.8	12.5
5	45.8	43.0	44.4	56.1
6	5.5	4.3	4.9	7.1
7	0.8	0.6	0.7	0.8
8	7.7	7.7	7.7	10.1
9	1.2	0.4	0.8	1.0
10	12.0	11.9	12.0	10.8
11	0.3	0.2	0.3	0.1
12	12.1	9.2	10.7	12.7
13	6.0	7.2	6.6	9.5
14	0.4	0.5	0.5	0.7
15	0.6	0.8	0.7	1.8
16	116.7	107.7	112.2	110.0
17	22.8	17.1	20.0	32.5
18	3.2	3.1	3.2	4.3
19	2.5	2.3	2.2	2.3
20	2.3	2.3	2.3	2.4

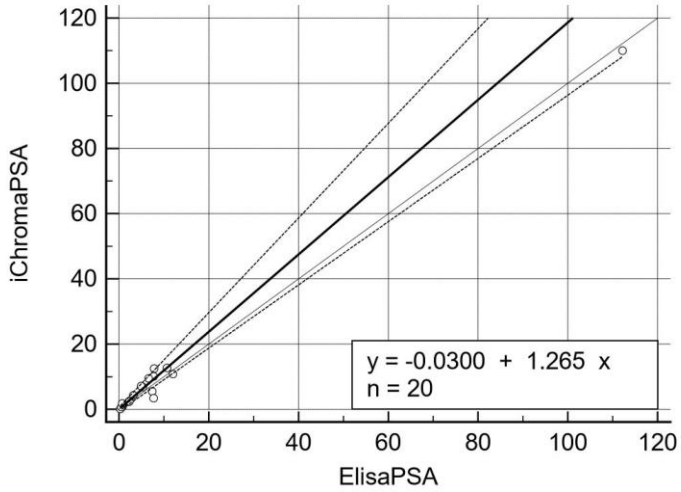

Figure 1: Passing Bablok regression curve.
The x-axis represents the PSA values (ng/ml) for the Accubind® ELISA method.
The y-axis represents the i-CHROMA® assay PSA levels (ng/ml).

Discussion

This study revealed a good correlation between the PSA values obtained with the i-CHROMA® PSA test and the traditional Accubind® ELISA method (r = 0.956). A study comparing the i-CHROMA® PSA with the automated Abbot Architect PSA assay showed a good correlation with $r^2 = 0.9841$. [7] Another study comparing the i-CHROMA® PSA with Cobas® e602 automated PSA assay revealed $r^2 = 0.9664$. [12] PSA watch, a POCT PSA assay method, also yielded an $r^2 = 0.88$ compared to the established automated third-generation immunometric assay employed in the laboratory. [8] The POCT assay Concile® Ω100 also gave $r^2 = 0.72$ and $r^2 = 0.63$ when compared with two standard laboratory assay methods. [9]

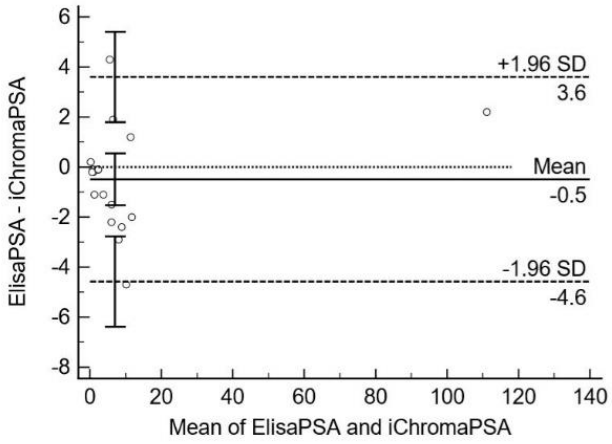

Figure 2: Bland Altman plot.
X-axis: mean of PSA value for the ELISA and i-CHROMA® assays (ng/ml).
Y-axis: the difference between PSA values of the ELISA and i-CHROMA® assays (ng/ml).
Horizontal dotted lines represent the mean and 1.96 standard deviations (2-tailed) of the differences.
Vertical boundary lines indicate 95% Confidence Interval limits of the parameters.

Agreement between the two methods tested in the present study met the criteria for acceptability, particularly at relatively low PSA levels (≤10ng/ml), as shown on the difference plot drawn after two outlier values with high PSA were removed. A proportional systematic error estimated at 27% from the linear regression equation suggests the presence of bias between the assays at higher PSA levels, with the i-CHROMA® returning higher values compared to the traditional ELISA method. This study estimated a positive bias of 1.6ng/ml between the two methods, with the i-CHROMA® PSA returning higher average PSA values. This may be due to the inclusion of high outlier PSA values in the dataset when estimating the bias parameter.

This positive bias was also found in a comparison of assay techniques on PSA quality control material between the i-CHROMA® and two quality assessment schemes (United Kingdom National External Quality Assessment Scheme: UKNEQAS and Randox International Quality Assessment Scheme: RIQAS) employing 9 and 12 assay methods respectively. The bias between the

i-CHROMA® and UKNEQAS assays ranged between +0.53 ng/ml and + 2.65 ng/ml with an average of +1.46 ng/ml, while bias between the i-CHROMA® and the RIQAS methods ranged from -2.99 ng/ml and +6.8 ng/ml with an average of +0.88 ng/ml. The quality control material used in this study was in the lower range PSA concentration (2.48 - 4.13ng/ml). [14]

PSA testing is still among the commonest biomarker test requests in the clinical chemistry laboratory. The point-of-care assays reduce pressure on the main laboratory. They often have lower turnaround times, relieving patient anxiety and enabling clinicians to make decisions regarding patient management quickly. Accurate and precise POCT PSA assays would significantly increase service efficiency in large centres with high-volume test requests. The i-CHROMA® PSA gives results about 40 minutes from sample collection per patient. The procedure manual is easy to understand, and the performance characteristics are spelt out apart from an omission of agreement studies with a reference method; this is unfortunately common with most manufacturers. The machine can be used after a relatively short period of training. The cost per unit is relatively inexpensive, and the sample volume required is small. The instrument is pre-calibrated from the factory. Hence, routine maintenance is simple. The equipment can assay up to 30 different analytes. However, owing to its small size, only a single test cartridge can be held in the cartridge chamber per time.

Conclusion

The i-CHROMA® automated immunofluorescence assay showed satisfactory correlation and agreement with the established ELISA method for PSA assay. The technique is recommended for monitoring patients with BPH or prostatic cancer, particularly those with PSA in the lower range (≤ 10ng/ml). Patients with higher PSA values should get a confirmatory result from the main laboratory pending further studies on the assay's performance. The technique should also not be used to detect cancer recurrence following radical prostatectomy due to its relatively high lower working range limit.

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