An Evaluation of the Point-of-Care Test i-CHROMA Prostate-Specific Antigen Method for Screening in the Community

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Background: This study evaluated and compared the performance of the i-CHROMA point-of-care testing (POCT) method for the quantification of prostate-specific antigen (PSA) against a traditional laboratory PSA method (Abbott Architect assay).

Materials and Method: Blood samples (venous [143] serum [143]) and finger prick (55) were collected from volunteers at a PSA screening campaign. Both venous and finger-prick samples were analyzed using the i-CHROMA PSA method and serum samples using the Abbott Architect method. Results were compared using linear regression and Red Amber Green analysis, a scoring system based on volunteer's age and PSA level. Red indicated a raised PSA, amber indicated a slightly raised PSA, and green indicated a normal PSA.

Results: The data showed that both the i-CHROMA PSA results using the venous samples ($r^2 = 0.9841$) and the finger-prick samples ($r^2 = 0.90845$) showed a good correlation when compared with the serum samples using the laboratory method. The Red Amber Green analysis showed the i-CHROMA' venous PSA method identified 15 reds, 13 ambers, and 115 greens compared with 9 reds, 8 ambers, and 126 greens identified by Abbot Architect method. The i-CHROMA finger-prick PSA method identified 3 reds, 3 ambers, and 49 greens compared with 3 reds, 1 ambers, and 51 greens identified by Abbot Architect method.

Conclusions: The i-CHROMA POCT PSA method showed good correlation with the Abbott Architect PSA method. Higher numbers of raised and abnormal PSA were identified by the i-CHROMA POCT PSA method due to the positive bias observed. The i-CHROMA POCT PSA method is a reliable method for total PSA within its limitations.

Key Words: point-of-care, i-CHROMA, POCT, PSA, PSA assay, prostate cancer

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Highlights:

- Point-of-care testing (POCT) of PSA for screening offers considerable benefits.
- The i-CHROMA PSA method is a novel fluorescence-based immunoassay.
- The i-CHROMA PSA method provides quantitative analysis of prostate-specific antigen (PSA).
- The i-CHROMA PSA method shows good correlation with the Abbot Architect PSA method.
- The i-CHROMA POCT provides a reliable measurement of total PSA in whole blood and finger-prick samples.

The quantification of prostate-specific antigen (PSA) in the blood is the most important biomarker in the diagnosis, monitoring, and management of prostate cancer.[1] Currently, PSA is measured using total PSA testing of serum, which is normally obtained from patients at a community location such as a hospital or a general practitioner surgery. The sample is then transported to the laboratory where the level of PSA in the blood is analyzed, and the results will be relayed to patients within 48 hours. Furthermore, a follow-up appointment with the patient is required to discuss the results.

More recently, PSA point-of-care testing (POCT) has come into play, which allows for same day results, and therefore eliminates the need for any further visits to the clinic. Furthermore, this would facilitate timely discussion, further investigations, or onward referral, if necessary, to urology.[2] A study carried out by Wilkinson et al[3] revealed that patients felt the ability to have an immediate discussion about the result and future management was advantageous. In addition, Jadhav et al[4] demonstrated that waiting for prostate cancer test results was an extremely stressful time for patients. Point-of-care testing would minimize this stressful waiting period, which may contribute to optimizing patient care.

At present, there are several POCT PSA assay systems available on the market. The NHS Centre for Evidence-Based Purchasing recently evaluated 3 quantitative methods, the Qualigen FastPack, VEDALAB PSA-CHECK-1, and Medi*watch* PSA*watch* and Bioscan systems, and 1 semiquantitative method, SureScreen PSA test.[5] These methods did not compare favorably with assays currently used routinely in the laboratory, and all of the systems demonstrated poor precision, with the exception of the FastPack and the VEDALAB PSA-CHECK-1. Furthermore, none of these POCT PSA tests satisfied the acceptable performance criteria for use when testing asymptomatic men as part of the NHS Prostate Cancer Risk Management Programme.[6] Therefore, in view of the poor performance of the POCT PSA assays and the incomparability between laboratory and POCT PSA methods, the report concluded that it was doubtful that the introduction of a POCT PSA testing service could offer any significant improvement in the diagnosis and monitoring of prostate cancer.

More recently, there have been developments in quantitative POCT PSA methods such as the FREND PSA Plus,[7] the OPKO 4Kscore Test,[8] and the i-CHROMA PSA system.[9] One study showed that the quantitative results obtained with the OPKO 4Kscore test using a finger stick of whole blood correlated extremely well with laboratory assays over the clinically relevant range of PSA, including at very low PSA concentrations.[10] Another article showed that the PSA*watch* and Bioscan systems demonstrated good correlation ($r^2 = 0.88$) with laboratory results.[11] Despite the availability of a range of POCT methods for the quantitation of PSA, there have been very few publications, and there

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remains very little information in the public domain with regard to their performance and clinical utility.

To address the lack of evidence base in this area, we set out to explore the possibility of incorporating the i-CHROMA PSA method into the screening of volunteers attending prostate cancer screening campaign. The aims of this study were to evaluate and compare the performance of the i-CHROMA POCT method for the quantification of PSA against the traditional Abbott Architect laboratory PSA method as a screening method.

MATERIALS AND METHODS

i-CHROMA Materials

i-CHROMA uses a sandwich immunodetection principle, such that the fluorescence-labeled detector antibody binds to the target protein in the sample. The sample is then applied onto a test strip, and the fluorescence-labeled antigen-antibody complex is captured by a second antibody embedded in the solid phase. The signal intensity of fluorescence of the captured complex is directly proportional to the amount of PSA present and thus allows for the calculation of sample PSA concentration, and the result is displayed on the reader as nanograms per milliliter. A fluorescence-labeled control protein is included in the reaction, and the intensity of the control line is measured as a quality check.

The assay was performed following the manufacturer's instructions. In brief, 35 µL of whole blood (capillary or venous) or 75 µL of serum were mixed with a premeasured volume of detection buffer containing fluorescence-labeled anti-PSA monoclonal antibodies and antirabbit immunoglobulin G, then 75 µL of the mixture was then loaded into the sample well of the test strip and the cartridge was incubated at room temperature for 15 minutes (Fig. 1). The intensity of the captured fluorescence-labeled PSA-antibody complexes was measured using the supplied meter, and the concentration of PSA in the sample was calculated.

Blood Samples

One hundred forty-three volunteers attending a PSA screening event had blood samples taken after giving informed consent. The following blood samples were then taken: 143 volunteers had 1 blood sample collected in a serum separator tube (sent to the laboratory at Worthing Hospital to carry out PSA estimation using the Abbott Architect PSA method) and another blood sample collected in a lithium heparin tube (Sent to JB Consulting Research Laboratory to carry out PSA estimation using the i-CHROMA PSA method). In addition, the first 55 volunteers had their capillary blood test (finger-prick) PSA estimated using the i-CHROMA PSA method at the screening centre.

METHODS

Evaluation of Correlation

Correlation analysis and Bland-Altman plots were carried out between PSA estimations obtained from the venous whole blood samples (i-CHROMA) and estimations obtained from the serum samples from the Worthing Hospital Laboratory method (Abbott Architect). Then correlation analysis and Bland-Altman plots were carried out between PSA estimations obtained from the finger-prick samples (i-CHROMA) and estimations obtained from the serum samples from the Worthing Hospital Laboratory method (Abbott Architect).

FIGURE 1. Prostate-specific antigen test strip and detection buffer containing fluorescence-labeled anti-PSA monoclonal antibodies and antirabbit immunoglobulin G.

TABLE 1. Red Amber Green Analysis

Age Range, y	Green, ng/mL	Amber, ng/mL	Red, ng/mL
<50	<2.0	2–3	>3.0
<60	<3.0	3–4	>4.0
<70	<4.0	4–5	>5.0
>70	<5.0	5–6	>6.0

Red Amber Green Analysis

The Red Amber Green (RAG) analysis correlated patients' PSA levels with their age, such that they were given a color according to the PSA level for their age. A normal PSA level for a man's age was labeled green, a slightly abnormal PSA level was labeled amber, and an abnormal PSA level was labeled red (Table 1).

RESULTS

Evaluation of Correlation (i-CHROMA Venous Whole Blood vs Abbott Architect Serum, N = 143)

The data showed that overall, the PSA estimations on the venous whole blood samples using the i-CHROMA method showed a good correlation with the PSA estimations from the serum samples using the Abbott Architect PSA method ($r^2 = 0.9841$) (Fig. 2). The graph shows bunching of results close to the origin, reflecting the generally low PSA values recorded during the screening event.

The Bland-Altman chart (Fig. 3) shows that most data points fell within the 2 SDs, and approximately 90% of data points are above the mean (solid line), demonstrating a biased mean of difference. This means that most the i-CHROMA PSA values are higher than that of the Abbott architect laboratory PSA values, creating a positive bias.

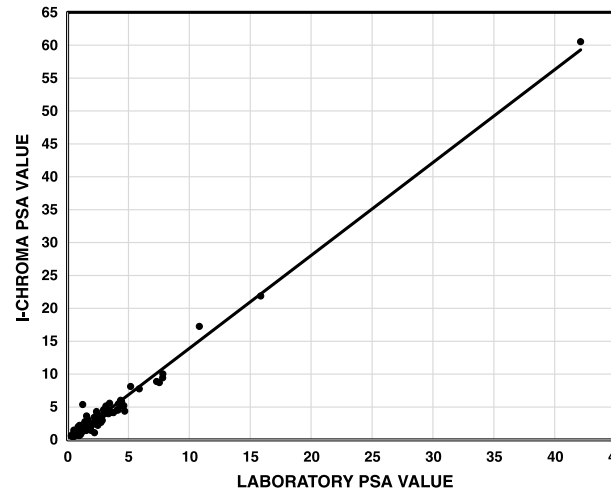

FIGURE 2. Correlation of the PSA estimations for venous whole blood samples using the i-CHROMA method and the serum samples using the Abbott Architect method for the measurement of total PSA (N = 143).

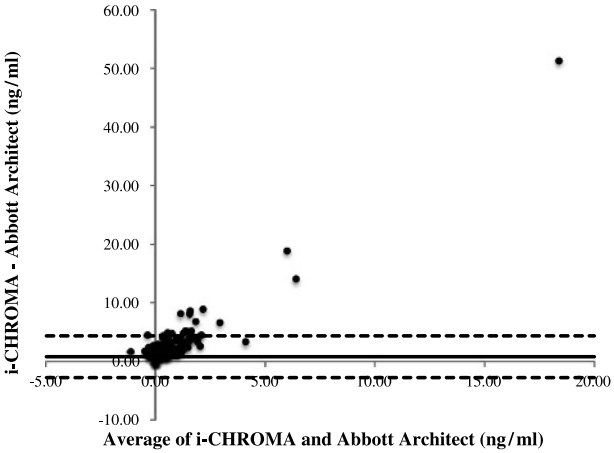

FIGURE 3. Bland-Altman difference plot comparing PSA concentrations obtained from the venous whole blood samples using the i-CHROMA method and the serum samples using the Abbott Architect method (N = 143). The x-axis represents the mean value of the duplicate results with the comparison method. The y-axis shows the deviation between the i-CHROMA (venous samples) PSA method and the Abbott Architect (serum samples) PSA method. The solid line represents the mean difference in measured PSA concentrations between the methods, and the dashed lines represent SD of 1.96.

Evaluation of Correlation (i-CHROMA Finger Prick vs Abbott Architect Serum, n = 55)

The data showed that overall, the PSA estimations on the finger-prick samples using the i-CHROMA method showed a good correlation with the PSA estimations from the serum samples using the Abbott Architect PSA method ($r^2 = 0.9084$) (Fig. 4).

The Bland-Altman chart (Fig. 5) shows that 90% of the data points fell within the 2 SDs, and data points are both above and below the mean (solid line), showing a very good means of difference.

Evaluation of RAG Analysis

Red Amber Green Analysis of i-CHROMA Venous Whole Blood Using the Abbott Architect as Standard, Serum, N = 143

The i-CHROMA method identified 15 individuals with abnormal PSA's (red) compared with 9 individuals identified by the Abbot Architect method. The i-CHROMA method identified 13 individuals with slightly abnormal PSA's (amber) compared with 8 individuals by the Abbott Architect method. The i-CHROMA method identified 115 individuals with normal PSA's (green) compared with 126 individuals by the Abbott Architect method.

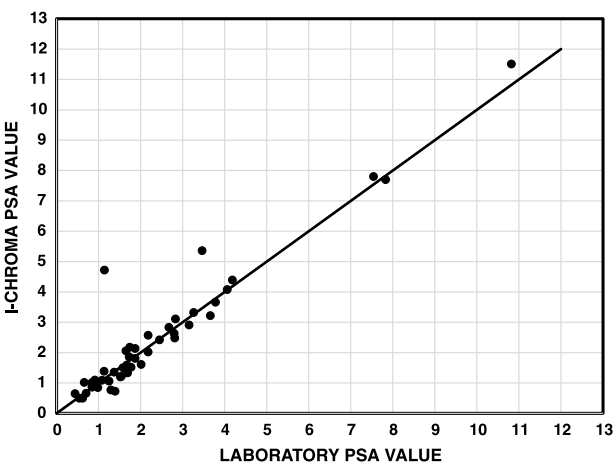

FIGURE 4. Correlation of the i-CHROMA finger-prick PSA assay and the Abbott Architect assay for the measurement of total PSA (n = 55).

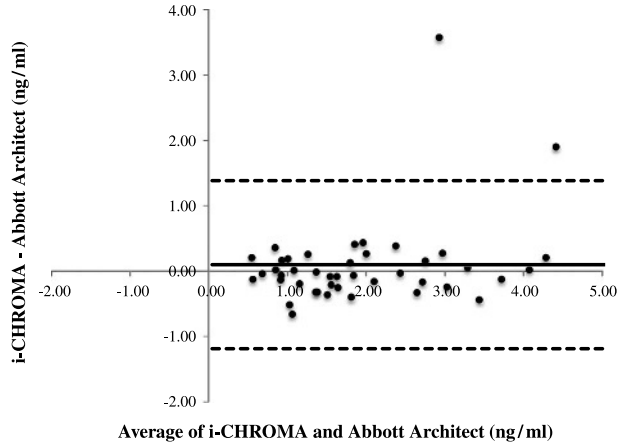

FIGURE 5. Bland-Altman difference plot comparing PSA concentrations obtained from the i-CHROMA (finger-prick) method and the Abbott Architect (serum) method (n = 55). The x-axis represents the mean value of the duplicate results with the comparison method. The y-axis shows the deviation between the i-CHROMA (finger-prick) PSA method and the Abbott Architect (serum) PSA assay. The solid line represents the mean difference in measured PSA concentrations between the methods, and the dashed lines represent SD of 1.96.

Overall, the i-CHROMA method identified 6 individuals with raised PSA levels and 5 individuals with slightly raised PSA levels more than Abbott Architect method (Table 2).

Red Amber Green Analysis of i-CHROMA Finger Prick Using the Abbott Architect as Standard, Serum, n = 55

The i-CHROMA method identified 3 abnormal PSA's (red), which were also the 3 identified by the Abbot Architect method. The i-CHROMA method identified 3 slightly abnormal PSA's (amber) compared with 1 by the Abbott Architect method. The i-CHROMA method identified 49 normal PSA's (green) compared with 51 by the Abbott Architect method.

Overall, the i-CHROMA method identified 2 individuals with slightly raised PSA levels more than Abbott Architect method (Table 3).

DISCUSSION

In this study, we demonstrated that the i-CHROMA PSA method correlated well with the Abbott Architect PSA laboratory method. Both the i-CHROMA PSA venous samples and the finger-prick samples demonstrated a good correlation with the serum samples of the Abbott Architect method, with values of r^2 value of 0.9841 and 0.90845 respectively. From the Bland-Altman plots although more than 90% of all venous sample data points were within the 2 SDs proving that both the i-CHROMA and Abbott Architect methods of PSA testing yield similar results, most the data points

TABLE 2. Red Amber Green Analysis of i-CHROMA Venous Whole Blood Using the Abbott Architect as Standard, Serum, N = 143

	Green	Amber	Red	Total
i-CHROMA PSA method (whole blood)	115	13	15	143
Abbott Architect PSA method (serum)	126	8	9	143

TABLE 3. Red Amber Green Analysis of i-CHROMA Finger Prick Using the Abbott Architect as Standard, Serum, n = 55

	Green	Amber	Red	Total
i-CHROMA PSA method (finger prick)	49	3	3	55
Abbott Architect PSA method (serum)	51	1	3	55

were above that of the mean, which demonstrates that on a whole, the i-CHROMA (venous) PSA values are higher than those seen in the serum samples using the Abbott Architect method, and this positive bias is more pronounced at PSA values greater than 10.0 ng/mL. These observations are comparable and consistent with correlations observed between i-CHROMA PSA method and other laboratory PSA methods enrolled in a PSA United Kingdom External Quality Assessment Scheme (UKNEQAS): Abbott Architect (r = 0.98), Beckman Access–Hybritech standard (r = 0.99), Beckman Access–WHO Standard (r = 0.99), Ortho Vitros (r^2 = 0.99), Roche COBAS EIA (r^2 = 0.99), Roche E-170 (r^2 = 0.99), Roche ELECSYS (r^2 = 0.99), SMS Immulite 2000 3rd Generation (r^2 = 0.98), and SMS Advia Centaur (r^2 = 0.99).[10] The positive bias demonstrated in this study was also comparable with the positive bias seen between the i-CHROMA PSA method and other laboratory PSA methods enrolled in a PSA UKNEQAS, which ranged between +0.29 and +1.46 ng/mL.[12] The correlations with the finger-prick samples were very good (r^2 = 0.9084) albeit in a much smaller sample size of 55, the bias was observed was very small, and a larger sample using the finger-prick sample would be required in the future to truly estimate the potential of this method of sampling in the community.

The RAG analysis evaluated the accuracy of the i-CHROMA in correlating a man's PSA levels with their age. The main observation is that the i-CHROMA PSA method detected the same individuals with raised and slightly raised PSA's that the Abbott Architect PSA method detected. However, overall, using the venous whole blood samples, the i-CHROMA method identified 6 individuals with raised PSA levels and 5 individuals with slightly raised PSA levels more than Abbott Architect method, and using the finger-prick samples, the i-CHROMA method identified 2 individuals with slightly raised PSA levels more than Abbott Architect method. The explanation for this could be because of the positive bias that we have observed with the i-CHROMA PSA method in this study and other comparative studies. In one of our previous studies, which compared with the performance of the i-CHROMA PSA method to the Abbot Architect PSA methods enrolled in a PSA UKNEQAS[12] and a similar study using the results of a Randox International Quality Assessment Service,[13] the i-CHROMA PSA method showed a bias of 1.7 and 1.2 ng/mL, respectively. This positive bias seen with the i-CHROMA PSA method would mean than when results are compared with the Abbott Architect PSA method, more individuals will be grouped into amber and red using the i-CHROMA PSA values, and this may warrant that these individuals will have to undergo further investigation, which might be unnecessary. It is of importance to bear in mind that this positive bias is seen in approximately 60% of the laboratory PSA methods.[13]

The i-CHROMA PSA method was altogether simple to use, requires no regular maintenance processes, and showed no performance issues throughout the study. The sample preparation protocol is simple to follow because all instructions are clearly outlined by the manufacturer. Furthermore, all reagents are supplied ready to use. However, one potential source for error is that the sample application well is not unambiguously labeled, and it is possible to apply this directly onto the cartridge membrane by mistake. The method is relatively straightforward to use, although there are some specific features that introduce some potential sources for error, which can be minimized with comprehensive operator training. In summary, the i-CHROMA PSA method showed a good correlation compared with the Abbott Architect PSA method, and although the i-CHROMA estimations tend to show a positive bias at higher PSA values, it provides a reliable measurement of total PSA in finger-prick and venous samples in a clinical setting as long its limitations are taken into appropriate consideration. There should be further studies that in the community testing the acceptability of the finger-prick sampling method and when making any conclusions from the current i-CHROMA PSA values, it is important to take into consideration the PSA method that it is being compared with because there is positive bias seen when compared with more than half of the laboratory methods in the UKNEQAS.

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