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SHORT REPORT

An evaluation of the performance of the Point of Care Test iCHROMA™ AFP method: Precision and accuracy

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Abstract

The estimation of serum alpha-fetoprotein (AFP) is useful in the diagnosis and monitoring of primary hepatocellular carcinoma, hepatoblastoma, non-seminomatous testicular germ cell tumours and other germ cell tumours. The iCHROMA™ AFP is a lateral flow chromatography, fluorescence immunoassay (FIA) for the quantitative determination of AFP in serum or plasma. In this study, the Boditech iCHROMA™ AFP assay had a very good precision of 9.8%. It showed a very good correlation with the following 12 methods: Abbott Architect (r^2 = 0.9705), BioMerieux VIDAS (r^2 = 0.9717), Roche Cobas 6000/8000 (r^2 = 0.9738), Siemens Centaur XP/XPT/Classic (r^2 = 0.9654), Siemens/DPC/Immulite 2000/2500 (r^2 = 0.9673), Siemens/DPC/Immulite 1000 (r^2 = 0.9670), Beckman Dxl 600/800 (r^2 = 0.9676), Roche Elecsys (r^2 = 0.9683), Roche Cobas 4000/e411 (r^2 = 0.9688), Roche Modular E170 (r^2 = 0.9692), SNIBE Maglumi (r^2 = 0.9457) and Ortho Vitros 3600/5600/ECi (r^2 = 0.9714). In summary, the iCHROMA™ AFP, a rapid point of care test method, has a within-run precision value of less than 10% and excellent correlations with traditional laboratory methods. There is a consistent overestimation with the iCHROMA™ method, which must be taken into consideration when setting a reference range.

Keywords: iCHROMA, AFP, RIQAS, comparison, accuracy

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Alpha-fetoprotein (AFP) is a carcinoembryonic glycoprotein present in abundance in foetal blood, with <15 ng/mL in normal adults. Its measurement can significantly increase the specificity at lower levels (i.e. between 10 and 500 ng/mL). These are available but have, to date, been too complex to be widely applied in clinical practice. Serum AFP estimation is useful in the diagnosis and monitoring of primary hepatocellular carcinoma, hepatoblastoma, non-seminomatous testicular germ cell tumours and other germ cell tumours. Estimation of AFP levels in maternal serum and amniotic fluid have been a useful tool in the pre-natal diagnosis of many foetal disorders, such as Down's syndrome, spina bifida, anencephaly and other neural tube defects [1–6]. There are a very few points of care test (POCT) methods for measuring AFP that have data in their literature. One of such methods shows the performance of a rapid quantitative method with a gold immunochromatographic strip, which showed a good correlation (r^2 = 0.961) with a chemiluminescent immunoassay analyser [7]. We have extensively evaluated the comparative performance of the qualitative point of care device Boditech iCHROMA™ for estimating prostate specific antigen (PSA) [8–10], vitamin D [11], human chorionic gonadotrophin (HCG) [12], luteinizing hormone (LH) [12], follicle stimulating hormone (FSH) [12], C-reactive protein (CRP) [13], microalbumin [13] and TSH [14], and found a very good correlation with other traditional laboratory methods. In this study, we set out to evaluate the precision and accuracy performance of the Boditech iCHROMA™ AFP method, a rapid POCT method that uses a very small amount of blood and can be performed on whole blood, with an analysis time of 15 min, and compared its performance with the traditional laboratory AFP methods enrolled in the Randox International Quality Assessment Scheme (RIQAS).

Materials and methods

The Boditech iCHROMA™ uses a sandwich immunodetection principle, such that the fluorescence-labelled detector antibody binds to the target protein in the

sample. The sample is then applied onto a test strip and the fluorescence labelled 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 AFP present and thus allows for the calculation of sample AFP concentration and the result is displayed on the reader as nanograms per millilitre (ng/mL). A fluorescence-labelled control protein is included in the reaction and the intensity of the control line is measured as a quality check.

AFP concentration method

The assay was performed following the manufacturer's instructions:

1. Transfer 30 µL (whole blood), or 15 µL (serum, plasma, control) using a pipette to the detection buffer tube.
2. Close the lid of the detection buffer tube.
3. Shake the tube up and down 10 times or more.
4. Transfer 75 µL of the mixture onto the sample well of the test device.
5. Wait 15 min.
6. Insert test cartridge into the Test Cartridge holder in the Boditech iCHROMA™ reader.
7. Press 'Select'
8. Read the result on the display screen.

Part 1

Precision tests the ability of the tests to be repeated on the same device; there are two types of precision tests, within- and between-run tests. In this study, the within-run precision was estimated using the universal control provided by the manufacturer. The control was made up with water and run 25 times on the iCHROMA™ using the AFP method described previously using 15 µL. The mean, standard deviation (SD) and coefficient of variation percent (CV%) were then estimated from the data. The coefficient of variation (CV) is defined as the ratio of the standard deviation to the mean. It is used to express the precision and repeatability of an assay.

Part 2

The accuracy study was carried out with external quality control material (EQA) samples 1–12 of cycles 40 purchased from the RIQAS. These samples (1–12) were constituted and run-in duplicate on the Boditech iCHROMA™ using the method described. The results were compared with the mean results of the following laboratory methods that were registered with the RIQAS database: Abbott Architect, BioMerieux VIDAS, Roche Cobas 6000/8000, Siemens Centaur XP/XPT/Classic, Siemens/DPC/Immulite 2000/2500, Siemens/DPC/Immulite 1000, Beckman Dxl 600/800, Roche Elecsys, Roche Cobas 4000/e411, Roche Modular E170, SNIBE Maglumi and Ortho Vitros 3600/5600/ECi. The results were plotted in a spreadsheet using an XY plot. The iCHROMA AFP method was plotted on the Y axis and the other laboratory methods on the X axis. A linear regression line was inserted through the data points, and the slope and Y intercept were calculated. The best-fit line will be defined by the equation: Y =m + b, where m is the slope and b is the intercept. The degree of association was measured by a correlation coefficient (R^2) on a scale that varies from +1 through 0 to –1.

Results

Part 1

Precision

Twenty-four (96%) of the 25 tests had values that fell within the range of the universal control (lower limit 16.25 ng/mL, an average 21.67 ng/mL, an upper limit 27.09 ng/mL), except for the fourth test with a value of 34.73 ng/mL, see Fig. 1. The Boditech iCHROMA™ AFP intra-assay results ranged from 22.25 to 34.73 ng/mL, with a mean value of 24.5 ng/mL, a SD of ±2.4 ng/mL and a coefficient of variation percent (CV%) of 9.8%.

Part 2

The mean values of the duplicate iCHROMA™ AFP results of the RIQAS samples, 1–12, were 21.10, 350, 88.27, 172.11, 193.39, 46.18, 277.93, 200.36, 95.67, 63.34, 155.09 and 15.83 ng/mL, respectively.

Accuracy

The iCHROMA™ AFP mean estimates for the RIQAS samples 1–12 were consistently overestimated (positive bias) compared with the mean estimates of the 12 other traditional laboratory AFP methods (see Table 1). The average percentage difference between the iCHROMA™ AFP and the methods was Abbott Architect (48%), BioMerieux VIDAS (52%), Roche Cobas 6000/8000 (38%), Siemens Centaur XP/XPT/Classic (39%), Siemens/

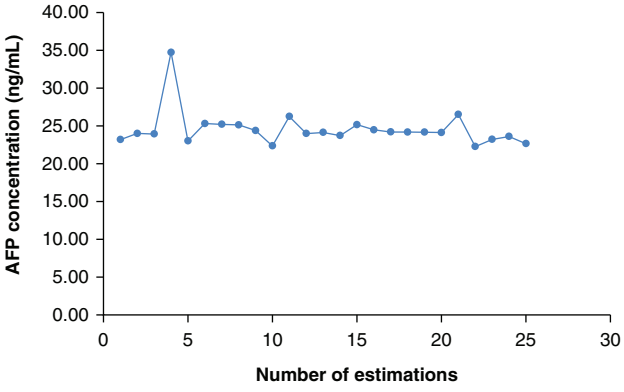

Fig. 1. Twenty-five AFP estimations run on the same day as the universal control.

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Table 1. AFP estimations of the methods in the RIQAS Samples 1–12.

Method	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Abbott Architect	9.096484	169.793	47.22094	108.2988	90.37115	28.30127
bioMerieux,Vidas/Mini Vidas	8.6294	161.4363	40.4248	96.00143	82.68017	25.894
Roche Cobas 6000/8000	10.62209	194.5909	52.99876	122.3518	106.4544	32.95655
Siemens Centaur XP/XPT/Classic	10.54653	198.0714	54.98807	124.2966	101.4718	31.64804
Siemens/DPC Immulite 2000/2500	9.353571	177.7557	48.34188	110.5056	96.30545	28.7102
Siemens/DPC Immulite 1000	9.83238	175.2	48.62	104.22	91.28	29.26
Beckman Dxl 600 /800	9.66406	177.6159	48.7631	114.0399	92.59606	27.82608
Roche Elecsys	10.75335	193.3664	50.33068	127.0314	103.9865	33.62196
Roche Cobas 4000/e411	10.38268	199.6713	54.31559	122.7732	104.2007	31.86843
Roche Modular E170	10.34424	191.835	52.54106	123.051	104.3345	32.80996
SNIBE Maglumi analysers	10.284	163.1595	50.566	119.8475	92.5605	26.74575
Ortho Vitros 3600/5600/ECi	10.33775	176.9886	49.10138	110.76	93.512	28.99356
Boditech iCHROMA™	21.10	350.00	88.27	172.11	193.39	46.18

Method	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
Abbott Architect	170.3701	107.8964	47.51272	28.3656	91.20265	9.096123
bioMerieux,Vidas/Mini Vidas	159.34	110.814	44.75229	27.238	87.00929	8.534
Roche Cobas 6000/8000	196.0695	123.7311	52.87621	32.564	105.4668	10.61144
Siemens Centaur XP/XPT/Classic	195.898	120.7891	54.77936	31.78392	102.4904	10.44253
Siemens/DPC Immulite 2000/2500	186.5882	111.1667	48.3979	29.11788	96.46091	9.6005
Siemens/DPC Immulite 1000	177.8	105.8429	49.67143	30.2125	91.9875	9.502857
Beckman Dxl 600 /800	176.0888	111.2946	47.54214	29.02904	92.9531	9.512804
Roche Elecsys	194.4745	124.1741	54.35563	31.88223	104.2455	10.54152
Roche Cobas 4000/e411	198.4786	121.1884	53.17305	32.54559	104.9461	10.25012
Roche Modular E170	197.1814	121.9721	53.54494	32.50451	106.391	10.48977
SNIBE Maglumi analysers	169.673	99.8305	39.1285	23.275	81.221	9.3005
Ortho Vitros 3600/5600/ECi	178.1725	114.8744	49.36608	29.10056	94.759	9.783636
Boditech iCHROMA™	277.93	200.36	95.67	63.34	155.09	15.83

DPC/Immulite 2000/2500 (46%), Siemens/DPC/Immulite 1000 (45%), Beckman Dxl 600/800 (45%), Roche Elecsys (38%), Roche Cobas 4000/e411 (39%), Roche Modular E170 (40%), SNIBE Maglumi (44%) and Ortho Vitros 3600/5600/ECi (43%), respectively.

Correlations

The coefficient of correlation measures the strength of a possible linear relationship between the other methods. The plots in Fig. 2A–L show the results of the various method estimation comparisons with the 12 quality control samples analysed using the iCHROMA™ AFP method. The coefficient of correlations ranged between + 0.9457 and + 0.9738.

Discussion

Most AFP measuring immunoassays have a high sensitivity and specificity but could be time consuming, expensive and complex. The value of a POCT to measure AFP is, therefore, invaluable. A simple and rapid point-of-care system that uses Eu (III) chelate microparticles with lateral flow immunoassay (LFIA) had been described to determine AFP in serum with an assay time of 15 min and excellent correlation (r = 0.9860) [15]. In this study, another POCT using LFIA, the iCHROMA™ AFP method showed a very good within-run precision of 9.8%, showing that the repeatability of the test performance is good with 95% of the estimated values falling within two standard deviations of the mean (19.7–29.3 ng/mL). There was a consistent overestimation with the iCHROMA™ AFP method compared with the other laboratory methods. The laboratory method's percent bias to the the iCHROMA™ AFP method ranged from 38% in Roche Cobas 6000/8000 and Roche Elecsys to 53% in BioMerieux Vidas. The difference (positive bias) observed between the values of the iCHROMA™ AFP method and the other traditional

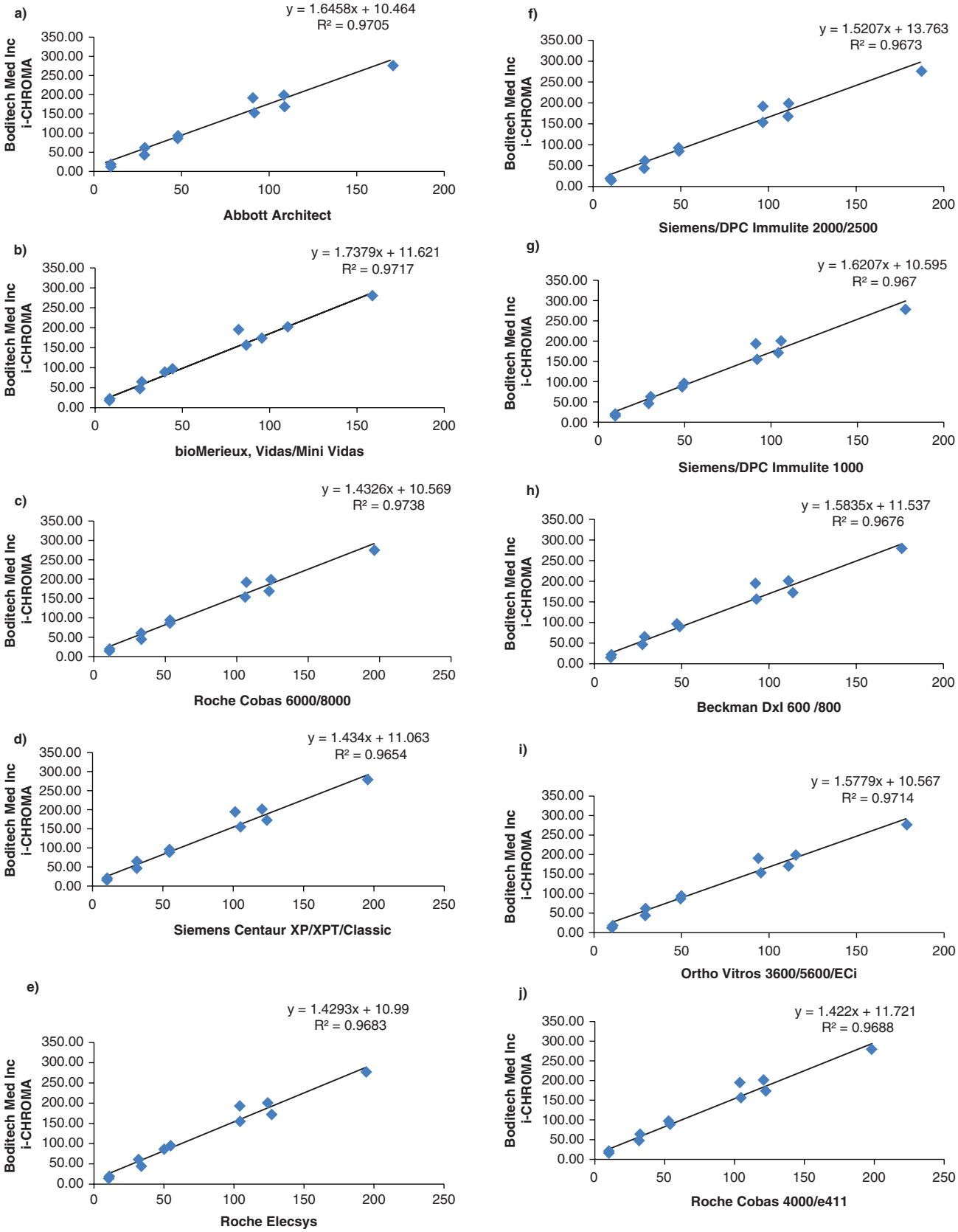

Fig. 2. a) Abbott Architect, b) bioMerieux, Vidas/Mini Vidas, c) Roche Cobas 6000/8000, d) Siemens Centaur XP/XPT/Classic, e) Roche Elecsys, f) Siemens/DPC Immulite 2000/2500, g) Siemens/DPC Immulite 1000, h) Beckman Dxl 600/800, i) Ortho Vitros 3600/5600/ECi, j) Roche Cobas 4000/e411, k) Roche Modular E170, l) SNIBE Maglumi analysers.

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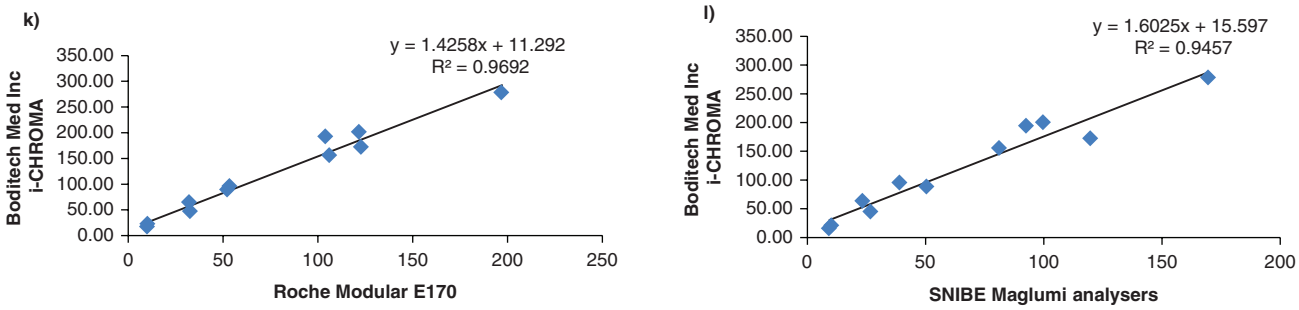

Fig. 2. (Continued) Showing iCHROMA™ AFP comparison (correlation) with other AFP methods a) Abbott Architect, b) bioMerieux, Vidas/Mini Vidas, c) Roche Cobas 6000/8000, d) Siemens Centaur XP/XPT/Classic, e) Roche Elecsys, f) Siemens/DPC Immulite 2000/2500, g) Siemens/DPC Immulite 1000, h) Beckman Dxl 600/800, i) Ortho Vitros 3600/5600/ECi, j) Roche Cobas 4000/e411, k) Roche Modular E170, l) SNIBE Maglumi analysers.

laboratory methods was consistent and could be due to calibration or the set point on the iCHROMA™ AFP method.

Despite the overestimations of the iCHROMA™ AFP method, there was a very good correlation with the methods ranging from $r^2 = 0.9457$ with SNIBE Maglumi to $r^2 = 0.9738$ with Roche Cobas 6000/8000 (Fig. 2). This confirms that the iCHROMA™ AFP method's results compare very well with those of the other traditional laboratory methods of measuring AFP, and the constant positive bias observed is probably as a result of a calibration or set point of the iCHROMA™ AFP method, thus suggesting that the reference range of the iCHROMA™ AFP method should be set up taking into consideration a proportional error of 38–52.

In summary, the iCHROMA™ AFP has excellent correlations with traditional laboratory methods, and the assay has a within-run precision value of <10%. These data here show that the iCHROMA™ is a very practical solution for laboratories that require to assay for AFP at the point of care.

Conflict of interest and funding

JB is a consultant advisor to Boditech Med Inc. Tae Kyum Kim is an employee of Boditech Med Inc. Boditech Med Inc. is the manufacturer of the iCHROMA.

Authors' contributions

JB concept, study development and initiator, OC and SB sample analysis evaluation and statistics, CA and TKK material and method support.

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