Validation and evaluation of eight commercially available point of care CRP methods

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A B S T R A C T

Background: There are several situations compelling to measure CRP with a point of care (POC) method. Assay performance of various available POC CRP methods were evaluated as analytical quality is important and should be known before clinical use.

Methods: We compared 2 semi-quantitative strips; Actim and Cleartest and 6 quantitative CRP tests; Afinion, QuikRead go, Smart, iChroma, Microsemi and AQT90 Flex to the Synchron CRP method, using the CLSI EP9 protocol. The coefficient of variance (CV) was determined. Various aspects of pre-analytical and analytical steps were evaluated.

Results: CRP strips showed 50–60% concordance with the Synchron CRP. The linear regression lines (95% CI) of the quantitative POC CRP methods compared to the Synchron CRP method were: y = [0.96–1.04]x + [−4.7 to −2.04] (Smart); y = [1.00–1.06]x + [1.65–4.99] (AQT90 Flex); y = [0.84–0.91]x + [1.13 to 3.95] (Afinion); y = [0.83–0.87]x + [0.25–1.5] (QuikRead go); y = [0.76–0.82]x + [−0.18 to 1.35] (iChroma) and y = [1.14–1.18]x + [−3.17 to −1.83] (Microsemi).

Conclusions: At best, the semi-quantitative CRP strips could be used to discriminate between normal and increased levels of CRP. Of the quantitative methods, when combining analytical with practical evaluation, the Smart and Afinion would be the preferred analyzers for POC.

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1. Background

C-reactive protein (CRP) is an acute phase reactant that can be used to monitor inflammation episodes and which is useful to distinguish between viral and bacterial infections. The measurement of CRP is widely used in central hospital laboratories and there are many automated methods available. In recent years qualitative and quantitative methods have been developed to measure CRP in a point of care setting. The patients can be sampled and analyzed in a one-stop visit giving a reduction in costs, turnaround time and number of visits. But the feasibility and patient can be sampled and analyzed in a one-stop visit giving a reduction in antibiotic prescription [2] and in the Netherlands the value of CRP has to be known before antibiotics are allowed to be given to patients with lower respiratory infections [3].

The beneficial effects of measuring CRP will be influenced by the quality of the results produced. There are many commercially available methods for POC CRP, with various pre-analytical handling and varying assay performance [4–8]. In one method the measurement of the white blood cell count is combined with that of CRP [9]. In all those studies except one [10] just one method or apparatus has been evaluated and compared with an automated routine method. In this study we investigated the various commercially available methods on their applicability for general practice. We compared 2 semi-quantitative strip methods and 6 quantitative methods, using capillary (finger-stick) or venous blood, with the Synchron CRP method and determined the coefficient of variance. The focus of this study was the assay performance and the necessary pre-analytical handling of various available POC CRP tests. Besides the analytical performance, the practical aspects of each method were evaluated.

2. Methods

The 8 POC CRP methods evaluated in this study can be divided into 2 groups; 1) semi-quantitative methods, i.e. strips using capillary blood and
2) quantitative methods, i.e. analyzers using either capillary or venous blood.

2.1. Semi-quantitative methods

- Actim® CRP strips (Medix Biochemica, Kauniainen, Finland), an immunochromatographic assay producing a semi-quantitative result subdivided into the following categories: <10, 10–40, 40–80, and >80 mg/L.
- Cleartest® CRP strips (Servoprax, Wesel, Germany), an immunochromatographic assay producing a semi-quantitative result subdivided into the following categories: <10, 10–40, 40–80, and >80 mg/L.

The results of these tests were interpreted by two independent observers after 5 min and after 15 minute incubation.

2.2. Quantitative methods

- QuikRead go (Orion Diagnostica, Espoo, Finland), CRP method based on immunoturbidimetric assay. The assay can be performed with capillary blood, and an automated Ht correction is applied.
- Smart analyzer (Eurolyser Diagnostica, Salzburg, Austria), CRP method based on immunoturbidimetric assay. The assay can be performed with capillary blood, and Ht correction can be applied manually (only with known Ht). We did not correct for Ht during this evaluation, because in practice the Ht will not be known either for most patients at the moment the CRP assay is performed.
- Affinon™ AS 100 analyzer (Axis Shield, Oslo, Norway), CRP method based on solid phase immunochemical assay. The assay can be performed with capillary blood, and an automated hematocrit (Ht) correction is applied.
- iChroma analyzer (Boditech, Med. Inc., Gangwon-do, Korea), CRP method based on fluorescence sandwich immunoassay. The assay can only be performed with capillary blood. Since Ht correction is not possible, we did not correct for Ht during the evaluation either.
- Microsem (Horiba Ltd., Kyoto, Japan), CRP method based on immunoturbidimetric assay. The assay can be performed with or without venous blood. CRP analysis can only be performed in combination with hematological parameters (measured: WBC, RBC, Hb, Ht, platelets, lymphocytes, monocytes, granulocytes. Calculated: MCV, MCH, MCHC, RDW, PDW, MPV). Automated Ht correction is applied.
- AQ790 Flex (Radiometer Medical ApS, Brønshøj, Denmark), CRP method based on solid phase sandwich immunoassay with timeresolved fluorometric detection. The assay can only be performed with venous blood. Automated Ht correction is applied.

2.3. Analytical validation

For comparison, all the samples used for the POC CRP evaluation were analyzed in the laboratory of Clinical Chemistry of the Medical Centre Alkmaar by means of the routine biochemical CRP method, on the Synchron® analyzer (Beckman Coulter, Krefeld, Germany). The analytical range of this method is between 1.0 and 500 mg/L, with an intra- and inter-assay variation (CRP 80 mg/L) of 1.1% and 5.5%, respectively.

The CLSI EP9 protocol was performed for each of the POC CRP methods, using 100 K2-EDTA blood samples for each method. All blood samples were from GPs’ patients, aged >18 years, with CRP concentrations ranging from 5 to 200 mg/L, determined with the

Table 1
Correlation of the CRP strips with the Synchron CRP method. Correlation is expressed with the kappa-index [11] as well as % discrepancy.

<table>
<thead>
<tr>
<th></th>
<th>After 5 min</th>
<th>After 15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kappa-index</td>
<td>% discrepancy</td>
</tr>
<tr>
<td>Actin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synchron vs observer 1</td>
<td>0.529</td>
<td>35</td>
</tr>
<tr>
<td>Synchron vs observer 2</td>
<td>0.633</td>
<td>27</td>
</tr>
<tr>
<td>Observer 1 vs observer 2</td>
<td>0.807</td>
<td>14</td>
</tr>
<tr>
<td>Clear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synchron vs observer 1</td>
<td>0.608</td>
<td>29</td>
</tr>
<tr>
<td>Synchron vs observer 2</td>
<td>0.561</td>
<td>33</td>
</tr>
<tr>
<td>Observer 1 vs observer 2</td>
<td>0.554</td>
<td>33</td>
</tr>
</tbody>
</table>

Observer 1
Kappa-index % discrepancy
5 min vs 15 min 0.638 26
n = 94
5 min vs 15 min 0.396 44
Clear
n = 82
5 min vs 15 min 0.601 29
n = 94
5 min vs 15 min 0.197 59
n = 82

Fig. 1. Correlation of the POC CRP strips, interpreted by observer 1 after 5 minute incubation, with the Synchron CRP method. Each dot represents one sample. The gray boxes represent the corresponding categories on the strips. A. Actim strips and B. Clear strips.
Synchron® analyzer. All samples were measured within 24 h after blood withdrawal. Because the analyzers were not tested simultaneously, the patient cohorts used for the CRP analysis on the various analyzers are not identical. Correlation criteria for acceptable correlation with the laboratory CRP method were defined as follows: the 95% confidence interval (CI) of the slope should include 1.0 with the 95% CI of the intercept including 0.0 and $r^2 > 0.95$.

To determine the coefficient of variation (CV), two patient samples with a CRP in the critical decision range (CRP 50–130 mg/L) were measured five times with each analyzer. The maximum allowable CV was set arbitrarily on 10%.

2.4. Statistical analysis

Statistical analyses were performed with Microsoft Excel (Microsoft Corporation) software combined with the software package Analyse-It (Analyse-It software) and EP Evaluator®. To calculate the correlation and bias with the routine CRP procedure, Passing and Bablok and Bland–Altman analyses were used, and Spearman for the correlation coefficient ($r^2$). For the semi-quantitative methods, the intra- and interobserver agreement was calculated with the kappa-index [11]. To be able to compare the quantitative method (Synchron) and the semi-quantitative method (strips), both results were translated into 4 categories (category 1: CRP 0–10 mg/L, category 2: CRP 10–40 mg/L, category 3: CRP 40–80 mg/L and category 4: CRP >80 mg/L).

2.5. Practical evaluation

Besides the technical performance, the practical aspects of the various POC CRP methods play a role in the reliability of the CRP result. A practical evaluation was performed in the laboratory, to assess the suitability of the different methods as a POCT method. The following aspects were considered:

Material and minimum amount of material necessary; method; analytical range; pre-analytical handling of the samples and estimated pre-analytical time; duration of the analysis; ht correction; robustness of the analyzer; size and weight of the analyzer; possibility of measuring other analytes on the same analyzer.

3. Results

3.1. Analytical validation

3.1.1. Semi-quantitative methods

The correlation of the semi-quantitative strips with the Synchron CRP method is shown in Fig. 1. The kappa-index for the Actim strips in comparison with the Synchron analyzer was 0.529 for observer 1 and 0.633 for observer 2. While observers 1 and 2 had a kappa-index of 0.807 when interpreting the Actim strips after 5 minute incubation, the kappa-index for the correlation with the Synchron CRP results decreased to 0.461 and 0.393 for observers 1 and 2 respectively after 15 minute incubation of the Actim strips. At 15 min, the inter-observer agreement between observers 1 and 2 was 0.828 (Table 1).

Similar results were found for the interpretation of the Clear strip after 5 minute incubation. In comparison with the Synchron CRP method, the Clear strips had a kappa-index of 0.608 for observer 1 and 0.561 for observer 2, but disagreement between observers 1 and 2 was greater (kappa-index of 0.554). After 15 minute incubation of the Clear strips, the kappa-index for the correlation with the Synchron CRP results decreased to 0.172 and 0.249 for observers 1 and 2 respectively, while the inter-observer agreement between observers 1 and 2 was 0.742 (Table 1).

Fig. 2. Correlation of the 6 POC CRP analyzers with the Synchron CRP method given as Bland–Altman plots with absolute and percentage differences. Each dot represents one sample. A: QuikRead go (n = 87), B: Smart (n = 86), C: Afinion (n = 98), D: iChroma (n = 111), E: Microsemi (n = 106), and F: AQT90 Flex (n = 86).
4.1. Evaluation of the semi-quantitative CRP strips

Evaluation of the semi-quantitative CRP strips regarding their interpretation of the lines was more difficult. About one third of the samples analyzed. The evaluation revealed that estimation as well as underestimation of the CRP value was observed for edge, this is the faults are met, POC CRP can be successfully implemented. To our knowledge, this is the first study to compare both capillary and venous blood CRP methods as well as semi-quantitative CRP strips regarding their analytical performance and their applicability for POC testing.

4.2. Evaluation of the quantitative CRP analyzers

POC requires a good quality assurance program. This program should contain a validation of the CRP method, training procedure for applicants, certification, and re-certification after performing an e-learning training. Distribution and storage of cuvettes and reagents and internal control should follow the user manuals of the distributor. A contact person from Distribution and storage of cuvettes and reagents and internal control should be advised to measure CRP with a quantitative method.

4.2. Evaluation of the quantitative CRP analyzers

The r² of all quantitative POC CRP analyzers was >0.95. Most probably, the r² of the Smart and iChroma CRP test was lower than the other analyzers due to the absence of an automated Ht correction. However, we did not manually correct for Ht, as this is in general not possible in POC situations. Several POC CRP analyzers have already been evaluated by others [4–10] but all except one [10] in a one to one comparison with a central laboratory method. The correlation coefficients are in general >0.95 comparable with our findings.

With regard to the correlation with the laboratory CRP test on the Synchron analyzer, there were some remarkable differences between the various POC CRP analyzers. The slope of Smart and AQT90 Flex CRP was around 1.00, and therefore showed the best correlation with the Synchron CRP method. The slope of QuickRead go and Afni, even underestimated the CRP value by 27% for the Clear strip. Moreover, the results seemed to indicate a higher CRP level than before. For example, CRP in the category 40–80 mg/L after 5 min became category >80 mg/L after 15 min. This phenomenon was observed for all levels of CRP.

During the practical evaluation we experienced a long hands-on time for the analysis (10–15 min) and difficult analytical handling for persons who does not perform the test on a regular basis. In addition, results are not automatically included in the patient record.

The main advantage of the strips is the low costs. Since no analyzer is required, the costs of performing a semi-quantitative CRP are much lower than the quantitative CRP tests.

In our opinion, the semi-quantitative CRP strips could at best be used to discriminate between no infection (CRP < 10 mg/L, only the control line is visible on the strip) and infection likely (CRP elevated, one or more blue lines are visible on the strip in addition to the control line). If one or more of the blue test lines appear on the strip, the user should be advised to measure CRP with a quantitative method.

Table 2

Linear regression equation, coefficient of correlation and coefficient of variation of the 6 POC CRP methods compared to the Synchron CRP method.

<table>
<thead>
<tr>
<th>Analyzers</th>
<th>N</th>
<th>Linear regression lines y = ax + b</th>
<th>Correlation</th>
<th>VC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a  (95% CI)</td>
<td>b  (95% CI)</td>
<td>r² (95% CI)</td>
<td>[mean CRP (n = 5) in mg/L]</td>
</tr>
<tr>
<td>QuickRead go</td>
<td>87</td>
<td>0.85 (0.83–0.87)</td>
<td>1.04 (0.25–1.5)</td>
<td>0.996 (0.994–0.997)</td>
</tr>
<tr>
<td>Smart</td>
<td>86</td>
<td>1.00 (0.96–1.04)</td>
<td>3.26 (−4.70 to −2.04)</td>
<td>0.970 (0.954–0.980)</td>
</tr>
<tr>
<td>Afni,</td>
<td>98</td>
<td>0.87 (0.84–0.91)</td>
<td>2.78 (1.13–3.95)</td>
<td>0.962 (0.973–0.988)</td>
</tr>
<tr>
<td>iChroma</td>
<td>111</td>
<td>0.79 (0.76–0.82)</td>
<td>0.59 (−0.18–1.35)</td>
<td>0.967 (0.953–0.976)</td>
</tr>
<tr>
<td>Microsemi</td>
<td>106</td>
<td>1.16 (1.14–1.18)</td>
<td>−2.5 (−3.17 to −1.83)</td>
<td>0.997 (0.996–0.998)</td>
</tr>
<tr>
<td>AQT90 Flex</td>
<td>86</td>
<td>1.01 (1.00–1.06)</td>
<td>2.68 (1.05–4.99)</td>
<td>0.992 (0.995–0.998)</td>
</tr>
</tbody>
</table>

n.e.: not established.

3.1.2. Quantitative methods

The results of the comparisons between the six quantitative POC CRP analyzers and the Synchron CRP method are graphically shown as Bland–Altman plots in Fig. 2, Table 2 summarizes the 95% CI of the linear regression lines, correlation coefficients and coefficients of variation found for all six POC CRP analyzers. None of the analyzers met all the correlation criteria determined beforehand. All analyzers had a r² > 0.95. However, the 95% CI for the slope of the linear regression equation compared to the Synchron CRP method only included 1.0 for the Smart analyzer and the AQT90 Flex analyzer. The linear regression equation of QuickRead go, Afni and iChroma revealed an underestimation of the CRP value compared to the Synchron CRP, while the Microsemi overestimated the CRP compared to the method used in our laboratory. The iChroma analyzer was the only analyzer with an intercept including 0.0 in the 95% CI.

The coefficients of variation, measured in patient samples with CRP values between 50 and 130 mg/L, varied among the analyzers (Table 2). Only the iChroma did not meet the criterion of VC < 10%.

3.2. Practical evaluation

Evaluation of the various POC CRP methods with regard to relevant points for successful implementation of POC CRP revealed that Afni required the least pre-analytical handling in combination with a capillary blood sample. The pre-analytical steps of both semi-quantitative strips, the QuickRead go and iChroma required 2–3 minute hands-on time compared to 30–45 s for Afni and Eurolyser. Both Microsemi and AQT90 Flex did not require additional handling after venous blood sampling. The strict timing of interpretation after 5 min is a disadvantage of the strips, while the analysis time of 13 min is the major drawback for the AQT90 Flex. All other analyzers produced a result within 5 min. Table 3 presents the results of all the items addressed in our evaluation.
Table 3: Practical aspects of the semi-quantitative strips and quantitative POC methods for the analysis of CRP.

<table>
<thead>
<tr>
<th>Semi-/quantitative strips</th>
<th>Quantitative CRP analyzers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actim (Medix)</td>
<td>Smart (Eurolyser)</td>
</tr>
<tr>
<td>Cleartest (Servoprax)</td>
<td>Afinnion (Axis Shield)</td>
</tr>
<tr>
<td>QuickRead go (Orion)</td>
<td>iChroma (Boditech)</td>
</tr>
<tr>
<td>Microsemi (Horiba)</td>
<td>AQ700 Flex (Radiometer)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analyzer (producer)</th>
<th>Material</th>
<th>Method</th>
<th>Minimum amount of material</th>
<th>Analytical range (full blood)</th>
<th>Preanalytical handling</th>
<th>Estimated preanalytical time</th>
<th>Analysis time</th>
<th>Ht correction Other tests on the analyzer</th>
<th>Estimated analysis time</th>
<th>Online connection with LIS</th>
<th>Analyzer size Analyzer weight</th>
<th>Practical aspect of the test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary blood</td>
<td>Capillary blood</td>
<td>Immunochromatographic assay</td>
<td>10 µL</td>
<td>0–80 mg/L</td>
<td>Draw blood in top to top capillary. Place the capillary in the cuvette, place cap on cuvette, shake, place the strip in the cuvette, remove the strip from solution and place horizontally.</td>
<td>2.5 min</td>
<td>Interpretaion between 2 and 5 min, no later than 10 min</td>
<td>No/5 min</td>
<td>n/a/Yes</td>
<td>4.5 min</td>
<td>n/a/Yes/No/PT-INR, HbA1c, hemoglobin, D-dimer, lipoprotein A, ferritin, homocysteine, FOB, microalbumin</td>
<td>Relatively complicated preanalytical handling, semi-quantitative, inter-observer variation, cut-off at 80 instead of 100 mg/L</td>
</tr>
<tr>
<td>Capillary blood</td>
<td>Capillary blood</td>
<td>Immunochromatographic assay</td>
<td>20 µL</td>
<td>5–240 mg/L</td>
<td>Draw blood in capillary. Empty the capillary in the cuvette with the plunger, place the cap on the cuvette, shake, place cuvette in the analyzer.</td>
<td>2.5 min</td>
<td>Yes/No</td>
<td>3.75 min</td>
<td>n/a/No</td>
<td>3 min</td>
<td>n/a/Yes/No/PT-INR, HbA1c, hemoglobin, D-dimer, lipoprotein A, ferritin, homocysteine, FOB, microalbumin</td>
<td>Relatively complicated preanalytical handling, semi-quantitative, inter-observer variation, cut-off at 80 instead of 100 mg/L</td>
</tr>
<tr>
<td>Capillary blood</td>
<td>Capillary blood</td>
<td>Immunoturbidimetric assay</td>
<td>5 µL</td>
<td>1–240 mg/L</td>
<td>Draw blood in capillary. Place the cap with integrated capillary on the cuvette and shake 3–4 times. Place cuvette in the analyzer.</td>
<td>45 s</td>
<td>Yes/No</td>
<td>30 s</td>
<td>n/a/No/Yes/PT-INR, HbA1c, hemoglobin, D-dimer, lipoprotein A, ferritin, homocysteine, FOB, microalbumin</td>
<td>Relatively complicated preanalytical handling, semi-quantitative, inter-observer variation, cut-off at 80 instead of 100 mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capillary blood</td>
<td>Capillary blood</td>
<td>Solid phase immunochromatographic assay</td>
<td>1.5 µL</td>
<td>8–200 mg/L</td>
<td>Draw blood in capillary. Place the capillary in the cassette. Place cartridge in the analyzer.</td>
<td>30 s</td>
<td>Yes/No</td>
<td>2 min</td>
<td>n/a/No/Yes/PT-INR, HbA1c, hemoglobin, D-dimer, lipoprotein A, ferritin, homocysteine, FOB, microalbumin</td>
<td>Relatively complicated preanalytical handling, semi-quantitative, inter-observer variation, cut-off at 80 instead of 100 mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capillary blood</td>
<td>Capillary blood</td>
<td>Fluorescence sandwich immunoassay</td>
<td>10 µL</td>
<td>2.5–300 mg/L</td>
<td>Draw blood in sample collector, place sample collector on buffer tube. Shake 10 times, discard the first 2 drops, fill sample well of cartridge with 2 drops. Place cartridge in the analyzer.</td>
<td>13 min</td>
<td>Yes/No/Yes/PT-INR, HbA1c, hemoglobin, D-dimer, lipoprotein A, ferritin, homocysteine, FOB, microalbumin</td>
<td>Relatively complicated preanalytical handling, semi-quantitative, inter-observer variation, cut-off at 80 instead of 100 mg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capillary blood</td>
<td>Capillary blood</td>
<td>Immunoturbidimetric assay</td>
<td>5 µL</td>
<td>10 µL</td>
<td>Draw blood in capillary. Place the cap in the cuvette.</td>
<td>2 min</td>
<td>n/a/Yes/Yes/PT-INR, HbA1c, hemoglobin, D-dimer, lipoprotein A, ferritin, homocysteine, FOB, microalbumin</td>
<td>Relatively complicated preanalytical handling, semi-quantitative, inter-observer variation, cut-off at 80 instead of 100 mg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capillary blood</td>
<td>Capillary or venous blood</td>
<td>Solid phase immunochromatographic assay</td>
<td>18 µL + dead volume in the tube: 100 µL 2.0–230 mg/L</td>
<td>5–500 mg/L</td>
<td>Draw blood with venous puncture, remove cap from the tube, place the tube in the analyzer.</td>
<td>13 min</td>
<td>n/a/Yes/Yes/PT-INR, HbA1c, hemoglobin, D-dimer, lipoprotein A, ferritin, homocysteine, FOB, microalbumin</td>
<td>Relatively complicated preanalytical handling, semi-quantitative, inter-observer variation, cut-off at 80 instead of 100 mg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n/a: not applicable.

* Only in serum/plasma, centrifuge step necessary.

b Urine/feces.
The advantages of the quantitative methods compared to the semi-quantitative strips are obvious: a quantitative result is obtained, there is less hands-on time and, importantly, there is no critical time of read-out, so the operator can walk away from the test after putting it into the analyzer. Most analyzers can be connected to the laboratory information system, thus facilitating automatic transfer of the results to the patient’s electronic lab dossier.

When comparing the different POC CRP analyzers with each other, the Afinion CRP had the most simple pre-analytical handling, with the Smart as second best. The AQ90 Flex CRP does not need any pre-analytical handling at all, but it requires a venous puncture, which makes it less suitable for POCT.

For QuikRead go and iChroma, the pre-analytical handling of the samples is much more complicated than for the other analyzers (Table 3, row pre-analytical handling). This might introduce variation if the test is not performed on a regular basis. Besides variation, incorrect pre-analytical handling can generate errors during the analysis. This might create spill of materials, having to use more than one cassette or cuvette per patient sample to gain a result. Simple pre-analytical handling is therefore an important matter in performing POC tests.

A drawback of the Afinion analyzer is that the sample volume is only 1.5 μL, which means that it is very important to place the cassette directly into the analyzer after adding the blood sample, otherwise an error is generated because the material will clot. A disadvantage of the Smart and iChroma CRP analyzers is the absence of automated Ht correction, which can introduce incorrect results, since in most POC settings the actual Ht of the patient will not be known before CRP measurement.

With an analytical time between 2 and 4 min, all analyzers are generating a result fast enough for POCT, with the exception of the AQ90 Flex, which has an analytical time of 13 min.

Taking into account both the analytical validation and the practical evaluation, we consider the Afinion and Smart CRP to be the most suitable analyzers for POCT. Although the correlation coefficient of the Afinion was better than the Smart, Afinion underestimated the CRP concentration by more than 10% and therefore our preferred method is the Smart analyzer.

The Smart analyzer showed the best correlation with the Synchron CRP method.

The CV-analytical of the various CRP methods varied, but only the iChroma did not pass our criterion of CV < 10%. This might be a direct consequence of the pre-analytical handling necessary for the iChroma method, which is relatively complex. We experienced that it was almost impossible to replace the cap on the tube after filling it with the material from the capillary without getting fluid on the outside of the cap, thereby inducing variation in the liquid volume. Besides, the presence of liquid containing human material on the outside of the vessel also is a negative aspect of this method with regard to hygiene and infection risk. Remarkably others found better results for the performance of the iChroma [4,8].

In general the agreement between the laboratory method and the POC methods is not very good but also the outcomes among various studies with the same POC method seems to be rather variable. First of all most POC methods are not calibrated before measurements and also no quality controls are available. Furthermore a lot-to-lot variation can be present and POC methods have mostly single cartridges for every measurement. Incorrect pre-analytical handling of the samples can also introduce considerable variation although POC devices should be user-friendly and full-proof. In our studies all pre-analytical handling and measurements were carried out by the same technician so the obtained CV’s are completely comparable with each other. Our study was conducted with fresh venous blood samples and differences between venous blood and capillary blood cannot be argued. For practical reasons, we performed no manual Ht correction which possibly could lead to better results.

4.3. Practical evaluation of the quantitative CRP analyzers

Pre-analytical handling necessary for the various methods was one of the items addressed in our practical evaluation of the suitability of the different POC CRP tests.

Acknowledgments

We would like to thank Erik Allon, Ria Baltus and the point of care technicians, of the Laboratory of Clinical Chemistry, Hematology and Immunology of the Medical Centre, Alkmaar, for their practical assistance and their critical views on various analyzers.

Competing interests

None declared.

Funding

All analyzers used in this research were provided free of charge for the purpose of validation and evaluation only. No other forms of funding were received for this research.

Ethical approval

Not required.

References


Assessment of an Affinity Based Point of Care System for HbA1c

Andrea Mosca; Renata Paleari; Gabriella Passerini and Ferruccio Ceriotti. Dept. Science and Biomedical Technology, University of Milano and Diagnostica e Ricerca S. Raffaele spa, Milano, Italy.

Abstract

Aim was to evaluate the analytical performance of a new Point of Care (POCT) system (Afinion™ AS100 Analyzer, Axis-Shield PoC AS, Oslo, N), based on boronate affinity chromatography. The system takes 3 min per test, requires 1.5 µl whole blood per analysis, does not suffer interference in presence of HbS and HBOC, and reports National Glycohemoglobin Standardization Program (NGSP) aligned values.

A total of 120 whole blood samples collected in EDTA and stored at +4 °C were analyzed within a 2 days from collection. Ten out of the 120 samples were from patients under constant dialysis regimen. Two POCT systems, one immunochromatographic (Siemens DCA 2000+) and one affinity chromatography based (NycoCard® READER II, Axis Shield PoC AS), and two HPLC systems (Bio-Rad Variant II and Tosoh G7), all NGSP aligned, were used as comparison. Twenty-one out of the 120 samples (HbA1c range 4.6–12.0 %) were measured in duplicate, in order to evaluate analytical imprecision by the method of the differences between duplicates. Three different batches of Afinion™ HbA1c reagents were evaluated.

Table 1 reports the results on the imprecision, calculated from the differences of duplicates performed over one month of routine use. Twenty-one duplicate measurements were performed per each method. The results obtained by the new POCT system meet the NACB guidelines for imprecision and accuracy. The method showed improved imprecision respect to the other POCT system (NycoCard®) produced by the same Company. The presence of carbamylated hemoglobin does not interfere in the measurement of HbA1c by the Afinion™ method.

Table 1. Imprecision of various HbA1c methods.

<table>
<thead>
<tr>
<th>Reference method (x-method)</th>
<th>Sx</th>
<th>Intercept ± ES</th>
<th>Slope ± ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>POCT #1 (NycoCard®)</td>
<td>0.976</td>
<td>0.667 ± 0.131</td>
<td>0.881 ± 0.019</td>
</tr>
<tr>
<td>POCT #3 (DCA 2000+)</td>
<td>0.989</td>
<td>1.205 ± 0.092</td>
<td>0.819 ± 0.014</td>
</tr>
<tr>
<td>HPLC #1 (Tosoh G7)</td>
<td>0.977</td>
<td>1.278 ± 0.082</td>
<td>0.783 ± 0.012</td>
</tr>
<tr>
<td>HPLC #2 (Bio-Rad Variant II)</td>
<td>0.974</td>
<td>0.931 ± 0.132</td>
<td>0.856 ± 0.019</td>
</tr>
</tbody>
</table>

Table 1 reports the results on the imprecision, calculated from the differences of duplicates performed over one month of routine use. Twenty-one duplicate measurements were performed per each method.

Table 1. Imprecision of various HbA1c methods.

Accuracy was assessed by analyzing simultaneously a number of blood samples (in EDTA) taken from the laboratory routine, and grouped as follows:

a) A total of 110 samples without renal disease.

b) A total of 10 samples from patients under constant renal dialysis.

From the analysis of the 110 samples from non-nephropatic patients the results of HbA1c by Afinion™ (y-method) showed good correlation with all other methods, with the following linear regressions: y=0.819HbA1c (DCA 2000+)=1.2 (r=0.989); y=0.818HbA1c (NycoCard®)=0.7 (r=0.978); y=0.899HbA1c (Bio-Rad)=0.9 (r=0.974); y=0.783HbA1c (Tosoh G7)=1.4 (r=0.977). On the average the Afinion™ results matched exactly the results from other methods at HbA1c of 6.0 %, were slightly overestimated (+0.3 % on the average) at HbA1c of 4.0 %, and were slightly underestimated (–0.4 %) at HbA1c of 9.0 %. Such differences were constant in the three tested reagent batches. On 1 out of 10 samples collected from patients under constant dialysis regimen the results obtained by the Bio-Rad system gave poor resolution of the carbamylated hemoglobin fraction, which did not affect the HbA1c results obtained by the other methods.

The results obtained by the new POCT system met the NACB guidelines for imprecision and accuracy. The method showed improved imprecision respect to the other POCT system (NycoCard®) produced by the same Company. The presence of carbamylated hemoglobin does not interfere in the measurement of HbA1c by the Afinion™ method.

Method comparison 1

Method comparison 2

The results obtained by the analysis of blood samples from group b (patients under constant dialysis regimen) are shown in Fig. 3. On one out of ten samples the results obtained by one of the HPLC methods used as reference (Bio-Rad Variant II) were not correlated, because carbamylated hemoglobin was not resolved in the HPLC chromatogram.

Method comparison 3

Summary

Afinion™ in our hands was found robust and reproducible for measuring HbA1c. The small bias was not significant to the clinical use, and within the actual goals of NACB guidelines. Error codes occurred only seldom, and were all related to the use of cartridges not brought to the room temperature before usage. We are glad to conclude that our evaluation meets the parallel evaluation recently performed by the Scandinavian evaluation of laboratory equipment for primary health care (SKUP), in which the Afinion™ test system was operated also by two nurses.

References

Six of Eight Hemoglobin \( A_{1c} \) Point-of-Care Instruments Do Not Meet the General Accepted Analytical Performance Criteria

Erna Lenters-Westra\(^1,2\)* and Robbert J. Slingerland\(^1,2\)

**BACKGROUND:** Hemoglobin \( A_{1c} \) (Hb \( A_{1c} \)) point-of-care (POC) instruments are widely used to provide rapid-turnaround results in diabetic care centers. We investigated the conformance of various Hb \( A_{1c} \) POC instruments (In2it from Bio-Rad, DCA Vantage from Siemens, Afinion and Nycocard from Axis-Shield, Clover from Infopia, InnovaStar from DiaSys, A1CNow from Bayer, and Quo-Test from Quotient Diagnostics) with generally accepted performance criteria for Hb \( A_{1c} \).

**METHODS:** The CLSI protocols EP-10, EP-5, and EP-9 were applied to investigate imprecision, accuracy, and bias. We assessed bias using 3 certified secondary reference measurement procedures and the mean of the 3 reference methods. Assay conformance with the National Glycohemoglobin Standardization Program (NGSP) certification criteria, as calculated from analyses with 2 different reagent lot numbers for each Hb \( A_{1c} \) method, was also evaluated.

**RESULTS:** Because of disappointing EP-10 results, 2 of the 8 manufacturers decided not to continue the evaluation. The total CVs from EP-5 evaluations for the different instruments with a low and high Hb \( A_{1c} \) value were: In2it 4.9% and 3.3%, DCA Vantage 1.8% and 3.7%, Clover 4.0% and 3.5%, InnovaStar 3.2% and 3.9%, Nycocard 4.8% and 5.2%, and Afinion 2.4% and 1.8%. Only the Afinion and the DCA Vantage passed the NGSP criteria with 2 different reagent lot numbers.

**CONCLUSIONS:** Only the Afinion and the DCA Vantage met the acceptance criteria of having a total CV <3% in the clinically relevant range. The EP-9 results and the calculations of the NGSP certification showed significant differences in analytical performance between different reagent lot numbers for all Hb \( A_{1c} \) POC instruments.

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Diabetes is one of the most challenging health problems of the 21st century. The International Diabetes Federation estimates that more than 250 million people around the world have diabetes (1). Currently diagnosis and follow-up are usually done in special diabetes care centers. Many patients have their blood drawn a week before they visit the physician to ensure that laboratory results are available for appropriate clinical action. By providing results rapidly following blood collection, point-of-care (POC)\(^3\) instruments could minimize patient inconvenience and possibly avoid an extra visit to the clinic. Studies have confirmed that immediate feedback of hemoglobin \( A_{1c} \) (Hb \( A_{1c} \)) results improves glycemic control in type 1 and insulin-treated type 2 diabetic patients (2–4).

Limited information is available regarding the analytical performance of POC instruments that measure Hb \( A_{1c} \) and whether National Glycohemoglobin Standardization Program (NGSP) certification ensures the accuracy of every instrument used in the field. The information provided by the manufacturers and the limited published data about the performance of POC Hb \( A_{1c} \) instruments suggest that some of these instruments can compete with clinical laboratory methods in terms of analytical performance (5, 6).

The aim of this study was to evaluate all available Hb \( A_{1c} \) POC instruments according to CLSI protocols and to check whether the instruments would pass the NGSP criteria with 2 different reagent lot numbers as judged by comparison with 3 certified IFCC and/or NGSP secondary reference methods.

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Nonstandard abbreviations: POC, point-of-care; Hb \( A_{1c} \), hemoglobin \( A_{1c} \); NGSP, National Glycohemoglobin Standardization Program.
measurement procedures. A manufacturer NGSP certification is performed by experienced technologists at the manufacturer’s site under ideal circumstances and may not reflect the analytical performance of the instruments in the field.

Materials and Methods

The 8 POC Hb A1c analyzers evaluated in this study were:

- The DCA Vantage™ (Siemens Medical Solutions Diagnostics, Tarrytown, NY), which is based on latex agglutination inhibition immunoassay methodology and provides results in 6 min. This is the successor of the DCA 2000™.
- The In2it™ (Bio-Rad, Hercules, CA), which is based on affinity separation, with results available in 10 min.
- The Afinion™ (Axis-Shield, Oslo, Norway), which is based on affinity separation, with results available in 5 min.
- The Nycocard (Axis-Shield), which is based on affinity separation, with results available in 3 min.
- The Clover (Infopia, Kyunggi, Korea), which is based on affinity separation, with results available in 5 min.
- The InnovaStar (DiaSys, Holzheim, Germany), which is based on agglutination immunoassay and provides results in 11 min. At the time of this study the InnovaStar was not yet launched on the market and the manufacturer considered the outcome of this evaluation as a starting point to further improve the method.
- The A1CNow+ (Bayer HealthCare, Sunnyvale, CA), which is an immunoassay with results available in 5 min.
- Quo-Test™ (Quotient Diagnostics, Surrey, UK), which is based on affinity separation and the use of fluorescence quenching, with results available in 3 min.

Apart from the Innovastar all methods were NGSP certified as of May 2009 (7).

We used the CLSI EP-10 protocol to become familiar with the instruments and to get an overall impression of performance (8). The results were sent to the manufacturers for their approval to continue with the evaluation. After we obtained manufacturer’s approval, we used the CLSI EP-5 protocol to further investigate assay imprecision (duplicate measurements twice per day on 2 samples for 20 days) (9). In contrast to the other instruments, the Afinion and the Nycocard do not work with hemolyzed material. Therefore, for this purpose with those 2 instruments we used the 2 controls supplied by the manufacturer.

The CLSI EP-9 protocol was performed twice with 2 different reagent lot numbers, and was used to investigate the bias between the POC instruments and the 3 different secondary reference measurement procedures (n = 40, 5 days, duplicate measurements) (10). Hb A1c value determination of the samples was performed with 3 certified secondary reference measurement procedures:

- Roche Tina-quant Gen.2 Hb A1c on Integra 800, immunoassay, IFCC and NGSP certified (Roche Diagnostics).
- Primus Ultra2, affinity chromatography HPLC, IFCC and NGSP certified (Primus Diagnostics, a Trinity Biotech Company).
- Tosoh G7, cation-exchange HPLC, IFCC certified (Tosoh Bioscience N.V./S.A.).

The secondary reference measurement procedures have documented good results in the IFCC and NGSP monitoring program and were calibrated by using the IFCC secondary reference material with assigned IFCC and derived NGSP values (11–13). To check overall calibration and bias independently of the chosen secondary reference method, the results of the POC instruments in the EP-9 procedure were compared to the mean of the 3 reference measurement procedures. The overall differences in slope and intercept of the regression lines with respect to the 2 reagent lot numbers used were tested by Chow statistics in SPSS version 16.0 with a univariate general linear model that incorporated an interaction-term (lot number * method). A P value of the interaction-term of <0.05 was considered as statistically significant (14).

The results of the EP-9 protocol were also used to calculate the NGSP certification criteria with 2 reagent lot numbers and 3 different reference measurement procedures. The 95% CI of the differences between methods (test method and reference method) should fall within ±0.85% Hb A1c to pass the NGSP criteria. We used the formula: Total error = bias ± 1.96 × SD of differences (15).

STATISTICS

We performed computations using Microsoft® Excel 2002 (Microsoft Corporation) software. Statistical analyses were also performed with the software package Analyse-It® (Analyse-It Software), EP Evaluator Release 8 (David G. Rhoads Associates) (16) and SPSS version 16.0 (SPSS).

Results

Two of the 8 manufacturers (local distributor of the A1CNow instrument, and Quotient Diagnostics of the
Quo-Test instrument) concluded that the EP-10 outcome data did not warrant progression to the EP-5 and EP-9 protocols and decided to discontinue the study (data not shown). At the time of this study, the Quo-Test was a prelaunch instrument and was still in development. The bias found with the EP-10 protocol of the A1CNow was probably due to EDTA interference problems. Normally Hb A1c POC instruments are used to measure Hb A1c directly in capillary blood. Both methods were NGSP certified.

The results of the EP-5 protocol are shown in Table 1. Imprecision ranged from 1.4% CV at an Hb A1c value of 6.3% for the Afinion to 5.3% CV at an Hb A1c value of 6.1% for the Nycocard.

The results of the EP-9 protocol are shown in Table 2, along with the calculations of the NGSP certification criteria and associated P values. The different POC instruments were compared to the 3 reference measurement procedures with 2 different reagent lot numbers. None of the instruments passed the NGSP criteria with 2 lot numbers compared with 3 reference methods. Only the DCA Vantage and the Afinion passed the current NGSP criteria with 2 different lot numbers when compared with just 1 reference method that had the same measurement principle. Based on the Chow-statistics testing for differences in regression lines with respect to the lot numbers used, all regression lines except In2it vs Tina-quant were statistically significantly different (Table 2).

The graphs of the comparisons between the different POC instruments with 2 reagent lot numbers and the mean of the 3 reference measurement procedures are shown in Fig. 1. In addition to the Chow-statistics, which demonstrated between-lot differences in the regression lines, the differences in mean bias between the lot numbers of all instruments seen in Fig. 1 reflected lot number instability, and were largest for the Clover (Clover 0.82, DCA Vantage 0.36, Nycocard 0.29, In2it 0.23, Afinion 0.18, InnovaStar 0.15).

Discussion

There is demonstrated benefit in using POC instruments for the measurement of Hb A1c in certain clinical situations (2–4), but recently concerns have been raised about the performance of NGSP-certified POC instruments compared with laboratory-based methods (17). The overall imprecision as determined by means of an EP-5 protocol is very important for interpretation of Hb A1c results (variability in the patient vs analytical variability). The Diabetes Complication Control Trial found that a 10% reduction in Hb A1c levels resulted in a 43%–45% lowering of risk of retinopathy (18). For optimal clinical monitoring and for effective differentiation of an Hb A1c of 7.0% from that of 7.6% an imprecision of <3% CV is required, assuming an intraindividual biological variation of 2% (19, 20). This criterion is very strict, however, and difficult to meet, even for certain laboratory-based methods (immunoassays). It would therefore seem inappropriate to impose this goal on POC testing devices measuring Hb A1c. Currently, an imprecision of <3% CV is a more realistic, though not optimal goal (21). Only the Afinion and the DCA Vantage were able to meet this criterion in the clinically relevant range (Table 1). The acceptable CVs of these 2 methods make them potentially equivalent to laboratory-based methods, if the problem of lot number instability is resolved and assured.

All of the instruments showed statistically significantly different regression lines for the different lot numbers compared to the mean of the 3 reference methods (Fig. 1). The calibration of the In2it is adequate but the variability of the instrument reflected by a high total CV in the EP-5 protocol, and a high standard error of estimates with the first lot number is still a matter of concern. The second lot number gave better

| Table 1. EP-5 total CV imprecision results from the different POC instruments. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | In2it           | DCA Vantage     | Clover          | InnovaStar      | Nycocard        | Afinion         |
| Patient sample 1| 4.9% (5.1%)     | 1.8% (5.1%)     | 4.0% (5.0%)     | 3.2% (5.2%)     | 4.8% (4.8%)     | 2.4% (4.7%)     |
| Patient sample 2| 3.3% (11.2%)    | 3.7% (11.2%)    | 3.5% (11.9%)    | 3.9% (11.5%)    |                 |                 |
| Nycocard normal control | 5.3% (6.1%) |                 |                 |                 |                 |                 |
| Nycocard abnormal control | 5.2% (11.6%) |                 |                 |                 |                 |                 |
| Afinion control CI |                 |                 |                 |                 | 1.4% (6.3%) |                 |
| Afinion control CII |                 |                 |                 |                 | 1.8% (8.2%) |                 |

*Hb A1c value of the sample/control are in parentheses.
<table>
<thead>
<tr>
<th>Linear regression lines</th>
<th>Lot number 1</th>
<th>Bias</th>
<th>SD of difference</th>
<th>Total error</th>
<th>NGSP criteria</th>
<th>Lot number 2</th>
<th>Bias</th>
<th>SD of difference</th>
<th>Total error</th>
<th>NGSP criteria</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>In2it (y)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>vs Ultra* (x)*</td>
<td>y = 0.95x + 0.26</td>
<td>-0.071</td>
<td>0.414</td>
<td>-0.88</td>
<td>Fail</td>
<td>y = 0.96x + 0.24</td>
<td>-0.040</td>
<td>0.265</td>
<td>-0.60</td>
<td>Pass</td>
<td>&lt;0.001</td>
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<tr>
<td>vs Tina-quant (x)</td>
<td>y = 0.93x + 0.36</td>
<td>-0.160</td>
<td>0.454</td>
<td>-1.05</td>
<td>Fail</td>
<td>y = 0.93x + 0.48</td>
<td>-0.112</td>
<td>0.338</td>
<td>-0.77</td>
<td>Pass</td>
<td>0.061</td>
</tr>
<tr>
<td>vs Tosoh G7 (x)</td>
<td>y = 0.93x + 0.22</td>
<td>-0.300</td>
<td>0.460</td>
<td>-1.20</td>
<td>Fail</td>
<td>y = 0.98x + 0.06</td>
<td>0.113</td>
<td>0.310</td>
<td>-0.72</td>
<td>Pass</td>
<td>&lt;0.001</td>
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<td>DCA V. (y)</td>
<td></td>
<td></td>
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<tr>
<td>vs Ultra* (x)</td>
<td>y = 0.92x + 0.59</td>
<td>-0.056</td>
<td>0.343</td>
<td>-0.73</td>
<td>Pass</td>
<td>y = 1.04x + 0.03</td>
<td>0.316</td>
<td>0.286</td>
<td>0.88</td>
<td>Fail</td>
<td>&lt;0.001</td>
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<tr>
<td>vs Tina-quant (x)*</td>
<td>y = 0.92x + 0.50</td>
<td>-0.141</td>
<td>0.298</td>
<td>-0.73</td>
<td>Pass</td>
<td>y = 1.00x + 0.24</td>
<td>0.244</td>
<td>0.248</td>
<td>0.73</td>
<td>Pass</td>
<td>&lt;0.001</td>
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<td>vs Tosoh G7 (x)</td>
<td>y = 0.97x - 0.01</td>
<td>-0.310</td>
<td>0.290</td>
<td>-0.88</td>
<td>Fail</td>
<td>y = 1.06x - 0.21</td>
<td>0.244</td>
<td>0.282</td>
<td>0.80</td>
<td>Pass</td>
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<tr>
<td>Afinion (y)</td>
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<tr>
<td>vs Ultra* (x)*</td>
<td>y = 0.88x + 0.66</td>
<td>-0.230</td>
<td>0.318</td>
<td>-0.85</td>
<td>Pass</td>
<td>y = 1.00x - 0.14</td>
<td>-0.122</td>
<td>0.213</td>
<td>-0.54</td>
<td>Pass</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>vs Tina-quant (x)</td>
<td>y = 0.83x + 0.94</td>
<td>-0.427</td>
<td>0.473</td>
<td>-1.35</td>
<td>Fail</td>
<td>y = 0.96x + 0.11</td>
<td>-0.176</td>
<td>0.258</td>
<td>-0.68</td>
<td>Pass</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>vs Tosoh G7 (x)</td>
<td>y = 0.87x + 0.63</td>
<td>-0.390</td>
<td>0.410</td>
<td>-1.19</td>
<td>Fail</td>
<td>y = 0.98x - 0.08</td>
<td>-0.224</td>
<td>0.284</td>
<td>-0.78</td>
<td>Pass</td>
<td>&lt;0.001</td>
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<td>Nycocard (y)</td>
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<tr>
<td>vs Ultra* (x)*</td>
<td>y = 0.94x + 0.89</td>
<td>0.405</td>
<td>0.406</td>
<td>1.20</td>
<td>Fail</td>
<td>y = 0.94x + 0.56</td>
<td>0.057</td>
<td>0.335</td>
<td>0.71</td>
<td>Pass</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>vs Tina-quant (x)</td>
<td>y = 0.88x + 1.18</td>
<td>0.212</td>
<td>0.505</td>
<td>1.20</td>
<td>Fail</td>
<td>y = 0.90x + 0.81</td>
<td>0.003</td>
<td>0.403</td>
<td>0.79</td>
<td>Pass</td>
<td>&lt;0.001</td>
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<tr>
<td>vs Tosoh G7 (x)</td>
<td>y = 0.93x + 0.83</td>
<td>0.240</td>
<td>0.440</td>
<td>1.10</td>
<td>Fail</td>
<td>y = 0.92x + 0.62</td>
<td>-0.050</td>
<td>0.380</td>
<td>-0.79</td>
<td>Pass</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clover (y)</td>
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<tr>
<td>vs Ultra* (x)*</td>
<td>y = 0.96x - 0.45</td>
<td>-0.792</td>
<td>0.251</td>
<td>-1.28</td>
<td>Fail</td>
<td>y = 0.98x + 0.12</td>
<td>-0.037</td>
<td>0.299</td>
<td>-0.62</td>
<td>Pass</td>
<td>&lt;0.001</td>
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<tr>
<td>vs Tina-quant (x)</td>
<td>y = 0.90x - 0.18</td>
<td>-0.985</td>
<td>0.345</td>
<td>-1.66</td>
<td>Fail</td>
<td>y = 0.94x + 0.38</td>
<td>-0.090</td>
<td>0.371</td>
<td>-0.82</td>
<td>Pass</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>vs Tosoh G7 (x)</td>
<td>y = 0.94x - 0.51</td>
<td>-0.950</td>
<td>0.310</td>
<td>-1.56</td>
<td>Fail</td>
<td>y = 0.96x + 0.20</td>
<td>-0.140</td>
<td>0.370</td>
<td>-0.86</td>
<td>Fail</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>InnovaStar (y)</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs Ultra* (x)</td>
<td>y = 0.89x + 0.57</td>
<td>-0.277</td>
<td>0.399</td>
<td>-1.06</td>
<td>Fail</td>
<td>y = 0.99x - 0.09</td>
<td>-0.158</td>
<td>0.374</td>
<td>-0.89</td>
<td>Fail</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>vs Tina-quant (x)*</td>
<td>y = 0.84x + 0.82</td>
<td>-0.470</td>
<td>0.490</td>
<td>-1.43</td>
<td>Fail</td>
<td>y = 0.96x + 0.13</td>
<td>-0.231</td>
<td>0.356</td>
<td>-0.93</td>
<td>Fail</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>vs Tosoh G7 (x)</td>
<td>y = 0.89x + 0.46</td>
<td>-0.437</td>
<td>0.372</td>
<td>-1.17</td>
<td>Fail</td>
<td>y = 0.98x - 0.06</td>
<td>-0.261</td>
<td>0.358</td>
<td>-0.96</td>
<td>Fail</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*a This row has the same measurement principle as investigated POC method.
Fig. 1. Hb A1c results for 2 different lot numbers from (A) the DCA Vantage, (B) Afinion, (C) In2it, (D) Clover, (E) Ny cocard, and (F) InnovaStar POC instruments compared to the mean Hb A1c results from 3 secondary reference measurement (SRM) procedures.

The P values of the regression lines between the 2 lot numbers of all POC instruments were <0.001, which confirmed the statistically significant differences between the regression lines.

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results. Unfortunately, it is impossible to predict whether the precision of a particular reagent lot number is acceptable because no duplicate measurements are run routinely with POC-instruments.
The first reagent lot number of the DCA Vantage showed slightly lower results in the clinically relevant range with a low variability (1.8% CV) and higher results in the high range with higher variability (3.7% CV). A recent evaluation of the DCA Vantage also showed lower results compared with the laboratory method; therefore adjustment of the calibration by the manufacturer was justified (22). However, Fig. 1A shows that the manufac-
The Nycocard system showed the worst imprecision of all the systems (Table 1) raising questions regarding its suitability for clinical use. The manual nature of this test may possibly explain the poor precision. The CVs presented here were obtained by the work of an experienced technologist and would likely be worse if the method were used by many different inexperienced personnel. However, the Nycocard passed the NGSP criteria with the second lot number compared with 3 reference methods. The bias of the second lot number was very small, which allowed a higher SD of differences.

The lot number dependency of the Clover was unacceptable (Fig. 1D), and the total imprecision was also too high for optimal clinical use. Because of the poor results seen with the first lot number, the software version of the instrument was successfully updated. All results of the controls were within the limits provided by the manufacturer, whereas the patient results of the first lot number proved to be too low. As a possible way to address such problems, manufacturers should be encouraged to narrow the range of acceptable values for provided QC materials sufficiently to enable users to meet the requirements for good clinical test results.

The InnovaStar method was still under development at the time of this study. The manufacturer regarded the outcome of this study as a starting point to further improve the method. In general lower results were obtained compared with the reference methods.

The measurement principle used with 5 of the 8 methods was affinity separation. This measurement principle is well accepted as being free of interference from hemoglobin variants, a very important attribute for use in areas of the world with a high prevalence of hemoglobinopathies. Healthcare professionals must be aware of potential interferences of rare hemoglobin variants, especially when they use immunoassay-based POC instruments (23, 24).

The NGSP uses 1 comparative secondary reference method for certification, which is usually the same method type. The NGSP also states that manufacturer certification is performed only once per year with 1 lot of reagent and it is up to the manufacturer to ensure consistency among different lots (7, 15). Passing or failing outcomes for NGSP certification of the tested POC methods are clearly dependent on lot number and reference method (Table 2). The NGSP criterion (which specifies that the 95% CI of the differences between methods should fall within ±0.85% Hb A\textsubscript{1c} by January 2010 (25). When this criterion is taken into account, only 9 of the 36 comparisons would pass NGSP criteria and only the DCA Vantage would pass it with 2 different lot numbers compared with just 1 reference method.

The reproducibility of the production of the different reagent lots of the POC instruments investigated appears inadequate at this moment for optimal clinical use of the test results. A manufacturer NGSP certification does not guarantee accuracy of a result produced in the field. We often observed significant differences between lots of reagents in this study. The Nycocard instrument data demonstrated that it is possible to pass the NGSP criteria while the total CV is >5%. Adjustments or additions to the criteria might be considered by the NGSP. For example, we believe the SD of differences should not exceed 0.30% Hb A\textsubscript{1c}. However, a manufacturer NGSP certification is still necessary and is an important tool to prove the optimal analytical performance of a method. In addition users of POC instruments should be required to run daily controls with tight ranges and, as with any Hb A\textsubscript{1c} method, users should participate in external proficiency-testing schemes.

It is important that the limitations of current POC instruments and laboratory methods be understood by healthcare professionals, because these limitations may have important clinical implications. Clinical chemists can play a valuable role by providing healthcare professionals with the information they need (measurement uncertainty) to properly interpret laboratory and POC Hb A\textsubscript{1c} results.

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**Role of Sponsor:** The manufacturers provided all instruments and reagents at no cost. The evaluation protocol was designed by the authors and sent to the manufacturers for comments when they were invited to join the evaluation. None of the manufacturers had comments on the design of the study. The manufacturers had the right to discontinue the evaluation after the EP-10 was finished. The manufacturers played no role in the review and interpretation of data or preparation or approval of manuscript, and had no rights to refusal for publication of the data. The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.
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