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Good correlation between the Afinion AS100 analyser and the ABX Pentra 400 analyser for the measurement of glycosylated haemoglobin and lipid levels in older adults in Durban, South Africa

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Background. The Afinion AS100 analyser is a small bench-top, multi-assay, point-of-care (POC) analyser that is able to measure glycated haemoglobin (HbA1c) and lipid levels.

Objective. To assess performance of the Afinion analyser compared with a reference laboratory test for the measurement of HbA1c and lipid levels.

Method. The study involved men and women enrolled in a cross-sectional study, Sexual health, HIV infection and comorbidity among older Persons (SHIOP), which was conducted from February to May 2016. Whole blood was drawn aseptically by a trained study nurse into a serum separator gel tube and an ethylenediaminetetra-acetic acid (EDTA) tube. The EDTA whole blood was used to measure HbA1c levels, and serum to measure total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglyceride levels. Lin’s correlation coefficient was used to assess the agreement between the Afinion and ABX Pentra 400 analysers for each marker.

Results. A total of 435 older individuals were included in the study. The proportion of HbA1c results that were correctly classified by the Afinion analyser was 92.2%. Bland-Altman analysis and linear regression analysis showed a very good agreement (correlation concordance 0.89) between the two analysers for the measurement of HbA1c. The two-way scatter plot for TC showed a substantial correlation (0.80). However, a total of 69 cholesterol results that were within the normal range on the Pentra were misclassified as abnormal on the Afinion. The readings obtained for HDL-C levels with the Afinion were shown to be slightly overestimated when compared with the Pentra. However, correlation for HDL-C on the two analysers was 0.93, indicating an almost perfect agreement. Seventy-four LDL-C results were erroneously classified as abnormal on the Afinion but were within the normal range on the Pentra, resulting in a substantial correlation of 0.75. An excellent agreement was observed between triglyceride measurements (0.99).

Conclusion. This study supports the use of the Afinion AS100 analyser in POC testing for the measurement of HbA1c, triglycerides and HDL-C in a South African setting.

Globally, 18 million people die annually from cardiovascular disease (CVD). Findings from the INTERHEART Africa study indicated that the highest number of premature acute myocardial infarctions in the world occur in sub-Saharan Africa, as a result of lack of early detection of CVD and effective management of risk factors. Various point-of-care (POC) tests (mainly finger-prick tests) are available that provide results ranging from total cholesterol (TC) alone to a full lipogram. However, the limitation with many of these tests is that the results obtained are not adequate to commit a patient to a lifetime of therapy. In addition, finger-prick testing that measures TC alone will not detect raised triglycerides.

Patients with diabetes mellitus (DM) are at high risk of developing CVD, with increased associated mortality. In many primary care settings, testing for glycaemic control involves sending a blood sample away for laboratory testing and waiting a number of days for the result to be returned. This delays patient counselling and treatment adjustments based on glycated haemoglobin (HbA1c) levels, and sometimes follow-up can be lost completely. Measurement of HbA1c levels using POC tests provides rapid results, improving patient management. Findings from a randomised controlled trial of POC testing in an Australian setting showed that POC tests of samples from patients with established hyperlipidaemia or established type 1 or type 2 DM, or taking anticoagulant therapy, had the same clinical effectiveness as testing in a pathology laboratory. Reports have also shown that access to a POC test is associated with improved treatment adherence.

The Afinion AS100 analyser (Alere, South Africa (SA)) is a small bench-top, multi-assay analyser for in vitro diagnostic POC testing using whole blood, plasma or urine samples. The unique feature of the analyser is that both the HbA1c and the lipid panel test can be done on one instrument. The turnaround time from collection of samples to all results being available to the patient is <30 minutes.
Preliminary results from a recent study on sexual health and chronic morbidities in people aged ≥50 years conducted in the Chatsworth and Botha’s Hill areas of Durban, SA, found that >70% of older adults had elevated blood glucose levels indicating prediabetes and diabetes, and that over half of the study participants had elevated blood pressure readings (unpublished data). In addition, >15% of the study participants were HIV-infected, which is higher than the national average.[9] There is therefore an urgent need to respond to the growing twin health challenges of HIV and non-communicable diseases among older adults.

**Objective**

To evaluate the performance of the lipid and HbA1c panels of the Afinion AS100 analyser compared with standard laboratory methods in a population of HIV-positive and HIV-negative older adults.

**Methods**

**Study participants**

The study was a sub-analysis of a cross-sectional study, Sexual health, HIV infection and comorbidity with non-communicable diseases among Older Persons (SHIOP). The SHIOP study enrolled both men and women and was conducted between February and May 2016. The primary aim of SHIOP was to describe sexuality, sexual health and the comorbidity of HIV and sexually transmitted infections with chronic non-communicable diseases in adults aged ≥50 years in a setting of high HIV prevalence. Participants were recruited from two sub-areas of Durban (Botha’s Hill and Chatsworth), SA. Botha’s Hill is a semi-urban area located west of Durban, and Chatsworth is an urban setting located south of Durban. The study eligibility criteria included age ≥50 years, being willing and able to provide written informed consent and able to communicate in English or isiZulu, and not being terminally ill or cognitively impaired.

**Ethical considerations**

As part of the study procedure, all study participants provided written informed consent prior to enrolment. Participants who were illiterate (i.e. unable to read or write English or isiZulu), were assisted by an impartial literate witness during the informed consent process. The participant used a thumbprint to mark the informed consent form and the impartial witness signed to confirm that the participant provided informed consent. We followed the usual procedure adopted in large-scale multi-site HIV prevention clinical trials. Ethical approval for SHIOP was obtained from the South African Medical Research Council (SAMRC) Ethics Committee (ref. no. EC030-9/2015).

**Sample collection and processing**

All eligible participants were requested to provide a venous blood sample for laboratory testing. Whole blood was drawn aseptically by a trained study nurse into a serum separator gel tube and an ethylenediaminetetra-acetic acid (EDTA) tube. The serum gel tube was centrifuged at the research clinics and the separated serum used for measuring lipid levels, and the EDTA whole-blood tube was used to measure HbA1c levels using the POC Afinion AS100 analyser, which was placed at the research clinics. The remaining serum and EDTA whole blood was transported on ice on the day of collection to the SAMRC HIV Prevention Research Unit’s central routine laboratory for measurement of blood glucose and lipid levels on the ABX Pentra 400 analyser (Horiba, USA). All testing was performed by trained medical technologists.

**POC testing using the Afinion analyser**

**HbA1c measurements**

HbA1c levels were measured using 1.5 µL of EDTA whole blood. The Afinion works on the boronic acid affinity test principle and is not affected by haemoglobin variants. The analyser claims a 4.0 - 15.0% (20.0 - 140.0 mmol/mol) HbA1c measuring range. The assay time was ~3 minutes, and controls with HbA1c-specific target values were included in the assay runs.

**Lipid measurements**

TC, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides were measured using 1.5 µL of serum. The assay time was ~8 minutes, and ready-to-use control material was included in the assay runs. The measuring ranges for lipids using the Afinion analyser were as follows: TC 2.59 - 12.95 mmol/L, HDL-C 0.39 - 2.39 mmol/L, and triglycerides 0.51 - 7.34 mmol/L. LDL-C was a computed value using the Friedewald formula:[10,11] LDL-C (mmol/L) = TC – HDL-C – triglycerides/2.2.

**Reference laboratory testing**

The Pentra analyser was used as the reference laboratory test according to the manufacturer's instructions. According to SA national guidelines, the reference (normal) range for testing and management of lipids is as follows: TC <4.5 mmol/L, LDL-C <2.5 mmol/L, HDL-C 1.0 mmol/L (men) and ≥1.2 mmol/L (women), and triglycerides <1.70 mmol/L. The diagnostic cut-off for HbA1c is 6.5% (or 48 mmol/mol). We used these reference ranges to compare participants categorised as normal v. abnormal on the Pentra and Afinion for each marker.

**Data analysis**

Lin’s correlation coefficient was used to assess the agreement between the Afinion and Pentra analysers for each marker. The mean bias was calculated, as well as the 95% limits of agreement to quantify the difference between a reading on the Afinion and the Pentra. Results were graphically depicted using Bland-Altman plots and two-way scatterplots. The coefficients were classified as indicating poor (<0.69), fair (0.70 - 0.79), good (0.80 - 0.89) or excellent (0.90 - 1.00) correlation.

**Results**

**Sample description**

We obtained data on 435 older adults from Durban, SA. The overall median age of the study participants was 61 years (interquartile range 12), with male participants being slightly older than female participants (62 v. 60 years). Using World Health Organization categorisations of clinically assessed weight and height, ~60% of study participants were overweight or obese, and ~40% (n=172) were hypertensive. Furthermore, 16.1% of participants (n=70) were HIV-positive, of whom 84.3% (n=59) knew their HIV status prior to study participation (data not shown).

**Diagnostic accuracy of the Afinion v. the Pentra analysers**

Table 1 describes the diagnostic performance of the Afinion analyser for the measurement of HbA1c, TC, LDL-C, HDL-C and triglyceride levels in comparison with the Pentra. In addition, the differences between the Afinion and Pentra measurements using Bland-Altman plots are described in Table 2 and Figs 1 - 5.

**HbA1c levels**

The proportion of HbA1c results that were correctly classified by the Afinion analyser was 92.2% (Table 1). We also observed high diagnostic
The accuracy of the Afinion in detecting results that were above target levels, as indicated by both sensitivity and specificity >90%. According to our analysis, higher values for HbA1c levels were detected with the Afinion than with the Pentra. Bland-Altman analysis and linear regression analysis showed an almost excellent agreement between the two analysers (Fig. 1). A correlation of 0.89 was observed (Table 2).

**Lipid levels**

The proportion of TC results that were correctly classified by the Afinion analyser was 79.3% (Table 1). The TC two-way scatter plot represented in Fig. 2 showed a good correlation (0.80) between the two analysers. However, a total of 69 TC results that were within the normal range on the Pentra were misclassified as abnormal on the Afinion, leading to a poor specificity of 63.9%. However, the Afinion correctly classified all 142 results that were in the abnormal range on the Pentra, resulting in a sensitivity of 100%. The mean bias observed for TC measurements between the Afinion and the Pentra analysers was 0.569, with 95% limits of agreement of –0.169 - 1.307 (Table 2).

The readings obtained for HDL-C levels with the Afinion were shown to be slightly overestimated compared with the Pentra (mean bias 0.089). The correlation between the two methods was 0.93, indicating excel-

**Table 1. Diagnostic accuracy of the Afinion analyser compared with the Pentra, using target levels as cutoffs**

|       | Pentra | Afinion |
|-------|--------|---------|
|       | Normal | Abnormal | Correctly classified, % | Sensitivity, % | Specificity, % |
| HbA1c |         |         |                        |                |                |
| Normal | 214    | 7       | 92.2                   | 90.9           | 92.6           |
| Abnormal | 17    | 70      |                        |                |                |
| TC     |         |         |                        |                |                |
| Normal | 122    | 0       | 79.3                   | 100            | 63.9           |
| Abnormal | 69    | 142     |                        |                |                |
| LDL-C  |         |         |                        |                |                |
| Normal | 126    | 1       | 76.1                   | 99.1           | 63.0           |
| Abnormal | 74    | 113     |                        |                |                |
| HDL-C, males | | | | | |
| Normal | 12    | 2       | 90.9                   | 97.5           | 63.2           |
| Abnormal | 7     | 78      |                        |                |                |
| HDL-C, females | | | | | |
| Normal | 76    | 5       | 89.9                   | 96.3           | 80.9           |
| Abnormal | 18    | 129     |                        |                |                |
| TG     |         |         |                        |                |                |
| Normal | 158   | 15      | 95.4                   | 91.1           | 100            |
| Abnormal | 0     | 154     |                        |                |                |

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**Table 2. Differences between the Afinion and Pentra measurements using BA plots**

|       | N     | Mean bias (SD) | 95% BA limits of agreement | Concordance correlation |
|-------|-------|----------------|-----------------------------|------------------------|
| HbA1c | 308   | 0.224 (0.840)  | –1.421 - 1.870              | 0.89                   |
| TC    | 333   | 0.569 (0.377)  | –0.169 - 1.307              | 0.80                   |
| LDL-C | 314   | 0.528 (0.385)  | –0.227 - 1.283              | 0.75                   |
| HDL-C | 327   | 0.089 (0.095)  | –0.097 - 0.275              | 0.93                   |
| TG    | 327   | –0.124 (0.127) | –0.373 - 0.125              | 0.99                   |

BA = Bland-Altman; SD = standard deviation; HbA1c = glycated haemoglobin; TC = total cholesterol; HDL-C = high-density cholesterol; LDL-C = low-density cholesterol; TG = triglycerides.
Discrimination of normal HDL-C levels by the Afinion was low in both males and females. The misclassification of HDL-C results was due to target levels on the Pentra being incorrectly set. 7 of 19 HDL results that were below the normal range on the Pentra, giving a specificity of 100%.

The Bland-Altman study and linear regression analysis showed a substantial agreement between the two analysers. (LDL-C = low-density lipoprotein cholesterol.)

Excellent agreement was observed between triglycerides measured on the Afinion and the Pentra, evidenced by the mean bias of 0.124 and almost perfect agreement between the two analyzers, with a correlation of 0.99 (Fig. 5). When categorising values according to target levels, excellent diagnostic accuracy was observed, with a sensitivity of 91.1% and specificity of 100%.

**Discussion**

The objective of this study was to evaluate the performance of the Afinion AS100 analyser compared with a standard laboratory method for the measurements of lipids and HbA1c in a population of HIV-positive and HIV-negative older adults. Overall, Bland-Altman plots revealed a good correlation between the Afinion and Pentra 400 analysers for the measurement of HbA1c levels. In addition, the Afinion was able to correctly classify >90% of the samples. With regard to the measurement of lipid levels, the results showed very good correlation between the Afinion and the Pentra, with almost perfect correlations noted for HDL-C and triglycerides.

HbA1c measurement is an important tool in the management of patients with DM. Evidence for the utility of HbA1c for the diagnosis and detection of DM in an SA population has been described by Hird et al. However, in SA primary healthcare facilities, random blood glucose measurement is commonly used to make clinical decisions with regard to glycaemic control, as HbA1c tests have not been done or the results are not yet available or are out of date. In addition, the DiabCare Africa study found that <50% of patients with DM had had an HbA1c test as part of their management during the previous year.

To this end, the availability of a rapid and accurate method of HbA1c evaluation is of paramount importance. According to Gaziano et al., since the late 1990s, deaths due to non-communicable diseases have increased steadily in adults aged ≥50 years. A number of risk factors contributing to mortality, such as hypertension, increased...
smoking prevalence, dietary changes and obesity, have been identified. These risk factors have also led to increases in symptomatic cardiovascular conditions such as stroke, ischaemic heart disease and DM, to which health policy makers have yet to provide an effective response. The global diagnostics industry is growing rapidly, since new diagnostic platforms have advanced modern healthcare in terms of the accuracy (sensitivity, specificity and reproducibility), speed and scope of diagnoses.\[16\]

The present study revealed a good correlation between the Afinion and Pentra analysers for the measurement of HbA1c levels. Our findings are supported by other published studies. A study by Lenters-Westra et al.\[17\] evaluated seven point-of-care analysers for HbA1c (DCA Vantage, Afinion, Innova Star, Quo-Lab, Quo-Test, Cobas B101, B-analyst). The Afinion passed the National Glycohemoglobin Standardization Program (NGSP) criteria with two different lot numbers. In addition, the Afinion remained unaffected by the common haemoglobin variants. The study concluded that the Afinion met the performance criteria for HbA1c.\[18\] Previous studies on the diagnostic accuracy of the Afinion have been conducted on paediatric samples, as described by Wood et al.\[19\] In this study, the Afinion's accuracy and precision was compared with high-performance liquid chromatography (HPLC) and DCA methods. The findings showed that the Afinion generated higher HbA1c results when compared with HPLC, while the DCA produced lower values. At high HbA1c levels, the DCA tended to read lower than HPLC, but the Afinion's accuracy did not vary according to HbA1c.\[20\] Jain et al.\[21\] evaluated the performance of the Afinion in a DM and CVD screening programme. In this study, the Afinion and the reference laboratory method identified similar numbers of new patients with suspected DM. In the present study, the researchers also found that the Afinion detected HbA1c levels that were above target levels. Similar findings were reported by Jain et al.,\[21\] with the Afinion being shown to overestimate HbA1c levels for certain samples. However, similar to the present study, the overestimation did not affect the overall diagnostic performance of the instrument. The Afinion is still recommended as a useful screening instrument for DM in a community setting, but diagnosis needs to be confirmed by an NGSP-certified method.\[21\]

In SA, since the prevalence of familial hypercholesterolaemia is as high as 1 in 100 in some communities, ideally everyone should undergo a full lipogram, or at minimum TC/LDL-C measurement, at least once in young adulthood (from 20 years of age).\[22\] Although the measurement of lipids in the blood is a widely accepted biochemical marker for cardiovascular risk assessment and management in primary healthcare,\[23,24\] testing performed in hospital laboratories may result in excess travel for the patient, sample loss and repeat clinic visits.\[25\] A survey of the literature showed a limited number of studies that have investigated the performance of the Afinion analyser in measuring lipid levels. Our study is one of two suggesting that the Afinion is an acceptable POC tool for the measurement of lipids. Our findings support those of Jain et al.,\[21\] who compared the Afinion with the Cholestech LDX for the measurement of TC, HDL-C and triglyceride levels. The Afinion was shown to meet the performance criteria recommended by the National Education Cholesterol Program in the UK for TC and HDL-C measurements.\[26\] In the present study, the readings obtained for HDL-C levels with the Afinion were shown to be slightly overestimated compared with the Pentra. Similarly, the Afinion was shown to overestimate HDL-C levels in the study by Jain et al.\[21\] Despite the overestimated values in the present study, overall the correlation between the two instruments used showed an excellent level of agreement. Our study adds to the growing body of evidence regarding the diagnostic performance of the Afinion for the measurement of lipid levels.

**Study limitations**

A limitation of this study is that the testing was performed in a laboratory setting by trained laboratory personnel and is not truly representative of a primary healthcare clinic setting. Field studies at primary healthcare facilities using healthcare workers (nurses) to perform the testing could be a future research direction. The study was also limited to a targeted population (older individuals). We have provided a strong rationale of why we chose to work with this population. However, future studies may need to be conducted in a more general population group. Cost-effectiveness studies on the use of the Afinion in resource-poor settings are needed.

**Conclusion**

The study is unique in that it provides the first report on the diagnostic performance of the Afinion AS100 analyser in measuring HbA1c and lipid levels in a population of HIV-infected and uninfected adults aged ≥50 years in KwaZulu-Natal Province, SA. In our study population there were no significant differences in HbA1c and lipid levels according to HIV status. In addition, there have been no reports demonstrating the role of HIV infection and antiretroviral use in affecting the diagnostic performance of point-of-care instruments for HbA1c and lipid levels. This study supports the use of the Afinion as a POC test for the measurement of HbA1c, triglycerides and HDL-C in an SA setting.

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**Conflicts of interest.** None.
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