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ARTICLE

The Accuracy and Precision of the Diazyme SMART 700-300 Analyzer for HbA1c and hs-CRP

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Abstract

Purpose: To assess the accuracy and precision of the Diazyme SMART analyzer through testing glycosylated hemoglobin (HbA1c) and high-sensitivity C-reactive protein (hs-CRP). ***Methods:*** Forty-five whole blood samples were collected from 15 participants (4/11m/f, 63 ± 8 yr). Two samples were used to determine precision by comparing repeated tests for both hsCRP and HbA1c. Accuracy was examined by comparing the analyzer results to the third sample measured by standard procedure in a hospital laboratory. ***Results:*** Test-retest revealed a mean difference of 0.07 ± 0.44% (p = 0.6), ICC = 0.72 for HbA1C, and 0.1 ±0.7mg/L (p=0.5), ICC = 0.99 for hs-CRP. Comparison of Diazyme SMART results with the lab results for HbA1C resulted in a mean difference of 1.6 ± 0.1%, p = 0.3; r = 0.75 and 1.9 ± 4.8 mg/L, p = 0.2; r = 0.98 for hs-CRP. ***Conclusion:*** The Diazyme SMART analyzer provides precise and accurate measures of hs-CRP while HbA1c values were less precise. The system may offer acceptable accuracy and precision for select screening in a research laboratory or clinic setting. **Health & Fitness Journal of Canada 2017;10(2):77-83.**

Keywords: Diabetes; Cardiovascular Disease; Accuracy; Precision; Clinical Research

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Introduction

Cardiovascular disease (CVD) causes one-third of deaths in Canada, more than any other illness Genest et al., 2009) . Therefore, cardiovascular risk assessment is an important routine clinical practice that helps clinicians identify patients most likely to benefit from primary or secondary prevention therapies (Sheridan et al., 2010). It has been shown that the benefits of risk assessment are maximized when the results are directly communicated to the patient with advised therapy, as compliance increases when patients are engaged in treatment decisions (Grover, 2007). Direct communication is enabled by the use of point of care diagnostics that can measure pathology tests on site within a short time frame, and test results can be discussed with the patient in the same visit. Certain benefits for rapid bench top analysis are also applicable in a human research setting, as point of care diagnostic devices reduce the expertise and time required to perform tests normally done in conventional reference laboratories. This provides research settings the benefit of quick and easy data acquisition, while minimizing the risk of human error in assay analysis.

The Diazyme SMART analyzer represents such a point of care diagnostic instrument that does not require extensive blood preparation or complex lab-based assays. The Diazyme SMART analyzer can be used for diabetes management and cardiac risk assessment and gives results for glycosylated hemoglobin (HbA1c), high-sensitivity C-reactive protein (hs-CRP), amongst others (CRP, homocysteine, microalbumin and D-

dimer). HbA1c has been recommended to be the primary test used in the diagnosis of pre-diabetes, type 1 and type 2 diabetes (International Expert Committee, 2009); whereas Hs-CRP is a inflammatory marker associated with many chronic disease states and by which risk can be assessed, with a pro-inflammatory state denoting a higher risk of CVD (International expert committee, 2009;·Pearson et al., 2003). This analyzer could also be used to detect changes from human interventional studies or provide baseline data, for the classification or inclusion of subjects, in the outcomes previously mentioned.

The Diazyme SMART analyzer quantitatively measures hs-CRPC and HgA1c in human venous whole blood. The hs-CRP automated process is based on a latex enhanced immunoturbidimetric assay using antigen-antibody reactions and monitoring absorbance changes following agglutination. The HbA1c assay is based on protease digestion and the release of glycated valines from the hemoglobin beta chains. The system has an auto-calibration for samples, which is checked using a known control sample of hs-CRP or HbA1c in reconstituted blood purchased from the manufacturer hs-CRP.

Potential advantages of the Diazyme SMART are a relatively low cost per test, ease of use, and high inter-operator standardization device that offers an advantage for frontline clinical use and human research based data acquisition. The accuracy and precision of this analyzer has yet to be shown, and the true value for front-line and research laboratory use depends on the trustworthiness of results. Therefore, the purpose of this study is to assess the accuracy and precision of the Diazyme SMART using the parameters HbA1c and hs-CRP, by way of repeated sample testing and compared to a hospital lab-grade analysis.

Methods

A total of 45 blood samples were collected from 15 independent subjects who were recruited from the greater Charlottetown area at the medical practice of one of the participating authors (GS). Participants were both male (n = 4) and female (n = 11) and of middle to older age (63 ± 8 yr) visiting their family physician for a regular check-up. Full study procedures were explained to all participants and informed written consent was obtained prior to the inclusion of any participant's data. All procedures were approved by the Human Ethics Research Board of the sponsoring University and were conducted in accordance with the declaration of Helsinki.

Design

In a repeated design, three samples of blood from each participant were analyzed for each of HbA1C and hs-CRP for a total of 90 samples (45 HbA1c, 45 hs-CRP). Two were completed using the Diazyme SMART and one using the standard laboratory measure at the local hospital (Queen Elizabeth Hospital). Laboratory analyses at this hospital are officially accredited by the accreditation division of Health Canada, with a coefficient of variation no greater than 0.02 for any parameter. Precision was determined using a repeated test of two like samples using the Diazyme SMART. Accuracy was examined by comparing the Diazyme SMART result to the gold standard lab results.

Procedure

A medical doctor oversaw all health screening and blood collections, with each

participant providing samples in standard 10 mL EDTA containing vacutainers collected by a registered nurse using standard venipuncture from the antecubital fossa. One blood sample was transported on ice to the Queen Elizabeth Hospital, while the other sample was transported on ice to the Human Health and Performance Lab at the University of Prince Edward Island, where it was analyzed within 3 hr. In the HHPL at UPEI, test-retest values for hs-CRP and HbA1c using the Diazyme Smart were calculated, and compared to the values obtained from the Queen Elizabeth Hospital.

Analytical Technique

Each Diazyme Smart assay kit contains a specific assay reference ID (RFID) card that is inserted into the analyzer that contains a pre-programmed specific calibration curve for the assay. It also contains a time stamp that ensures the assays are used within a pre-determined time period of viability from the time of manufacture. Figure 1 shows the contents of a HbA1c assay kit. Using a 100 µL fixed volume pipette; blood was placed in a microtube containing blood lysis buffer. This microtube was left to lysate for 10 min. In the mean time, 100 µL of Reagent B was transferred to a cuvette containing Reagent A. With a new pipette tip, 100 µL of lysated blood was transferred into the cuvette now containing Reagent A&B. This Cuvette was covered using a reagent C containing Cap and placed in the cuvette holder of the Diazyme SMART.

Figure 2 shows the contents of a hs-CRP assay kit. Using a 20µL fixed volume pipette, blood was added to a cuvette that contains Reagent I. This cuvette was covered with a cap, containing reagent II and placed in the cuvette holder of the Diazyme SMART.

Upon placing the sample in the analyzer and closing the door, the automated mixing of reagents and analysis occurs, for both types of assay. Assay results were displayed on screen in approximately 7 min for HbA1c assay and 4 min for the hsCRP assay.

Figure 1: The hbA1c assay kit contents used for the Diazyme Smart Analyzer.

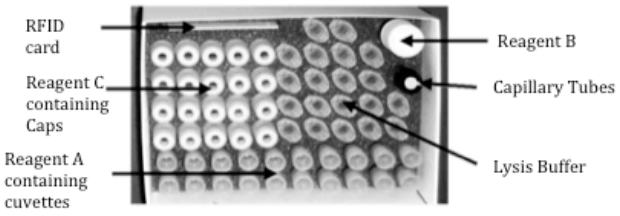
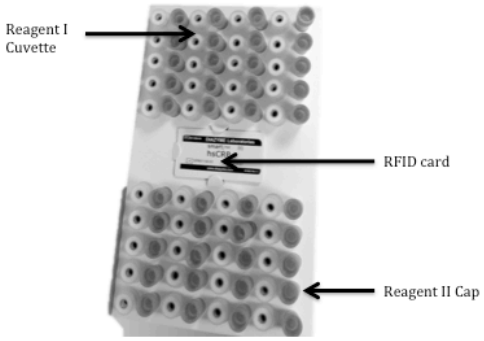


Figure 2: The hs-CRP assay kit contents used for the Diazyme Smart Analyzer.



Statistical Analysis

Data were analysed using SPSS version 21.0 (SPSS Inc, Chicago, IL, USA). The mean difference between the test-retest data and an intra-class correlation coefficient (ICC) was used for precision analysis. Paired t-tests were performed to determine statistical differences between test-retest values. To analyze accuracy, the repeated test values were averaged and compared against the gold standard using an independent samples t-test. Pearson correlation was also performed to evaluate the linear relationship

between laboratory and Diazyme analyzer. An alpha-value of $p < 0.05$ was selected for all comparisons, *a priori*.

Results

The tests are expressed as means and standard deviations (Table 1). The patient samples were distributed evenly over the range of 6.4-7.8% for HbA1C and 0.5-15.3 for hs-CRP. The test-retest using the Diazyme revealed a mean difference between tests of 0.07 ± 0.44% ($p = 0.6$), ICC = 0.72 for HbA1C. The Bland-Altman plot for the HbA1C tests is presented in Figure 3. For hs-CRP the mean difference between tests was 0.1 ± 0.7 mg/L ($p = 0.5$), ICC=0.99. The Bland-Altman plot for the hs-CRP tests is provided in Figure 4. Comparison of results between the analyzer and the hospital lab for HbA1C resulted in a mean difference of 1.6 ± 0.1%, $p = 0.3$; $r = 0.75$. For hs-CRP, the mean difference was 1.9 ± 4.8 mg/L, $p = 0.2$; $r = 0.98$.

Table 1: Test, retest, and gold standard values, and relative and absolute reliability of HbA1c and hp-CRP.

	Test	Re test	ICC
hs-CRP (mg/L)	4.4±3.8	4.3±3.8	0.99
HbA1c (%)	7.0±0.5	6.9±0.4	0.72

Figure 3: Bland-Altman plots representing comparisons between the tests, re-test values for the HbA1c values measured by the Diazyme SMART. The mean line represents the mean difference between the test, with the upper and lower lines representing the limits of agreement (2SD).

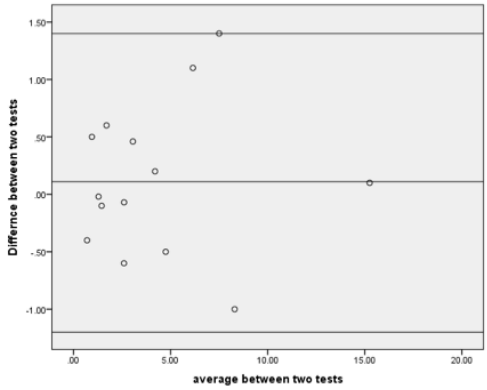
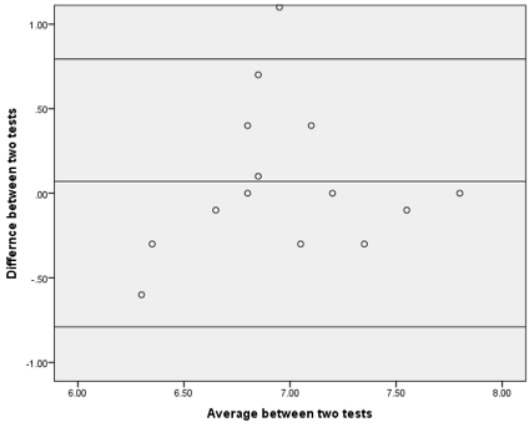


Figure 4: Bland-Altman plots representing comparisons between the test, re-test values for the hs-CRP values measured by the Diazyme SMART. The mean line represents the mean difference between the test, with the upper and lower lines representing the limits of agreement (2SD).



Discussion

The ability for clinicians and researchers to assess HbA1c and hs-CRP at the point of care using a portable, inexpensive, reliable and valid system could provide important benefits.

Hs-CRP

A high ICC (0.99) was found for the hs-CRP test-retest values measured by the Diazyme SMART, in addition to no statistical difference between mean test results by time (p = 0.5) indicating a very high reliability of measurement. As is evident in Figure 4, there is no proportional bias, as there are no more points above or below the mean difference line. There appears to be excellent agreement (r = 0.98) and no difference in means between the Diazyme SMART values and hospital laboratory values for hs-CRP (p = 0.20). These results provide evidence that, in an ostensibly healthy population undergoing routine screening or a research intervention, the Diazyme SMART analyzer can provide a reliable and valid measurement of hs-CRP.

The Center for Disease Control and American Heart Association recommendations for the interpretaion of hs-CRP levels in clinical practice indicate normal levels to be <1.0 mg/L, with extremely high levels (>10 mg/L, which were not observed in the present investigation) warranting the investigation of an obvious cause of inflammation. Cut points of risk are: low <1.0 mg/L; average: 1.0-3.0 mg/L; high: >3.0 mg/L (Pearson et al., 2003). This study observed 79% of participants classified as low risk (<1.0 mg/L), while 21% fell within the average risk cutpoints (1.0-3.0 mg/L). Our study observed an average SMART analyzer hs-CRP value of 0.44 ± 3.8 mg/L, compared to a retest value of 0.43 ± 3.8 mg/L and a mean hospital lab value of 0.62 ± 5.58 mg/L. Although this suggests a slight underestimation of CRP by the SMART analyzer (0.18 mg/L) this discrepancy was not statistically different (p = 0.6, p = 0.2), and such an error is far from altering the clinical risk classification from optimal to average and even further from high risk. These results suggests the measurement of hs-CRP by the Diazyme SMART is highly reliable, and clinically valid, and thus could be useful for CV risk factor assessment in a frontline clinical setting.

HbA1c

Moderate to high ICC (0.71) was found for HbA1c test-retest values measured by the Diazyme SMART, with no difference between mean test-retest values (p = 0.6). As can be observed in Figure 3, there again seems to be no proportional bias, as there are no more points above or below the mean difference line. Moderate to good agreement (r = 0.75) was found between the Diazyme SMART and hospital laboratory values for HbA1c, with no test-group mean difference (p = 0.30). These results suggest suitable percision and accurate for the Diazyme SMART when measuring the parameter HbA1c.

Comparison of the HbA1c results between the SMART analyzer and the hospital lab revealed a non-significant difference (1.6 ± 0.1%, p = 0.30) that may be clinically significant given its magnitude. For people without diabetes, the normal range for HbA1c is between 4 and 5.6%. HbA1c levels between 5.6 and 6.0% indicate a substantially increased risk of diabetes, while levels from 6.0-6.5% indicate a very high risk of diabetes (Zhang et al., 2010). HbA1c levels of >6.5% indicate diabetes. The SMART

analyzer showed a mean HbA1c of 7.0% compared to the mean lab value of 5.5%, which meaningfully inflated the category of risk. The SMART analyzer value is over the diabetes diagnostic cut point whereas the lab test value borders on the cut-point between "normal" and elevated risk. This difference is of obvious clinically importance, as a difference in characterization as such could lead to incorrect management and action, and would at the very least require further testing. This result detracts from the device's applicability in the clinical setting, as accuracy of the HbA1c assay may not be good enough for diagnosis/risk assessment. That being said, the SMART analyzer may still be appropriate in a research setting when measuring inter-individual changes in HbA1c, as precision seems to be suitable.

Limitations

There are some notable limitations to the current study, including the observed absence of statistical difference between Diazyme SMART test-retest values or between the device and the hospital laboratory value. It is important to note that a lack of statistical difference does not mean the values are thus the same, as this could be affected by other factors including group mean differences (which were small), individual variance in the data (which was larger) and the number of observations (which was relatively small at n = 30 samples). That said, group mean differences in the current investigation were a secondary analysis and do not take away from the main findings, which offer important insight for laboratory and point of care use. Another potential limitation for broad generalizability of these results is the range of scores across which the device was tested, which did not include persons with known existing cardiovascular or cardiometabolic disease. As the purpose of our study was to include persons who would regularly be screened in a medical clinic or characterized for participation in our human exercise, it is unknown if the same reliability and accuracy would be found at all ranges of test values, but this offers an important area for future investigation.

Conclusion

Measurements made using the Diazyme SMART Analyzer showed moderate test-retest precision and accuracy for the HbA1C test, but showed very high test-retest precision and accuracy for the measurement of hs-CRP. The results suggest that the Diazyme SMART analyzer can be a reliable and valid measurement tool for hs-CRP and reliable, but less accurate for measures of hbA1c. These considerations should be made when determining the utility of this device as a screening tool or for characterization of participants in research studies.

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Conflicts of Interest

None

Authors' Qualifications

The authors' qualifications are as follows: Joshua Slysz MSc, Garth Slysz MD, Andrea Slysz RN, and Jamie Burr MSc, PhD, CEP

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