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Smartphone-based digital point-of-care panel assay with enzymatic catalytic reaction

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ABSTRACT

The recent pandemic improved awareness amongst the public of the need for rapid blood tests for community and home settings. In this work, we evaluated the performance of a digital, lipid panel test in microfluidic assay format which can be read using a smartphone camera. The PocDoc Lipid test is embedded within a cardiovascular screening application that utilizes the QRISK3 risk prediction algorithm to determine an individual's risk of having a cardiovascular event in the next 10 years and their healthy heart age. The test can be used to screen for individuals at risk of hyperlipidemia (e.g. high total cholesterol or triglycerides) and for individuals at high risk of cardiovascular disease at home or in community or surgery settings. The device was evaluated in a performance evaluation study, using 125 whole blood samples, following CLSI guidelines. Performance evaluation of the PocDoc device demonstrated accuracy that meets international NCEP guidelines and that is on par with other point-of-care tests. Sensitivity and specificity analysis supports the use of PocDoc to identify patients with hyperlipidemia or at high risk of cardiovascular disease. Bland-Altman analysis suggests that this point-of-care device can be used as an alternative to venous blood collection. This single-step model for cardiovascular disease risk measurement which can be done at home or in community settings may improve cardiovascular disease prevention.

1. Introduction

1.1. Clinical needs

The Covid pandemic accelerated a trend for consumers to seek alternative healthcare solutions outside of the traditional surgery setting (Lee and Lee, 2021). On the one hand, patients have become increasingly comfortable with performing tests such as nasal swabs and fingerprick blood tests on themselves. On the other hand, the increasing demands of a growing population and shrinking workforce on the primary care service have increased waiting times for appointments, at least within the UK's National Health Service.

Cardiovascular disease (CVD) is the leading cause of death globally, yet 80% of premature deaths caused by CVD are preventable by risk factor modifications, including diet and lifestyle changes (Vaduganathan et al., 2022; WHO, 2024). One of the most significant modifiable risk factors is having high lipid levels (dyslipidemia) (Joseph et al., 2017; Kopin and Lowenstein, 2017). The increasing incidence of dyslipidemia, obesity, high blood pressure and diabetes is causing an epidemic of CVD (Movsisyan et al., 2020; Reddy and Yusuf, 1998). This increasing burden of CVD calls for an evidence-based approach by healthcare professionals to manage and decrease CVD risk effectively.

As an example, in the UK, the NHS Health Check is a publicly funded program of health checks designed to identify people at high risk of preventive diseases such as CVD (Tanner et al., 2022). However, the program has a limitation in that less than 50% of those between the ages of 40 and 75 eligible for the program have been tested. Furthermore, the program has been more successful in engaging people in higher-income areas compared to deprived areas where the CVD burden is higher (NHS, 2022). Individuals from low socioeconomic status have been shown to disproportionately suffer high CVD morbidity and mortality and are more likely to have CVD events with poorer outcomes (Schultz et al., 2018). Therefore, solutions that can support patients in these groups with CVD prevention and screening are sought after.

Community screening of CVD has potential to identify individuals at high risk of CVD who are unaware of that risk (Lang et al., 2016). Supporting those individuals at risk for CVD in making positive lifestyle changes and/or offering them advice on lipid lowering therapies

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promises to reduce their risk and decrease the mortality and morbidity of CVD in the community. Schemes such as the NHS community pharmacy hypertensive advanced service (NHS, 2023), aiming to prevent screen for hypertension in community settings such as pharmacies, community centers, sports grounds, and places of worship have been received well and have succeeded in engaging the community outside of traditional surgery settings (Anderson and Sharma, 2020) (Mahase, 2023). Screens in community settings also are attractive to people who cannot make or attend GP appointments due to childcare (Alvarez et al., 2022), shift working (Costa, 2010) or transportation issues (Syed et al., 2013), offering a more accessible option for those facing logistical challenges in accessing traditional healthcare services. At-home screening services whereby individuals can elect to have tests sent to them at home are also very attractive to this demographic.

CVD risk assessment tools are used to determine the risk of an individual suffering a cardiovascular event over the next decade. This in term determines whether that individual should be offered lipid-lowering therapy. In the UK the QRISK tool, currently encompassing the QRISK3 algorithm (Hippisley-Cox et al., 2017), is used routinely in clinical practice to do this. One important contributor to QRISK is an individual's lipid levels, specifically, the ratio of Total Cholesterol (TC) to High-Density Lipoprotein (HDL). This metric has a high predictive value of cardiovascular disease (Millán et al., 2009).

Although point-of-care devices measuring lipid panel biomarkers have been around for decades, the devices are not digitally integrated with risk assessment tools and patient records (Jiang et al., 2019). This results in a high administrative burden of using the data using risk assessment tools and high potential for user error when inputting the values. Therefore point-of-care lipid screening is not routinely used in clinical practice as part of an integrated CVD screen. Further, the individual devices are expensive, preventing testing in the home environment, or scaling in the community, especially in lower socioeconomic areas.

The PocDoc Lipid test represents an affordable lipid panel test in single-use microfluidic assay format (Fig. 1). The diagnostic reader comprises a smartphone application containing a full QRISK3 assessment. This enables a user to measure a full lipid panel and at the same time to receive the results of the QRISK assessment in the context of a full cardiovascular risk assessment. The test can be conducted at home, in a community setting or in a surgery setting.

1.2. Detection of lipids from whole blood

Existing point of care tests that measure lipids utilize a simple enzymatic reaction which, when combined with dyes, produce a color change (Nirala et al., 2018; Sharma et al., 1987; Sperry and Brand, 1943). Cholesterol and Cholesterol esters are hydrolysed by cholesterol esterase into cholesterol, which is then oxidized by cholesterol oxidase into the ketone cholest-4-en-3-one. H_2O_2 formed as a bi-product reacts with 4-aminophenazone and a dye in the presence of horseradish peroxidase (HRP) to generate a visible colour that can be recorded with a regular camera (Scheme 1) (Böck et al., 2020; Menezes et al., 2015).

In recent years the quality of smartphone cameras has improved so that they are able to quantify color change of colorimetric reactions (Fan et al., 2021; Shen et al., 2012). Both TC and HDL are detected using this method. The HDL reaction includes an additional step to separate the non-HDL fragments within the cassette before detection (Schriewer et al., 1984).

The presented lipid panel test works in a microfluidic assay format, using a fingerprick of blood. Algorithms contained in the PocDoc mobile application control for different lighting conditions using different mobile phone models and enable the colorimetric reaction within the microfluidic assay test to be quantified. In this way the lipid test can quantify levels of TC, HDL and TG in the test. The levels of Non-HDL, LDL and the TC: HDL ratio are then calculated within the app. The PocDoc app also feeds the lipid results into the QRISK3 CVD assessment

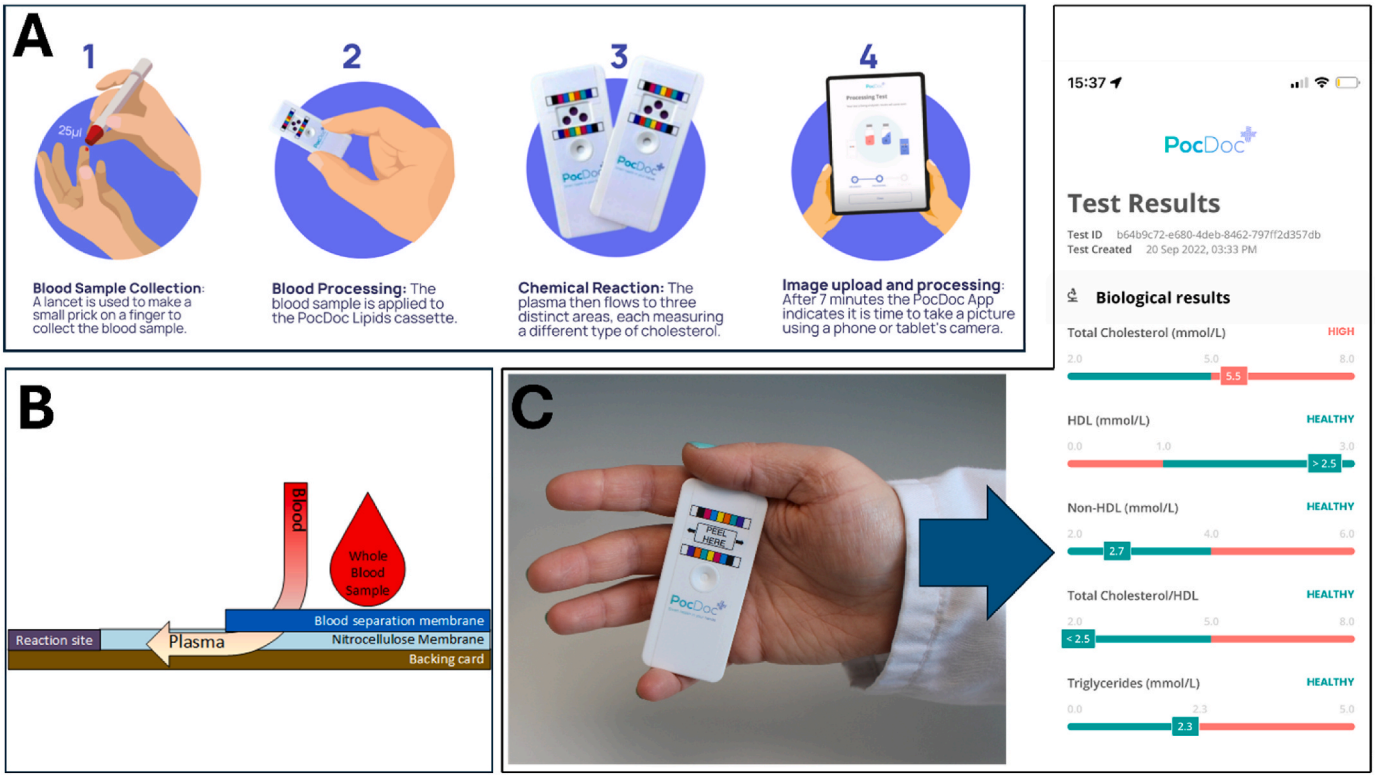
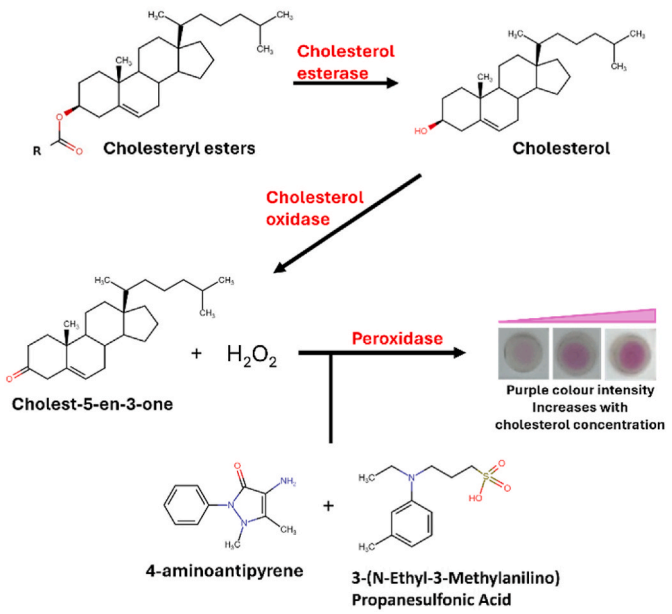


Fig. 1. **A –** 4-step process involved in the blood cholesterol test; **B –** illustration of the individual components that are assembled together to create the microfluidic assay device; **C** – the lipid test is just slightly wider than a standard microscope slide (35 mm × 79 mm), it fits into a palm of a hand and requires no electronic components, the smartphone serves as an image reader, analyzer and a results display.



Scheme 1. An example of colorimetric principle of cholesterol detection. The resulting dye complex gives a visible purple colour, distinguishable from the background.

tool along with other data collected in an initial health questionnaire such as BMI, BP and medical history. The PocDoc application displays the lipid level results next to the NHS recommended values as well as the person's 10-year risk of having a cardiovascular event and their healthy heart age. In this way PocDoc can be used as a screen to identify individuals with high risk of CVD (in the UK this is defined as those with greater than 10% risk of CVD). Integrations with electronic medical records support clinical actioning of the results. In this work, we present a complete performance evaluation of the lipid test to support clinical utility of PocDoc in CVD screening in the community.

2. Methodology

2.1. Ethical considerations

This study was conducted in accordance with the Declaration of Helsinki. The study was registered and approved by the Integrated Research Application System (IRAS study number 322100). Ethical approval and written informed consent were obtained prior to enrollment.

2.2. The PocDoc technology

The PocDoc Lipid Test (Fig. 1A and B) comprises a microfluidic assay device with 3 reaction zones capable of measuring enzymatically total cholesterol, HDL cholesterol and triglycerides. Non-HDL, LDL and the TC:HDL ratio are inferred calculations derived from these direct measurements. The basic components of the microfluidic assay device are a) nitrocellulose membrane (Cytiva, FF80HP); b) blood separation membrane (Whatman, MF1); c) backing card (Kenosha, KN-PS10265.279); d) cassette (Europlaz, custom made). The assembly of layers a-c is illustrated in Fig. 1B. The user enters personal information required for the CVD risk assessment into the PocDoc mobile application. A fingerprick blood sample is then applied onto the disposable PocDoc microfluidic assay device. The blood travels through a blood separation membrane, where the blood cells are separated from plasma. Plasma continues flowing along the membrane microchannels to the point where it is mixed with enzymes and organic dyes to produce a visible, colorimetric output. After 7 min, the app guides the user to take a picture of the lateral-flow cassette through the PocDoc Pro App. An app-built algorithm then quantifies the levels of colour change. The PocDoc algorithms contain pre-programmed calibration curves constructed using Human Blood Samples measured in UKAS ISO15189:212 accredited laboratory by Roche Cobas Photometry method. The PocDoc Results Dashboard within the mobile application displays the results next to NHS recommended targets. Non-HDL, LDL and the TC:HDL ratios are calculated from these levels, and the App reports the results on-screen and by email to the user who optionally provided an email address during testing.

2.3. Performance evaluation methodology

The analytical performance of PocDoc was investigated through a performance evaluation study conducted between September 2022 and July 2023. The specific experimental designs were adapted from guidelines published in Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2016).

2.4. Blood measurement procedure example

Healthy individuals and patients with dyslipidemia and/or diagnosed cardiovascular disease aged >18 years were included in this study. Whole blood specimens from participants were collected in Lithium Heparin tubes collected from a venipuncture site using an accepted phlebotomy technique avoiding excessive blood cell trauma causing lysis of the cells.

2.4.1. Candidate measurement procedure

A single replicate per specimen was needed for the candidate measurement procedure. This was to ensure that the resulting analytical error estimate reflects the accuracy of the candidate measuring procedure under typical use and fairly represents analytical accuracy for patient testing. One half of the tested blood sample was sent to the comparative analytical laboratory (a full lipid panel being measured using a Roche Cobas c702 system).

2.4.2. Comparative measurement procedure

The comparison measurement procedure was tested over the clinically meaningful range, i.e., where medical decisions are made. 25 μl of the venous blood was placed directly onto a PocDoc chip. After 7 min the PocDoc Pro app was used to take a picture of the lateral-flow cassette in ambient lighting conditions. The lipid levels were calculated by the PocDoc App and used to determine the characteristics of the device versus the reference lab values. The comparative measurement procedure was repeated in multiple replicates, as required by the individual study designs.

2.5. Study designs

The following characteristics of the device were investigated in the performance evaluation:

2.5.1. Limits of quantification: start/end of linear colorimetric reaction

The specific experimental design for the analysis of LoQ were adapted from published guidelines (Armbruster and Pry, 2008; CLSI, 2012). The LoQ measurement procedure was tested over a clinically meaningful range, i.e. where medical decisions are made. Several independent specimens close to the quantifiable limits of PocDoc were measured across two lots of PocDoc tests. Each concentration level was measured in triplicate to provide statistically relevant data set.

2.5.2. Precision: %CV – repeatability

Precision was evaluated according to published standards(CLSI, 2014a). In this study, 2 whole blood samples were measured repeatably in 2 × 20 days/independent observations, each sample measured in two duplicates, one in the morning, one in the afternoon to test for random

error in the device and repeatability.

2.5.3. Accuracy: total analytical error estimate

Estimation of accuracy and the related Total Analytical Error of the measurement followed guidance from the published material by CLSI (CLSI, 2016). Minimum experimental design: i) Candidate measuring procedure – Roche Cobas c702 analyser at TDL laboratory, one calibrator lot; ii) Comparative measuring procedure – PocDoc Lipid Test, minimum one PocDoc batch per triplicate; iii) Minimum 5 testing days; iv) Minimum 120 patient specimens (full clinically relevant range); iv) One measurement per specimen per candidate measurement procedure – i.e., to establish the ground true value; v) Three replicates per specimen per comparative measurement procedure; vi) One testing location – standard room lighting conditions. Overall, 125 whole blood specimens were tested in triplicate, the total duration of the study was 17 days. The total number of analysed PocDoc tests was $125 \times 3 = 375$, across 10 different batches/lots.

2.6. Statistical analysis

Data analysis was performed according to the requirements of CLIA. Regression analysis and other data were evaluated using the mean, standard deviation, and coefficient of variation (CV). All statistical analyses were performed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and SPSS v29.0.1.0 (IBM Corp. Released, 2023. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp) at a significance level of 0.05.

2.6.1. Clinical sensitivity: ability of PocDoc to correctly identify patients with hyperlipidaemia and/or high CVD risk

The data from the 125 participants was used to allocate patients to being healthy or having hyperlipidaemia, using the UK's NHS guidelines. The results obtained using PocDoc were compared to the results obtained using the reference lab measurement in order to determine whether any patients would have been incorrectly classified as being healthy when they have hyperlipidaemia or vice versa.

2.6.2. Interchangeability with reference lab tests

A Bland-Altman analysis was performed to examine limits of accuracy between the PocDoc point of care test (i.e., comparative measurement) and the reference lab measurement (i.e., candidate measurement).

3. Results and discussion

3.1. Limits of quantification

The limit of quantitation describes the smallest/highest concentration of an analyte that not only can be observed but can also be observed and measured with consistent accuracy. Several independent levels of lipids, as close as realistically possible to the lower and upper limits of the PocDoc measuring intervals were tested to provide a quantitative sense of the detection capability of PocDoc Lipid Test. Each concentration level was measured in triplicate to provide statistically relevant data (Figure S1, ESI). In the LoQ study, measured on samples with clinical concentrations close to the PocDoc quantification limits, the variation of the CV (%) was between 0 and 14.9%. The average CV% for total cholesterol across both Low and High levels was 3.5%, whilst for HDL it was 4.3% and for triglycerides the average CV% was 3.4%.

Limits of quantification were confirmed to be:

Total Cholesterol (TC) 2.5–7.0 mmol/L.

High Density Lipid (HDL) 1.0–2.5 mmol/L.

Triglycerides (TG) 1.0–2.5 mmol/L.

3.2. Precision

Determination of precision is done optimally on at least ten replicates of a single specimen. In addition, reproducibility experiments are performed to demonstrate the closeness of agreement between independent test results of successive measurements of the same measurand carried out under the same conditions of measurement. Imprecision is a quantitative value indicating the extent of disagreement of a set of replicates. For the number of replicates (n), the average, standard deviation (SD) and percentage coefficient of variation (%CV) are calculated. Two independent levels of lipids, representing 'healthy' and 'unhealthy' individuals, were tested to determine the precision. At least 20 independent observations were performed, with samples most realistically resembling the real-life scenario for which the test is intended to be used. In the present precision study, measured on samples with clinical concentrations determined as 'healthy' and 'unhealthy', the CV (%) was 2.1% and 3.9% respectively. For HDL it was 1.99% and 3.02% respectively. And finally for TG the imprecision was determined to be 0% and 3.3% CV. The full statistical analysis is summarized in Table 1.

3.3. Accuracy

Although Bias and precision are important performance attributes of quantitative measurement procedures, the most meaningful parameter that integrates influence of other sources of errors is accuracy. The approach to estimate TAE is based upon evaluation of the differences in patient specimen results between the candidate and a comparative measurement procedure. As such, the resulting TAE estimate incorporates multiple sources of testing errors that commonly arise in a medical laboratory, or in case of PocDoc Lipid test, in the field.

In laboratory medicine, different methods define the required quality and specifications for quality control of analytical results. The most common requirements are based on maximum allowable deviation in variance (i.e., standard deviation– > imprecision) and maximum allowable systemic deviations of measurement (i.e., Bias).

A comparison between the PocDoc result and the Reference Method result was calculated in pairwise fashion as: *PocDoc Result - Reference Method Result* = Bias. The Bias percentages for each run are averaged, giving the averaged difference values (CLSI, 2014b). The imprecision (expressed as coefficient of variation) and Bias are expressed as a percentage of the reference result. The two parameters, imprecision and Bias are fitted to a Root Mean Square model (Eq (1)) and expressed in terms of Total Error (TE).

$$\text{model TE} = \overset{\text{RMS}}{\sqrt{CV^2 + Bias^2}} \quad \text{(Eq 1)}$$

The RMS model was chosen because it has the advantage of being concerned with only a single control rule for a quantity, which is directly important for the metrological quality of results. This is particularly attractive in highly regulated fields of biomedical diagnostics and medicine. A clear advantage is that the user has more flexibility to fulfil the requirements, since a larger systematic error can be accepted if the standard deviation is smaller and vice versa (Macdonald, 2006). On the other hand, the widely use Westgard model suffers from significant real-world complications, including an assumption that the errors are normally distributed, which is not always true, especially, e.g., when manufacturing defects in unit-use devices result in grossly aberrant results in a small minority of devices (CLSI, 2016).

Of the 125 blood donors included in this study, 44% were females, 56% were males. The blood donors were volunteers, no special considerations regarding the healthy/unhealthy levels of cholesterol, HDL or triglycerides were requested from the donors and therefore the blood donors represent the population that is willing to donate blood for research and clinical purposes.

PocDoc device is intended to be used with 25 μL of capillary blood sample (i.e., fingerpick). It is important to highlight that reference

Table 1
Summary of statistical results demonstrating the test repeatability, imprecision and accuracy.

Repeatability and imprecision as Coefficient of Variation [%]							
		Estimate of repeatability (Sr)	Between-days SD (Sdd2)	Between-run SD (Srr2)		Precision (ST)	Coefficient of variation [%]
Total Cholesterol	Level 1	1.0	−2.0	3.6		0.9	2.1
	Level 2	1.3	−9.4	19.8		1.1	3.9
HDL Cholesterol	Level 1	0.58	−0.24	0.65		0.50	3.02
	Level 2	0.72	−0.55	0.84		0.62	1.99
Triglycerides	Level 1	0.6	−0.2	0.6		0.5	0.0
	Level 2	0.7	−0.5	1.12		0.6	3.3
Accuracy as Total Analytical Error [%]							
		PocDoc Lipid Test			Recommended NCEP limits[a]		
Total Cholesterol		6.9%			<9%		
HDL Cholesterol		7.7%			<13%		
Triglycerides		5.7%			<15%		

[a] From ref P. C. Fallest-Strobl, 1997.

methods (i.e., candidate measurement procedure) to obtain accurate values of lipids from non-anticoagulated fingerprick blood do not exist. Because blood coagulates so quickly, any non-anticoagulated measurements must be done by a point-of-care machine which do not have the same levels of accuracy as reference laboratory machines (which all use venous blood draws, or large volume fingerprick blood draws in tubes treated with anticoagulants). Therefore, our performance evaluation study was done with the same material used in the reference lab machines, which is venous heparin treated blood. Although some analytes have differences in capillary blood as compared to venous or arterial blood specimens, lipids in fingerprick and venous blood values are interchangeable. Riccioni et al. have conducted a study to ascertain a statistical variance between cholesterol levels in fingerpick blood and venous blood (Sblendorio et al., 2008). The Bias obtained in the capillary blood samples was only slightly higher (e.g., 2.87%) than the venous sample measurements. Moreover, the total variance was statistically similar for venous and capillary measurements (F value = 1.199, where the upper critical value of the F distribution is 2.124, $p < 0.05$). The results agreed with other studies (Ansari et al., 2021; Greenland et al., 1990), concluding that cholesterol and lipid testing can be performed safely and accurately in either venous or capillary specimens.

It is also worth discussing small differences between capillary and venous blood levels in the much larger context of biological variation. According to the National Cholesterol Education Program guidelines, there are notable day-to-day variations in lipid levels meaning your lipid levels will change from one day to the next (Bookstein et al., 1990; Matthews et al., 2001, 2022).

The normal day-to-day variations for total cholesterol were 5%, triglycerides 20%, HDL-Cholesterol 10%, and calculated LDL-Cholesterol 8% levels. These levels mean that the small difference that may be seen between venous and capillary levels are clinically insignificant compared to normal biological variation. Studies have also shown a seasonal effect on blood cholesterol levels. This variation in blood lipid levels has shown cholesterol levels up to 5% higher in winter than in summer. Studies have also shown that lipid levels, specifically total cholesterol, LDL-cholesterol and HDL-cholesterol, fluctuate over the menstrual cycle by 5–8% due to changing reproductive hormone levels (Mumford et al., 2008).

The analysis of the 125-blood specimen revealed that the standard deviation for cholesterol, and HDL/triglycerides was 0.3 and 0.1 mM respectively. This is quite remarkable for a rapid test that measures the concentration from 25 μL of whole blood and provides the results in 7 min. The measurements of total cholesterol show a small positive Bias of +0.7 mM. HDL cholesterol has a slight negative Bias of −0.1 mM. The Bias for triglycerides was −0.1 mM. All values are referred to as the average Bias across the 125 specimens (375 total tests). Interestingly, because the 125 samples were not used in one single day but have been added over the 11 testing days, it was possible to map the evolution of the key parameters such as CV%, Bias% and TAE% as a function of increased number of samples being tested. The trends are summarized in Fig. 2.

Applying the calculated imprecision (represented as CV%) and Bias% to a root mean square model for the calculation of total analytical error, the final results meet the recommended limits by NCEP, as seen in Table 1.

3.4. Sensitivity analysis

PocDoc results were compared to reference lab results for the directly measured biomarkers. The values were highly correlated with R squared values of 0.8 for total cholesterol and triglycerides and 0.7 for HDL. A sensitivity analysis was performed to evaluate whether the same clinical judgement would occur using the PocDoc result compared to the reference lab method.

For total cholesterol, there was good correlation with cholesterol level banding in that none of the 125 patients in the accuracy study would have likely been classified incorrectly as healthy when they had high cholesterol; or as having high cholesterol if they were healthy (Fig. 3A). The majority of patients were correctly classified although some patients with reference lab values near the category limits would have moved into borderline high or vice versa e.g. 5 vs 5.1 or 6.1 vs 6.2. However, this would not have resulted in any altered clinical outcomes.

For triglycerides, there was good correlation with the NHS's recommended range – 5 out of the 125 patients in the accuracy study would have likely been classified incorrectly as either unhealthy when healthy (1) or healthy when unhealthy (4). To note that all 4 individuals misclassified in this setting had values of 2.1 or 2.2 (Fig. 3B).

For HDL, >98% of individuals (123 out of 125) have had healthy HDL levels (>1.0 mmol/L) with both PocDoc and reference lab values. The remaining 2 patients had HDL values by reference lab of <1.0 mmol/L but measured 1.0 and 1.1 mmol/L with PocDoc (Fig. 3C).

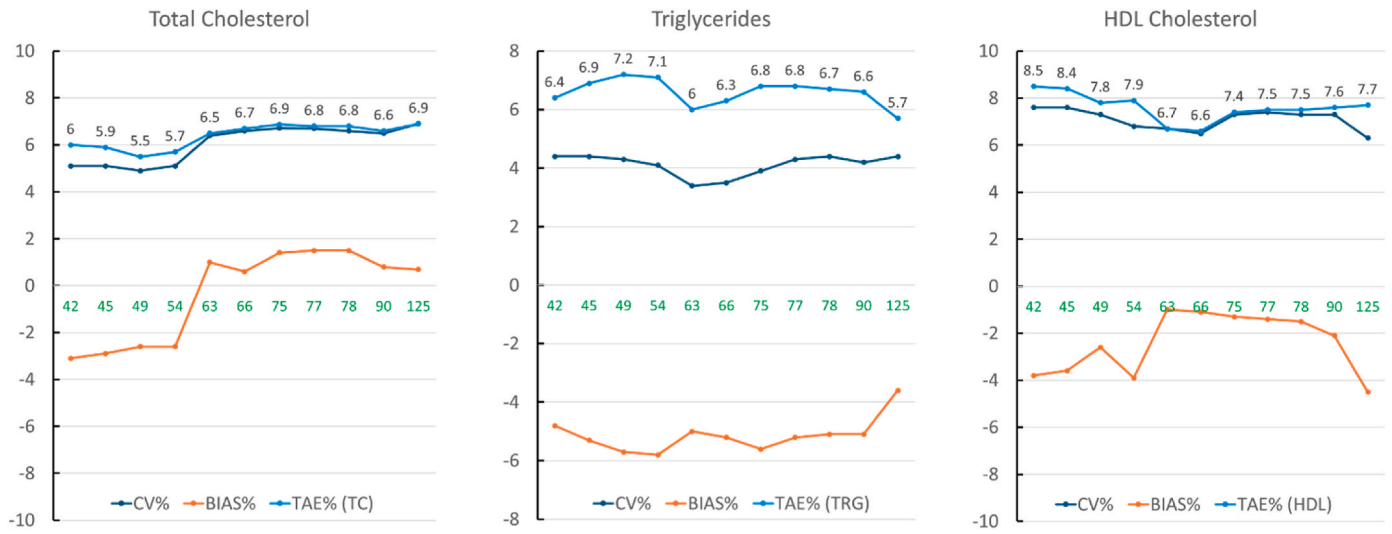
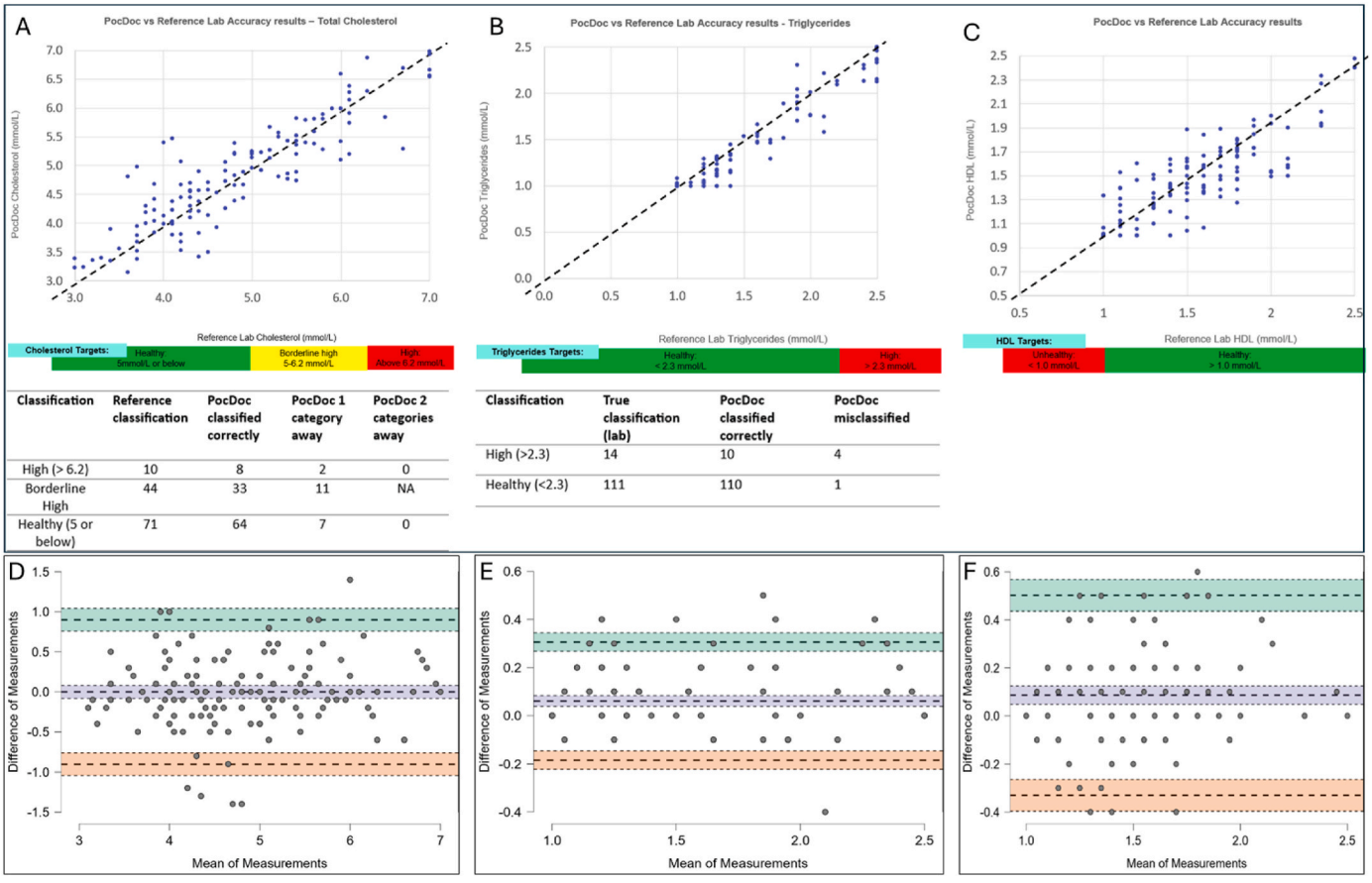


Fig. 2. Graphs summarising the evolution of key performance parameters such as CV% (blue line), Bias% (red line) and TAE% (yellow line). The horizontal axis, numbers in green 42, 45, 49, 54, 63, 66, 75, 77, 78, 90, 125 represent the number of samples tested at each time point. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



Classification	Reference classification	PocDoc classified correctly	PocDoc 1 category away	PocDoc 2 categories away
High (> 6.2)	10	8	2	0
Borderline High	44	33	11	NA
Healthy (5 or below)	71	64	7	0

Classification	True classification (lab)	PocDoc classified correctly	PocDoc misclassified
High (>2.3)	14	10	4
Healthy (<2.3)	111	110	1

Fig. 3. PocDoc results correlated with reference values; 4A - > cholesterol; 4B - > triglycerides and 4C - > HDL cholesterol. Classification sensitivity analysis for cholesterol and triglycerides are in the tables under the graphs, HDL analysis showed no misclassification using 125 patient samples. Bland-Altman plots for total cholesterol (4D), HDL cholesterol (4E) and triglycerides (4F).

3.5. Interchangeability with reference lab values

A Bland-Altman plot analysis was conducted to evaluate the differences between the two datasets (reference vs PocDoc) and to assess the differences between two sets of data over the entire value scale, focusing on systematic Bias and proportional Bias (Kaur and Stoltzfus, 2017). It is common practice to calculate 95% limits of agreement for each comparison (average difference ±1.96 standard deviation of the difference), which is indicative of how far apart measurements by the two methods were more likely to be for most individuals.

The Bland-Altman plot analysis revealed that there was no clinically significant absolute or proportional Bias between PocDoc and reference lab values and they can be used interchangeably, considering the limit of agreement. For total cholesterol, the limit of agreement is 0.9 mmol/L between PocDoc and reference lab method. Virtually all data points lie within the Bland Altman limits of agreement with no worrying outliers or influential cases suggesting on the whole agreement between methods is good. It is most likely that a PocDoc value is not significantly different from a reference value but with 95% certainty, this is within the ±0.9 mmol/l limit. For HDL, there is a Bias of 0.09 mmol/L for PocDoc compared to ref lab values which is not clinically significant. The Bland Altman limit of agreement between PocDoc and lab is +0.5 and - 0.3 mmol/L, with 95% certainty. For triglycerides, there is a Bias of 0.06 mmol/L which is also not clinically significant. The Bland Altman limit of agreement between PocDoc and lab is +0.3 and - 0.2 mmol/L and in also in this case, it is most likely that a PocDoc value is not significantly different from a reference value. With a significance level of 0.05, this is within +0.3 and - 0.2 mmol/l. The individual Bland-Altman plots are shown in Fig. 3D–F.

3.6. Clinical utility – QRISK®3 risk calculator assessment

The QRISK®3 risk calculator is a modelling algorithm that calculates a person's risk of developing a heart attack or stroke over the next 10 years. The software was derived using data from over 10 million patients registered in UK primary care (Hippisley-Cox et al., 2017). The QRISK®3 is based on the input of various risk factors such as age, sex, ethnicity, postcode (UK specific, analogy of local area where the person lives), some clinical information and importantly for CVD risk, cholesterol/HDL ratio and systolic blood pressure, among others.

To demonstrate clinical utility of the PocDoc lipid test, we performed a comparison between the estimated QRISK®3 values for 42 patients with a known cholesterol/HDL ratio. In Model 1, we compared the QRISK®3 calculation difference between the cholesterol/HDL ratio obtained by PocDoc and the reference lab method. We kept all clinical risk factors for the individual patients identical, except for systolic blood pressure was set to 120 mmHg for each patient. In Model 2, the same patient input was modelled but the systolic blood pressure was set to elevated 150 mmHg. The rationale was that with elevated blood pressure, the QRISK®3 increases and it was important to understand if this would have any impact to QRISK®3 calculation when inputting the PocDoc results vs reference lab results. The summary, shown in Fig. 4 clearly demonstrates an excellent correlation between the PocDoc results and the reference lab results when the QRISK®3 algorithm calculates the CVD risk. The R^2 values in both models, Model 1 and Model 2 are >0.9 and the intercept in both cases is very close to 0. As expected, modelling the elevated blood pressure increased the patient's overall calculated risks but the correlation between the reference results and PocDoc results remained the same.

3.7. Closing remarks

The PocDoc device utilizes the power of a smartphone camera to quantify enzymatic colorimetric reactions. This technology has been successfully applied to produce a 6-marker lipid panel in a microfluidic assay format, the PocDoc Lipid test. Lipids profiles are an important component of cardiovascular disease prevention and management. The PocDoc platform integrates the lipid profile results into QRISK3 and provides the user with their risk of a cardiovascular disease event in the next 10 years and their healthy heart age.

The results of the 42-patient analysis showed that the deviation between PocDoc derived measurements and the reference laboratory derived measurements would not have resulted in a differing clinical outcome for any patients. In the UK, patients with a QRISK3 score of

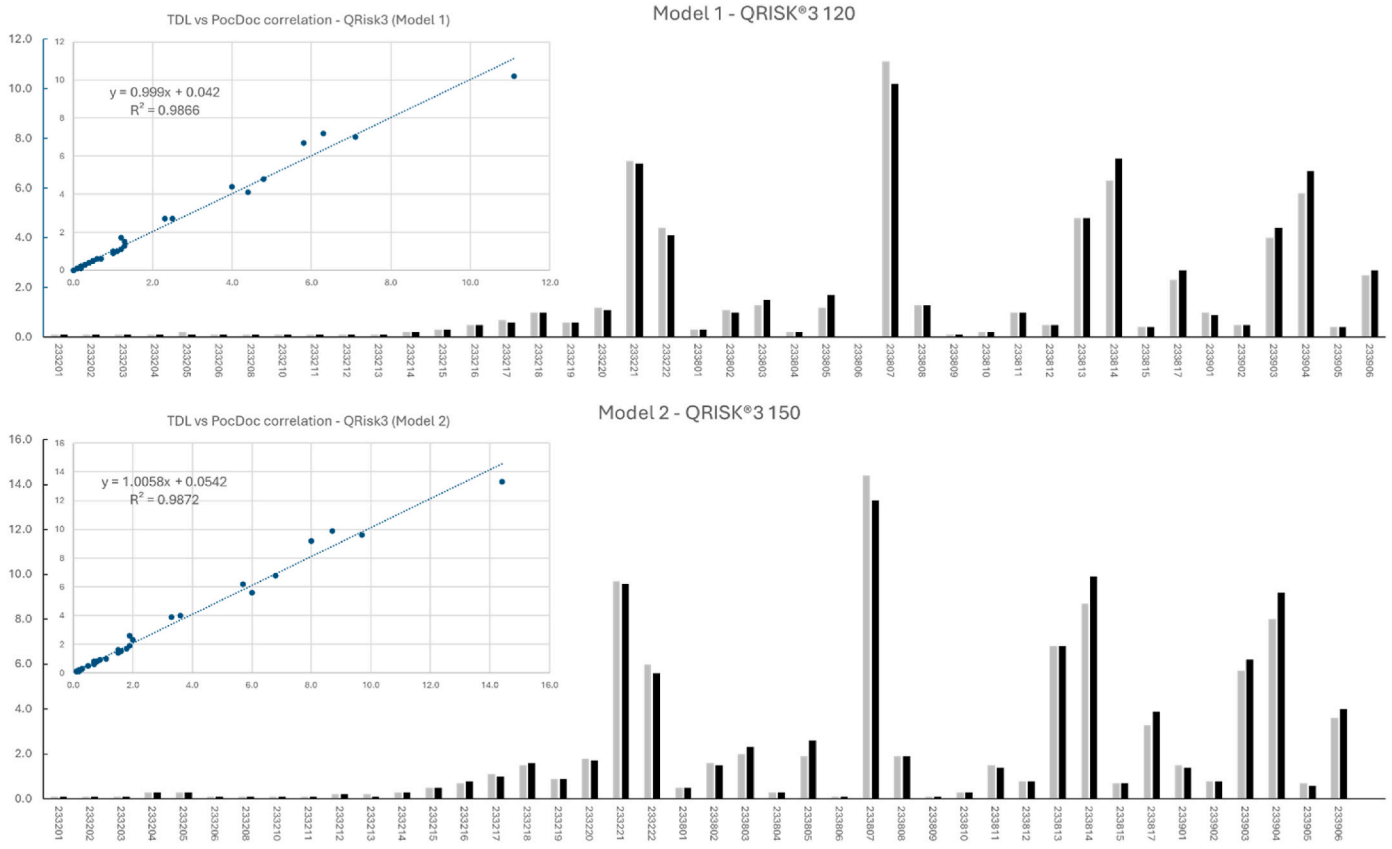


Fig. 4. The modelled QRISK®3 calculation comparison when the cholesterol/HDL ratio input was based on either the PocDoc results (grey bars) or the reference laboratory results (black bars). The two plot insets show the correlation between the calculated QRISK®3.

over 10% are classified at high risk of cardiovascular disease and receive clinical support including lifestyle advice and lipid lowering therapies. Our analysis shows that all patients were either above or below this 10% threshold regardless of whether the cholesterol/HDL ratio was derived using PocDoc or a reference lab.

Clinical utility – international guidelines.

The accuracy and precision of the PocDoc device falls within international limits recommended by NCEP. Further statistical analysis suggests accuracy compared to venous blood reference lab measurement methods makes the test suitable for community screening events. In this setting, PocDoc can be used to evaluate an individual's risk of cardiovascular disease. Individuals at high risk of disease (>10% chance of a CVD event in the next 10 years ago) can then be referred for clinical follow-up.

Some international guidelines, including the American Heart Association, recommend that individuals over the age of 20 have regular lipid profile monitoring along with other traditional risk factors at least once every 4–6years (Grundy et al., 2017). In the UK individuals over the age of 40 who have not already been diagnosed with CVD, diabetes mellitus, or chronic kidney disease should offered lipid profile measurement through the NHS Health Check-NICE (NICE, 2023). Systems such as PocDoc, which provide digital lipid profiling in the context of cardiovascular risk assessment can be a powerful tool in the community, especially in lower socio-economic areas, to identify individuals at high risk of CVD. As a screening option, it may offer particular value in areas of deprivation and/or in areas with high proportions of ethnic minorities where the CVD burden is high and engagement with traditional in-surgery healthcare is low.

CRediT authorship contribution statement

Kiran N. Roest: Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Matthew Lee:** Writing – review & editing, Writing – original draft, Validation. **Jon Rees:** Writing – review & editing, Writing – original draft, Formal analysis. **Vladimir Gubala:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Vladimir Gubala reports financial support was provided by PocDoc. Vladimir Gubala reports a relationship with PocDoc that includes: employment. Kiran Roest, Matthew Lee and Vladimir Gubala are all employees of PocDoc Jon Rees is an academic employed by the University of Sunderland If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biosx.2024.100504.

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