ORIGINAL ARTICLES

Point-of-care testing of cholesterol and triglycerides for epidemiologic studies: evaluation of the multicare-in system

STEFANO RAPI, CRISTINA BAZZINI, CAMILLA TOZZETTI, VALENTINA SBOLCI, and PIETRO AMEDEO MODESTI

FLORENCE, ITALY

Cardiovascular disease is the leading cause of death in the world with 80% of cardiovascular events that occur in low- and middle-income countries. Reliable data on the prevalence of risk factors in developing countries can be obtained in door-to-door epidemiologic studies with the use of automatic instruments. This study was performed to assess the sensitivity and specificity of a low-cost and manageable point-of-care testing (POCT) instrument (HPS MultiCare-in, Italy) for cholesterol and triglyceride assays. Plasma blood samples were obtained from consecutive subjects referred to our clinic for diagnostic evaluation. The analyzer currently used in our central laboratory (ADVIA 2400; Siemens, Deerfield, Ill) was used as comparison method. The inter-assay imprecision (expressed as variation coefficient) of the MultiCare POCT system was 4.51% (range, 2.38%–8.54%) and was 3.29% (range, 1.06%–7.45%) for cholesterol and triglycerides systems, respectively. The mean percent bias for capillary samples was 3.5 ± 4.3% for total cholesterol and −2.4 ± 4.9% for triglycerides. The difference in results obtained by nonprofessionals compared with professionals (practicability testing) was 0.28 ± 7.61% and 1.26 ± 9.86%, respectively (*P* value was nonsignificant for both). Sensitivity and specificity measurements were 95.7% and 61.9% (threshold value of cholesterol 190 mg/dL) and 98% and 93.5% (threshold value of triglycerides 170 mg/dL), respectively. POCT instruments are essential to perform epidemiologic studies while avoiding transportation and storage of biologic material. The characteristics of sensitivity and specificity as well as diagnostic accuracy make the POCT instrument useful for obtaining an accurate stratification of a study population. (Translational Research 2009;153:71–76)

Abbreviations: CI = confidence interval; CV = cardiovascular; EDTA = ethylenediaminetetraacetic acid; NCEP = National Cholesterol Education Program; POCT = point-of-care testing; WHO = World Health Organization

From the Central Laboratory of Clinical Biochemistry, Careggi Hospital, and the Department of Critical Care Medicine, University of Florence, Florence, Italy.

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Reprint requests: Pietro Amedeo Modesti, MD, PhD, Department of Critical Care Medicine and Don Carlo Gnocchi Foundation, University of Florence, Viale Morgagni 85, 50134 Florence, Italy; e-mail: pamodesti@unifi.it.

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AT A GLANCE COMMENTARY

Background

The importance of plasma lipids to stratify global cardiovascular risk is well recognized. However, because of epidemiologic transition, most cardiovascular events occur in developing countries where the prevalence of risk factors is derived from patients referred to clinical centers.

Translational Significance

The possibility of having a low-cost device for cholesterol and triglycerides assay used in epidemiologic door-to-door studies represents a clear example of translational medicine. The device may be used to obtain directly reliable epidemiologic data in the field, which is necessary to plan effective actions to deal with noncommunicable diseases.

Cardiovascular (CV) disease is the leading cause of death worldwide.[1] The World Health Report 1999 estimated that in 1998, 85% of the CV burden developed from low- and middle-income countries.[2] This contribution is projected to grow, given the consistent population growth rates.[3,4] Demographic transition and urbanization also contribute to the increased prevalence of risk factors.[5] Both cholesterol and triglycerides plasma levels are important parameters for assessing risk factors of a population in epidemiologic studies.[6,7]

Although the developing world bears most of the burden of world CV deaths, there are still no signs of success in halting the CV disease epidemic in developing countries.[4,8,9] Actions to deal with noncommunicable diseases require consistent data on the prevalence of risk factors to address large-scale prevention programs. This goal can be pursued only with door-to-door epidemiologic studies, which use point-of-care testing (POCT), as suggested by the World Health Organization (WHO).[10] In recent years, semiautomatic POCT instruments that can measure blood levels of cholesterol and triglycerides have been developed. This study was performed to assess the sensitivity and specificity of a new low-cost and manageable POCT instrument (HPS MultiCare-in, Biochemical System International, Arezzo, Italy) for cholesterol and triglyceride assays.

METHODS

Blood samples. The research conforms to the relevant ethical guidelines for human research (Declaration of Helsinki). Informed consent was obtained, and the study was approved by our Institutional Review Board. According to the manufacturer, determinations with the MultiCare system can be performed in ethylenediaminetetraacetic acid (EDTA)-venous blood samples or fresh capillary blood applied directly to the test strip. Blood samples were then collected into K_2EDTA-containing tubes from outpatients (n = 636; age range, 22–69 years) referred to our unit for diagnostic evaluation. Fresh capillary blood samples (n = 66) were obtained immediately before assay in a practicability test.

Device used and quality control. The comparison method involved daily quality controlled with sets of cholesterol and triglyceride solutions at 2 medical decision levels (106 ± 2 and 262 ± 5 mg/dL for cholesterol and 88 ± 2.5 and 173 ± 3.5 mg/dL for triglycerides). In addition, reference instruments were submitted to external quality assurance services (EQAS November 2007 to April 2008; Bio-Rad Laboratory, Hercules, Calif). Bias and imprecision were 0.79 and 1.73 for cholesterol and 2.69 and 2.39 for triglycerides, respectively.

Ten MultiCare devices and 5 different lots of reactive strips for cholesterol (MultiCare, CH) and triglycerides (MultiCare, TGL) were tested during the study. All measurements were performed at room temperature, between 22°C and 28°C. Each MultiCare System device was checked twice per day using the control solution. The MultiCare systems were considered technically reliable if the values obtained were within the control range set up by the manufacturer. Instrument absorbance was reconsidered at the end of the study to confirm instrumental efficiency. The reliability of each lot of reactive strips was tested using reference solutions (expected range of variability set up by the manufacturer for control materials was ±25% for both cholesterol and triglycerides).

Analytical data analysis. ***Imprecision study.*** The interassay imprecision was calculated by performing 12 runs of the same fresh venous plasma-EDTA sample on the same instrument. Different levels of cholesterol and triglyceride concentrations were studied. The intra-assay imprecision was calculated by performing 4 runs with the same sample on 3 different days. The venous plasma-EDTA used for intra-assay imprecision profiles was stored at 4°C, and hematocrit, cholesterol, and triglycerides levels were tested with reference methods at the beginning and at the end of the study. Intra-assay imprecision was also calculated on control solutions provided by the manufacturer. Imprecision values are exressed as coefficients of variation.

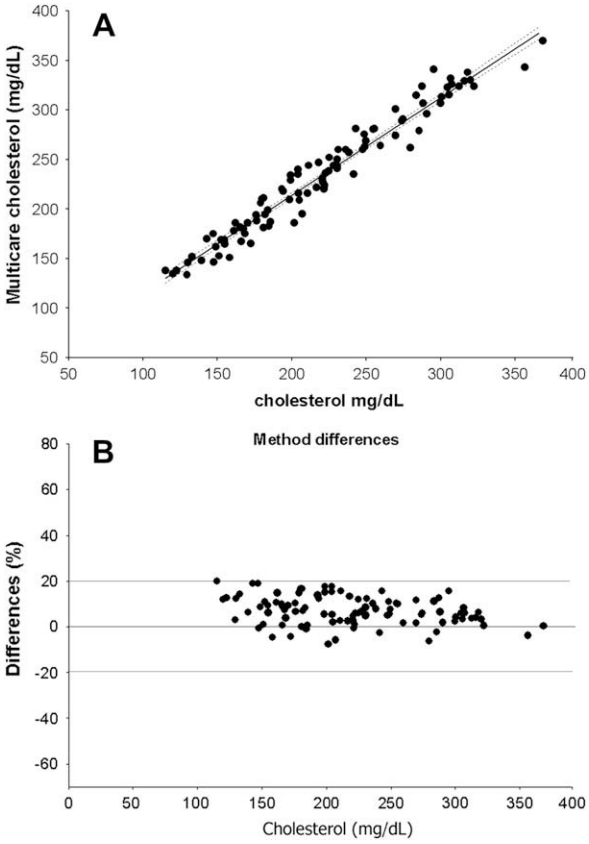

Fig 1. Passing-Bablok comparison of methods for cholesterol (n =103; cholesterol range, 120–370 mg/dL) (y = 0.9637x + 22.547; r = 0.942) (**A**). Difference between methods (**B**).

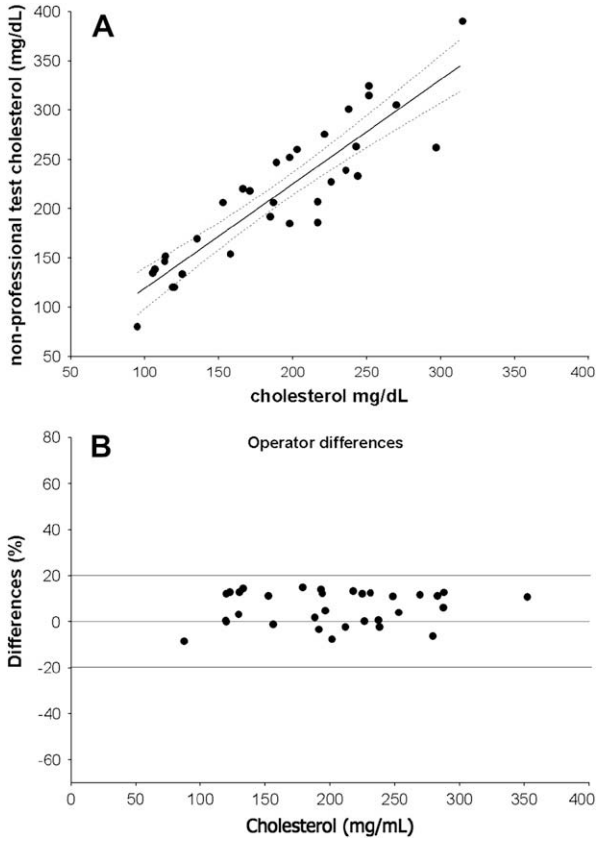

Fig 2. Passing-Bablok regression for cholesterol in practicability test (n = 32) (y = 1.1208x – 20.677) (**A**). Differences in results obtained by nonprofessionals compared with professionals (**B**).

Accuracy. The reference method was the ADVIA Analyzer (2400; Siemens). According to the manufacturer's instructions, subjects with hematocrit values beyond the 35–50 range were preliminarily excluded. The comparison between methods was performed according to the National Committee for Clinical Laboratory Standards NCCLS EP9[10] using samples with a wide range of cholesterol and triglycerides. Each sample was tested twice with both the MultiCare system and the comparison method. Duplicate results with variability >15% were retested, and aberrant data were excluded from additional analysis.

Practicability test. In a practicability test, patients self-measured their triglycerides (n = 34) or cholesterol (n = 32) with the MultiCare system, on capillary blood according to the instruction manual. Immediately afterward, a professional operator conducted a second determination with the same instrument on a second capillary sample. The pairs of results were then compared.

Sensitivity and specificity. The sensitivity and specificity of the MultiCare system (ROC curve data) were obtained by performing measurements with both the POCT device and the comparison method on the same venous EDTA blood sample (n = 340). To reproduce the conditions of epidemiologic studies, ROC curves were performed in the total group of subjects independently of hematocrit values.

Statistical analysis. Data shown are mean ± standard deviation (SD). For statistical analysis, regressions of the method comparisons were calculated according to the method of Passing and Bablok.[11] Method differences are presented as Bland-Altman bias plots.[12] All tests were performed using SPSS statistical software (SPSS Inc., Chicago, Ill).

RESULTS

Cholesterol. *Imprecision study.* The inter-assay imprecision of the MultiCare Cholesterol system was 4.51% (range, 2.38%–8.54%) with cholesterol concentrations ranging between 132 and 368 mg/dL.

The range of intra-assay imprecision on venous-EDTA blood samples was 4.72%–10.17%. The intra-assay

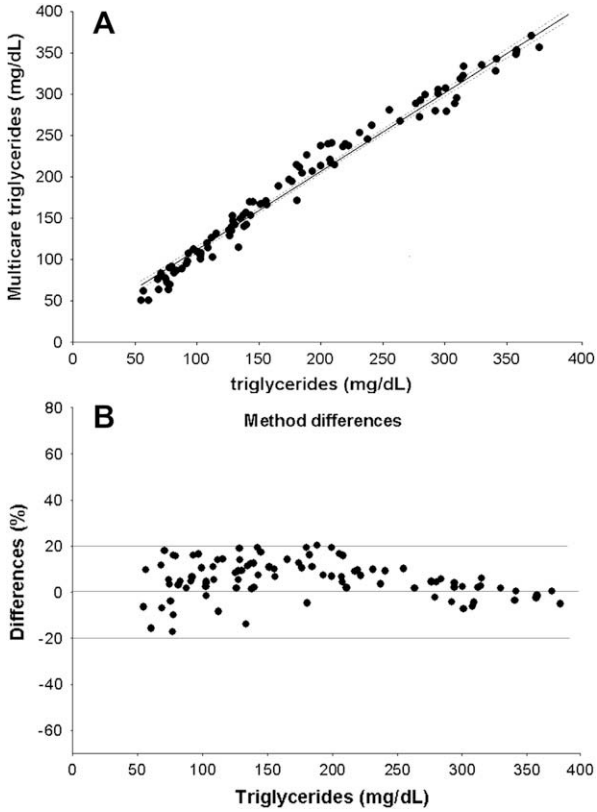

Fig 3. Passing-Bablok comparison of methods for triglycerides (n = 103; range of triglycerides, 55–429 mg/dL) (y = 0.9678x + 7.883; r = 0.995) (**A**). Differences between methods (**B**).

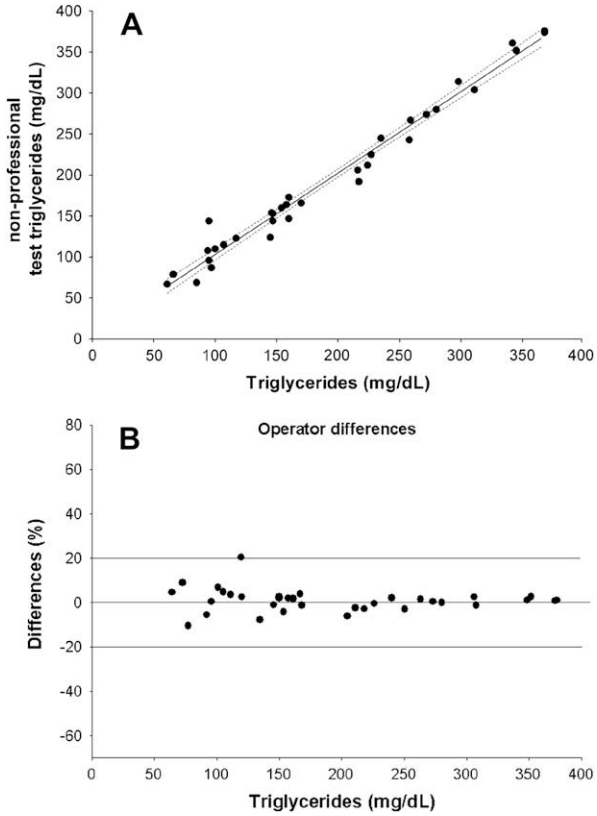

Fig 4. Passing-Bablok regression for triglycerides in practicability test (n = 34) (y = 0.8787x + 16.777; r = 0.993) (**A**). Differences in results obtained by nonprofessionals compared with professionals (**B**).

imprecision calculated on control material was between 1.27% and 1.72%.

Accuracy. Three of 103 (2.91%) samples were retested for aberrant data (insufficient sample on the strip). When blood samples (n = 103; cholesterol range, 120–370 mg/dL) were examined within the framework of the comparison method, the Passing-Bablok regression equation was y = 0.9637x + 22.547 (95% confidence interval [CI] for the slope, 0.89–1.031; r = 0.942) (Fig 1, *A*). The mean difference between methods was 3.53 ± 4.29% (Fig 1, *B*).

Practicability test. In practicability testing (n = 32), the Passing-Bablok regression equation was y = 1.1208 x – 20.677 (95% CI for the slope, 0.9626–1.279; r = 0.978) (Fig 2, *A*). The difference in results obtained by patients compared with professionals was 0.28%± 7.61% (Fig 2, *B*).

Sensitivity and specificity. When a cut-off value of 190 mg/dL was considered, the ROC value for MultiCare was 95.7% for sensitivity and 61.9% for specificity.

Triglycerides. ***Imprecision study.*** The inter-assay imprecision of the MultiCare triglycerides system was 3.29% (range, 1.06–7.45) with triglyceride concentrations between 79 and 323 mg/dL. The range of intra-assay imprecision was 2.4%–7.7% on the venous-EDTA blood samples and 1.15%–1.54% on the control solution.

Accuracy. Two samples of 103 (1.95%) were retested for aberrant data (insufficient sample on the strip). The method comparison performed on capillary blood samples (n = 103; range of triglycerides 55–429 mg/dL) showed a Passing-Bablok regression equation of y = 0.9678x + 7.883 (95% CI for the slope, 0.89–1.031; r = 0.995) (Fig 3, *A*). The mean difference between methods was –2.36 ± 4.95 (Fig 3, *B*).

Practicability test. In practicability testing (n = 34), the Passing-Bablok regression equation was y = 0.8787x + 16.777 (95% CI for the slope, 0.9626–1.279; r = 0.993) (Fig 4, *A*). The difference in results obtained by nonprofessionals compared with professionals was 1.26 ± 9.86 (Fig 4, *B*).

Sensitivity and specificity. When a cut-off value of 170 mg/dL was considered, the ROC values for MultiCare were 98% for sensitivity and 93.5% for specificity.

DISCUSSION

The MultiCare system is easy to learn to use by both professionals and laypersons. A drop of capillary blood

Table I. Analytical performance of POCT device for determination of cholesterol and triglycerides

Assay	Device	Inter-assay Imprecision (%)	Bias (%)	Year (reference)
Cholesterol				
	Cholestech LDX	4.0	2.1	1998[13]
	Cholestech LDX	3.0	—	2007[14]
	Cholestech LDX	—	−0.6 ± 6.9	2002[15]
	CardioChek PA	4.4	—	2007[14]
	Accumeter	5.3	−1.0	1998[13]
	Accutrend	2.5	−5.6–16.6	2000[16]
	Accutrend	<5	−3.2–2.5	1995[17]
	MultiCare-in	4.5	3.5 ± 4.3	
NCEP goals		<3	<3	1988[18]
Triglycerides				
	Cholestech LDX	2.6	—	2007[14]
	Cholestech LDX	—	22 mg/dL	2006[19]
	Cholestech LDX	—	−19 ± 9.4	2002[15]
	CardioChek PA	4.8	—	2007[14]
	Accutrend	1.4–6.1	0.9 ± 12.9	2000[20]
	MultiCare-in	3.3	−2.4 ± 4.9	
NCEP goals		<5	<5	1988[18]

is applied to the test strip, and results are obtained after 3 min for cholesterol and 2 min for triglyceride assays. The necessary minimum volume is 10 μL. The handling procedure is much the same as those used with most blood glucose meters, but certain precautions should nevertheless be taken. The user's hands must be washed with warm water before measurement to avoid possible contamination with glycerol from hand lotions, soaps, or disinfectants. In addition, the blood drop must be obtained strictly according to the instructions. When device quality control was correct and these simple precautions were followed, laypersons achieved results comparable with those achieved by professionals.

The MultiCare systems are pocket-sized reflectance photometers, in which the intensity of the color developed from a chromogen reaction being proportional to the concentration of the cholesterol or triglycerides in the blood. The results of the MultiCare method compared with the reference method demonstrated good agreement between the 2 methods, with a mean difference of 3.5% and –2.3% for cholesterol and triglycerides, respectively.

The availability of POCT lipid monitors has increased in recent years (see Table I).[13-20] Any POCT must be validated for bias and imprecision to ensure that appropriate medical decisions and population screenings are made.[21-23] Bias (inaccuracy) is defined as disagreement between the monitor and the standardized laboratory. The National Cholesterol Education Program (NCEP) in the United States recommended bias goals of 3% and 5% for cholesterol and triglycerides, respectively.[18,24] Imprecision refers to the reproducibility of a test result. Imprecision goals of 3% and 5% are the desirable specification for total cholesterol and triglyceride laboratory methods, respectively, according to the NCEP.[18,24] Overall, the analytical goals for POCT should be equivalent to those used for laboratories to ensure that POCT use does not compromise standards of patient care and clinical decision making. POCT methods are not designed to replace lipid determinations in professional laboratories. In addition, it is important to acknowledge that particularly in rural areas, the POCT environment is different from the laboratory setting. Therefore, the most recent analytical goals recommended for POCT instruments used for diabetes management in the public health setting in Australia considered a minimum imprecision goal of 5% for cholesterol and 7.5% for triglycerides.[25]

Cholesterol, triglyceride, and blood glucose assays are the 3 laboratory investigations required by the WHO Stepwise approach to Surveillance, which is a simple, standardized method for collecting, analyzing, and disseminating data in WHO member countries.[26] Other currently available POCT devices can measure high-density lipoprotein and low-density lipoprotein directly, which avoids the need for testing while the patient is fasting. However, fasting is usually required in epidemiologic studies to obtain reliable data on diabetes prevalence. It must be noted that multiple operators and instruments involved in our study simulated a true picture of the usual clinical setting. The good results of the practicability test as well as the small amount of blood needed to perform measurements make the MultiCare instrument useful in epidemiologic

studies. The investigator may test the subject at home thus obtaining reliable data in the field. This aspect is crucial in developing countries in which the transfer of biologic material from remote areas to a clinical center may introduce a significant source of variability because of unpredictable problems in sample storage and refrigeration. Some potential advantages of this advice for developing country screening are that it or the test strips do not require refrigeration, and that is small, uses long-life batteries, and it is not costly in terms of the device or the strips. In particular, the cost of the tested device (Multicare-in, $48) is less compared with other available instruments.

The instrument might also be useful for monitoring responses to therapy in a single patient. The easy operation of the system enables patients to monitor their own blood lipids in response to lifestyle changes, such as modified eating and exercise patterns or other therapeutic means adopted.

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