

Performance of the Abaxis Piccolo® Analyzer for Lipid Measurements

B Robertson¹, C Levtzow¹, J.F. Chapman².

¹McLendon Clinical Laboratories, UNCH, Chapel Hill, NC ²
Department of Pathology and Laboratory Medicine,
University of North Carolina, Chapel Hill, NC.



Abstract

Introduction: Ongoing assessment of blood lipid levels is an essential component in the management of patients being evaluated for coronary artery disease (CAD). The use of point-of-care (POC) instruments for lipid assessment is becoming an increasingly popular in outpatient settings where real-time test results can contribute significantly to medical decision-making and efficiency of care. The Piccolo® (Abaxis, Inc., Union City, CA) is a compact POC system capable of performing a wide range of electrolyte, metabolite and enzyme analyses.

Objective: We sought to assess the agreement between total cholesterol, HDL cholesterol and triglycerides test results performed on the Piccolo® POC instrument with lipid measurements from the central lipid laboratory at UNC Hospitals.

Methods: The comparison was based on a total of 44 patient samples. Of these, 29 were tested as serum collected in gel separation tubes and 15 were run as plasma samples collected in lithium heparin tubes. Lipid measurements conducted in the central lipid laboratory were performed on a Roche Hitachi 911® (Roche Diagnostics, Indianapolis, IN) system using Roche Cholesterol, Triglycerides/GB and HDL-C plus reagents. In addition, Piccolo imprecision was assessed using two levels of commercial lipid control material run twice a day for 5 days.

Results: The Deming regression results follow:

	Slope	Intercept	r
Cholesterol			
Serum	0.959	2.7	0.998
Plasma	0.973	2.1	0.994
HDL Cholesterol			
Serum	0.997	-8.9	0.987
Plasma	0.948	-2.4	0.977
Triglycerides			
Serum	0.946	9.4	0.999
Plasma	1.027	-5.7	0.998

Piccolo total CV's were 1.9% at 160 mg/dL and 3.2% at 196 mg/dL for total cholesterol; 5.0% at 26 mg/dL and 2.7% at 41 mg/dL for HDL-cholesterol and 1.3% at 115 mg/dL and 1.4% at 177 mg/dL for triglycerides.

Conclusions: The Piccolo analyzer was found to provide suitably accurate lipid measurements as compared with values obtained from our central lipid laboratory. Imprecision was also found to be within acceptable limits. Overall, the results provided by this point-of-care analyzer are comparable to the values obtained on our automated, high throughput clinical chemistry analyzer, although some systematic bias was observed for HDL Cholesterol.

Introduction

- The objective of this study was to assess the agreement between total cholesterol, HDL cholesterol and triglycerides test results performed on the Piccolo® point-of-care (POC) instrument with lipid measurements from the central lipid laboratory at University of North Carolina Hospitals.
- The ongoing assessment of blood lipid levels is an essential component in the management of patients being managed for coronary artery disease (CAD) risk.
- The use of POC instruments for lipid assessment is becoming increasingly popular in outpatient settings where real-time test results can contribute significantly to medical decision-making and efficiency of care.
- The Piccolo® (Abaxis, Inc., Union City, CA) is a compact POC system (CLIA classification: moderately complex) capable of performing a wide range of electrolyte, metabolite, and enzyme analyses.



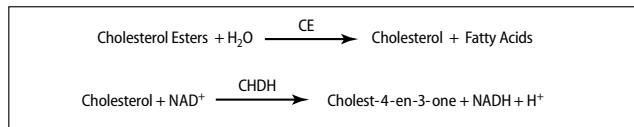
We appreciate Abaxis providing necessary reagents for this evaluation.

Materials and Methods

A. Principles of Procedures: Abaxis Piccolo*

- **Cholesterol (CHOL)**

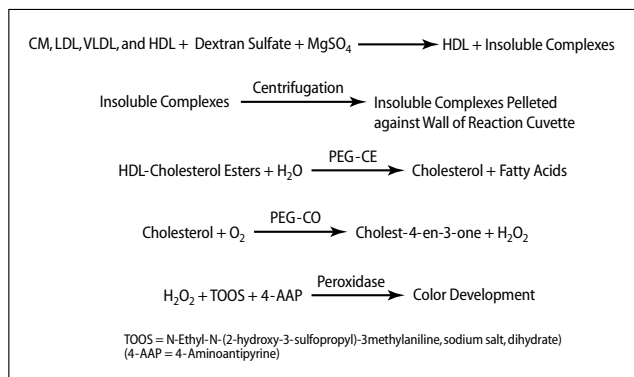
The CHOL assay is an enzymatic end-point method that uses cholesterol esterase (CE) and cholesterol dehydrogenase (CHDH).



CE hydrolyzes cholesterol esters to form cholesterol and fatty acids. The CHDH reaction converts cholesterol to cholest-4-en-3-one. The NADH is measured bichromatically at 340 nm and 405 nm. NADH production is directly proportional to the amount of cholesterol present. An assay-specific blank is also monitored to ensure no extraneous reactions interfere with the calculations of CHOL levels.

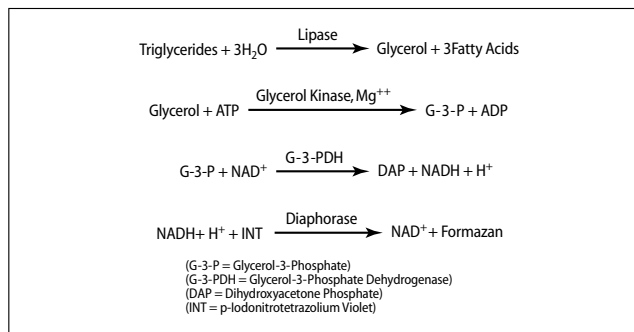
- **High-Density Lipoprotein Cholesterol (HDL)**

The HDL assay is a precipitation method that utilized polyethylene glycol-modified cholesterol esterase (PEG-CE) and cholesterol oxidase (PEG-CO) for additional specificity. The reaction mechanism follows:



The precipitating agents dextran sulfate and magnesium sulfate (MgSO_4) specifically form insoluble complexes with chylomicrons (CM), VLDL, and LDL in plasma or serum. The insoluble complexes are pelleted to the wall of the reaction cuvette within the analyzer. The remaining HDL is hydrolyzed by PEG-CE to make cholesterol and fatty acids. Cholesterol reacts with PEG-CO to produce cholest-4-en-3-one and peroxide (H_2O_2). The peroxidase reaction results in the production of a purple colored product that has an absorbance maximum at 550 nm and is referenced to absorbance at 630 nm. HDL cholesterol concentration is directly proportional to the absorbance maximum in this end-point reaction. A sample blank is also monitored to ensure no extraneous reactions interfere with the calculations of HDL levels.

- **Triglycerides (TRIG)**



Materials and Methods (cont.)

In the first step the triglycerides are hydrolyzed into glycerol and fatty acids in a reaction catalyzed by lipase. Glycerol is then phosphorylated in an ATP-requiring reaction catalyzed by glycerol kinase (GK). The glycerolphosphate is then oxidized to dihydroxyacetone phosphate with the simultaneous reduction of NAD^+ to NADH in a reaction catalyzed by glycerol-3-phosphate dehydrogenase (G-3-PDH). The NADH is then oxidized with the simultaneous reduction of INT in a reaction catalyzed by diaphorase. The intensity of the highly colored formazan is measured bichromatically at 500 nm and 850 nm and is directly proportional to the concentration of triglycerides in the sample. An assay-specific blank is also monitored to ensure no extraneous reactions interfere with the calculations of TRIG levels. The results provide a measure of total triglycerides without a glycerol blank.

- **LDL (Calculated)**

The Piccolo automatically calculates the estimated concentration of LDL in each sample using the directly determined values for total cholesterol, HDL, and triglycerides and the standard Friedewald equation. This equation is not valid for triglyceride concentrations above 400 mg/dL, non-fasting patients, and patients with Type III hyperlipoproteinemia (dysbetalipoproteinemia). An LDL value is not reported for samples with triglycerides greater than 400 mg/dL or if any of the directly measured analyte values is unavailable.

- **VLDL (Calculated)**

The Piccolo automatically calculates the estimated concentration of VLDL in each sample using the standard triglycerides/5 (if units in mg/dL) equation. This equation is not valid for triglyceride concentrations above 400 mg/dL, non-fasting patients, and patients with Type III hyperlipoproteinemia (dysbetalipoproteinemia). Of course, no VLDL value is calculated if no triglyceride value is available.

- **Total Cholesterol/HDL Ratio (Calculated)**

The Piccolo automatically calculates the total cholesterol/HDL ratio (abbreviated as TC/H) for each sample. If the directly measured total cholesterol or HDL value is missing, no ratio is provided.

B. Effects of Endogenous Substances

- The sample indices are printed on the result card to inform the operator about the level of interferents present in each sample.
- The Piccolo suppresses any results that are affected by greater than 10% interference from hemolysis, lipemia or icterus.

C. Specimens

- Comparison was based on a total of 44 patient samples.
 - 29 were serum samples collected in a gel separation tubes.
 - 15 were anticoagulated whole blood samples collected in lithium heparin tubes.

D. Central lipid laboratory measurements were performed on a Roche Hitachi 911 (Roche Diagnostics, Indianapolis, IN).

- Roche Cholesterol, Triglycerides/GB and HDL-C Plus reagents.

Note: This laboratory maintains accuracy of lipid measurements through ongoing participation in the CDC-NHLBI Lipid Standardization Program.

E. Piccolo* imprecision assessed using 2 levels of commercial lipid control material (Randox Laboratories Ltd, San Diego, CA).

- Control material run twice a day for 5 days.

F. Linearity was demonstrated for total cholesterol, HDL Cholesterol and Triglycerides through the analytical measurement range (AMR).

- Prepared by diluting serum sample with 5% Plasmanate (Bayer Healthcare, Biological Products Division, Research Triangle Park, NC).

Results

Table 1. Imprecision

	Randox Lipid Level 1			Randox Lipid Level3		
	Lot# 1237CH Exp: 7/05			Lot# 1158CH Exp: 7/05		
	Cholesterol (mg/dL)	HDL (mg/dL)	Triglycerides (mg/dL)	Cholesterol (mg/dL)	HDL (mg/dL)	Triglycerides (mg/dL)
	137-191	22.6-33.4	97-135	166-236	33.5-53.7	148-206
Mean	159.5	26.2	114.8	195.5	40.7	177.3
SD	3.0	1.3	1.5	6.3	1.1	2.5
CV (%)	1.9%	5.0%	1.3%	3.2%	2.7%	1.4%

Figure 1. Linearity Total Cholesterol (mg/dL)

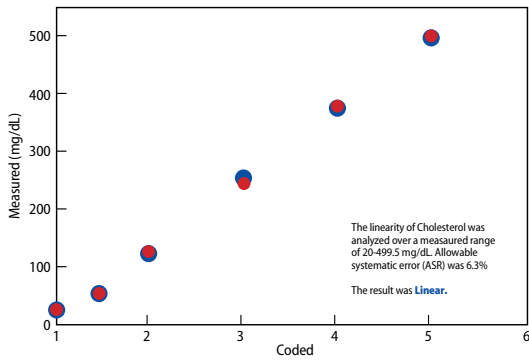


Figure 2. Linearity HDL Cholesterol (mg/dL)

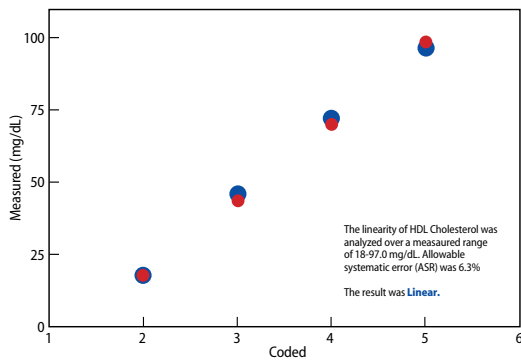
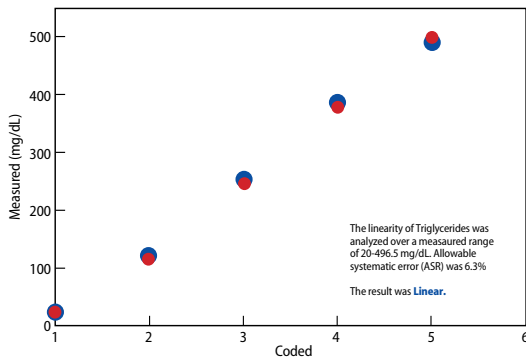


Figure 3. Linearity Triglycerides (mg/dL)



Results (cont.)

Figure 4. Method Comparison Total Cholesterol (mg/dL)

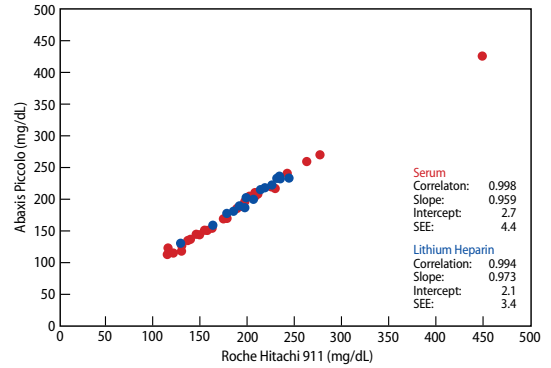


Figure 5. Method Comparison HDL Cholesterol (mg/dL)

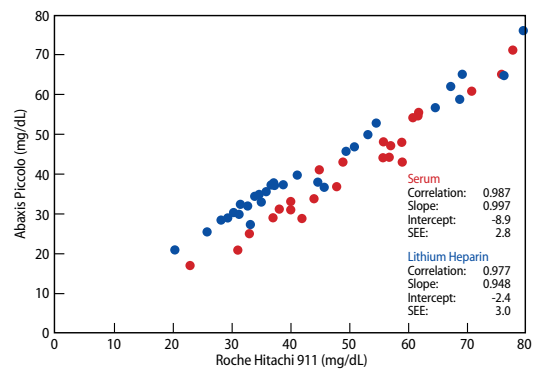
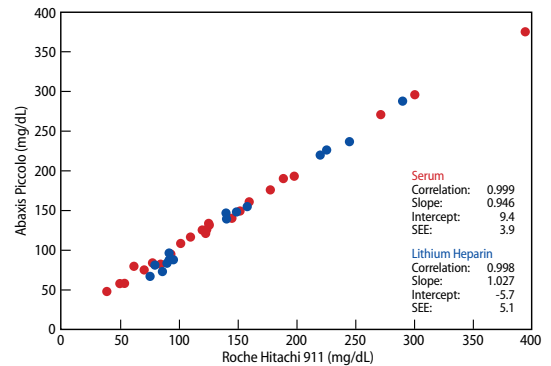


Figure 6. Method Comparison Triglycerides (mg/dL)



Conclusions

- Imprecision was also found to be within acceptable limits.
- Piccolo[®] lipid values demonstrated adequate linearity.
- Method bias, as determined by comparison to our reference lipid methods, was generally acceptable, although HDL cholesterol values exhibited a negative bias.
- In summary, if care is taken to determine and correct any significant bias relative to reference methods, the results of our study suggest that the Piccolo[®] analyzer may be suitable for use for lipid testing in a point-of-care settings.