Lipid Panel Analysis on the ABAXIS Piccolo® Compared to CDC-Standardized and Conventional Laboratory Methods

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Abstract

Reagents for total cholesterol (CHOL), high-density lipoprotein cholesterol (HDL), and triglycerides (TRIG) have been developed by ABAXIS for its Piccolo® instrument, a point-of-care chemistry analyzer that uses centrifugation and capillary flow to accomplish measuring and mixing functions. The Piccolo methods were compared to CDC-standardized methods and conventional methods in three separate laboratories using frozen aliquots of 37 serum specimens tested in duplicate. The Core Laboratory for Clinical Studies of Washington University operated ABAXIS Piccolo instruments and their usual CDC-standardized methods on a Hitachi 917 analyzer with Bayer reagents for CHOL and TRIG and Roche homogenous HDL reagent. Southwest Washington Medical Center assayed CHOL and TRIG with both the Ortho Vitros® 950 and the Roche Integra® and HDL by Roche homogenous reagent on Integra. Oregon Health and Science University measured all three analytes on a Beckman LX-20 analyzer. Agreement with the CDC-standardized methods (x-axis) by linear regression and % biases at specific cutpoints (mg/dL) are as follows:

Y	X	R^2	slope	int	bias
CHOL					@200
Piccolo	Bayer	0.989	1.011	-1	1%
Beckman	Bayer	0.989	1.000	3	1%
Ortho	Bayer	0.992	0.977	2	-1%
Roche	Bayer	0.979	0.904	11	-4%
HDL					@40
Piccolo	Roche	0.975	0.935	1	-4%
Beckman	Roche	0.992	0.961	-6	-18%
Roche	Roche	0.989	0.917	3	-1%
TRIG					@200
Piccolo	Bayer	0.997	0.960	4	-2%
Beckman	Bayer	0.998	0.923	-5	-10%
Ortho	Bayer	0.996	0.910	-1	-9%
Roche	Bayer	0.998	0.925	-1	-8%

Correlations were excellent for all methods and analytes. For CHOL, the biases, determined from linear regression analysis at specific cutpoints, were similar, except the Roche Integra had the largest bias (-4%) versus the CDC-standardized method. For HDL, the Piccolo and Roche/Integra were in good agreement with the CDC-standardized Roche/Hitachi method, but Beckman demonstrated a substantial bias (–18%). For TRIG, Piccolo agreed well with Bayer non-blanked (including free glycerol) results. However, results for non-blanked Beckman, Ortho and Roche were approximately 8-10% lower, suggesting a compensation for free glycerol in their calibrations. In summary, the Piccolo lipid panel assays appear to be accurate, which was confirmed by the CHOL and HDL assays qualifying for certification through the CDC Cholesterol Reference Method Laboratory Network by demonstrating acceptable agreement with reference methods.

Introduction

Objective: To validate the ABAXIS Lipid Panel Reagent Disc on the Piccolo® Point-of-Care Blood Analyzer

A. The Lipid Panel Reagent Disc is intended for quantitative determination of total cholesterol (CHOL), high-density lipoprotein cholesterol (HDL), and triglycerides (TRIG).

  1. Low-density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL), and a total cholesterol/high-density lipoprotein ratio (TC/H) are calculated by the analyzer.

  2. Heparinized whole blood, heparinized plasma, or serum may be used.

B. The ABAXIS Piccolo system consists of (see picture):

  1. The Piccolo Point-of-Care Blood Analyzer

   • It is 24.2 cm (9.5 in) high, 15.3 cm (6 in) wide, and 29.2 cm (11.5 in) deep.

   • It weighs 6.9 kg (15 pounds).

  2. Single-use disposable reagent discs

   • A clear plastic disc, 8 cm in diameter and 2 cm in depth

   • The reagent disc contains an aqueous diluent in the center and dry reagent beads in cuvettes around the disc periphery.

   • Plasma separation, mixing, and critical volumetric measurements are performed by the disc.

   • Fluid flow within the disc is controlled by centrifugal and capillary forces.

   • The test system requires approximately 90 µL of sample (whole blood, serum, or plasma).

C. Current instruments available for point-of-care (POC) testing have limited capability for analyzing multiple analytes, simultaneously. However, the Piccolo analyzer can provide up to 14 simultaneous results, depending on the reagent disc selected.

D. The Piccolo analyzer provides push button, walk-away convenience making the system highly suitable for POC applications.

E. Lipid panel reagents were validated in external and internal clinical trials for precision, accuracy, linearity, stability, and freedom from interferences and a reliable calibration protocol was developed.

F. Guidelines from the National Cholesterol Education Program (NCEP) encourage measurement of the lipid panel for screening, assessment, and monitoring of cardiovascular disease risk in patients.

G. The ABAXIS Lipid Panel Disc has received Cholesterol Reference Method Laboratory Network (CRMLN) certification for accuracy using accepted reference methods for CHOL and HDL.

H. A multi-center comparison of the ABAXIS lipid panel versus conventional high-throughput methods in three separate hospital laboratories was organized.
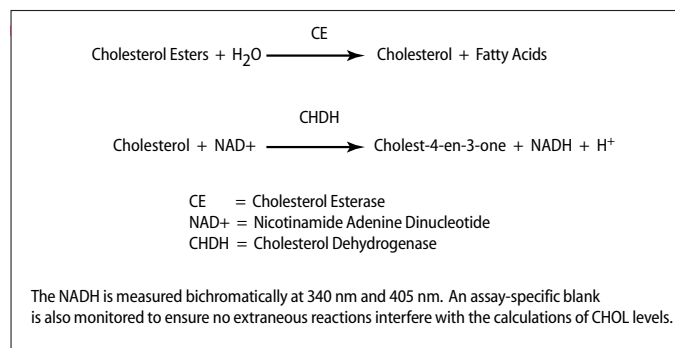
Principles of the Assay

Figure 1

$$CE$$
$$\text{Cholesterol Esters} + H_2O \longrightarrow \text{Cholesterol} + \text{Fatty Acids}$$

$$CHDH$$
$$\text{Cholesterol} + NAD+ \longrightarrow \text{Cholest-4-en-3-one} + NADH + H^+$$

CE = Cholesterol Esterase
NAD+ = Nicotinamide Adenine Dinucleotide
CHDH = Cholesterol Dehydrogenase

The NADH is measured bichromatically at 340 nm and 405 nm. An assay-specific blank is also monitored to ensure no extraneous reactions interfere with the calculations of CHOL levels.

Figure 2

$$\text{CM, LDL, VLDL, and HDL} + \text{Dextran Sulfate} + MgSO_4 \longrightarrow \text{HDL} + \text{Insoluble Complexes}$$

$$\text{Centrifugation}$$
$$\text{Insoluble Complexes} \longrightarrow \text{Insoluble Complexes Pelleted against Wall of Reaction Cuvette}$$

$$PEG\text{-}CE$$
$$\text{HDL-cholesterol Esters} + H_2O \longrightarrow \text{Cholesterol} + \text{Fatty Acids}$$

$$PEG\text{-}CO$$
$$\text{Cholesterol} + O_2 \longrightarrow \text{Cholest-4-en-3-one} + H_2O_2$$

$$\text{Peroxidase}$$
$$H_2O_2 + TOOS + 4\text{-}AAP \longrightarrow \text{Color Development}$$

PEG-CE = Polyethylene glycol-modified cholesterol esterase
PEG-CO = Polyethylene glycol-modified cholesterol oxidase
TOOS = N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline, sodium salt, dihydrate
4-AAP = 4-Aminoantipyrine

The production of a purple colored product that has an absorbance maximum at 550 nm and is referenced to absorbance at 630 nm is measured. A sample blank is also monitored to ensure no extraneous reactions interfere with the calculations of HDL levels.

Figure 3

$$\text{Lipase}$$
$$\text{Triglycerides} + 3H_2O \longrightarrow \text{Glycerol} + 3\text{Fatty Acids}$$

$$GK, Mg++$$
$$\text{Glycerol} + ATP \longrightarrow \text{G-3-P} + ADP$$

$$G\text{-}3\text{-}PDH$$
$$\text{G-3-P} + NAD^+ \longrightarrow DAP + NADH + H^+$$

$$\text{Diaphorase}$$
$$NADH + H^+ + INT \longrightarrow NAD^+ + \text{Formazan}$$

ATP = Adenosine 5'-triphosphate
GK = Glycerol Kinase
G-3-P = Glycerol-3-Phosphate
NAD+ = Nicotinamide Adenine Dinucleotide
G-3-PDH = Glycerol-3-Phosphate Dehydrogenase
DAP = Dihydroxyacetone Phosphate
INT = p-Iodonitrotetrazolium Violet

The intensity of the highly colored formazan is measured bichromatically at 500 nm and 850 nm. An assay-specific blank is also monitored to ensure no extraneous reactions interfere with the calculations of TRIG levels.
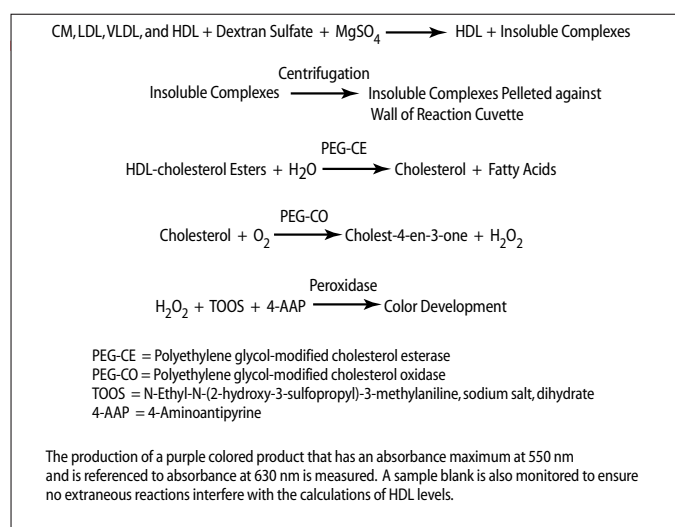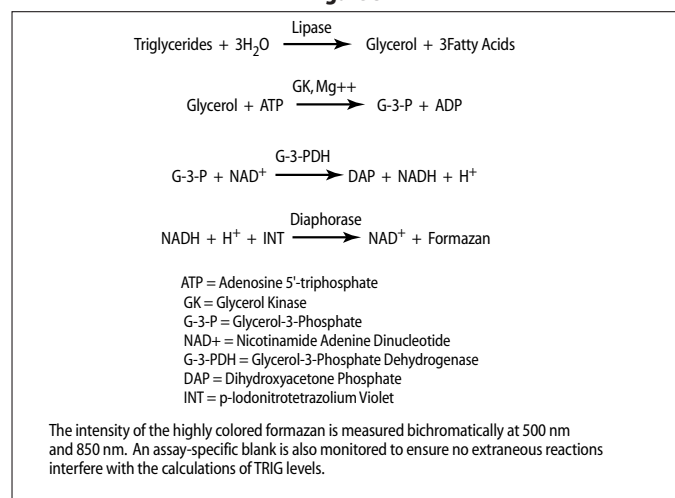
Materials and Methods

We evaluated the assays on the ABAXIS Piccolo Lipid Panel Disc by assessing:

A. Precision

1. Within-run and total precision was evaluated in accordance with NCCLS EP5-A guidelines with modifications based on NCCLS EP18-P for unit-use devices.

2. Results from two sites involving use of two reagent disc lots and four Piccolo analyzers across multiple days were combined.

3. Testing was done at the Core Laboratory for Clinical Studies, Washington University School of Medicine, St. Louis, MO, and ABAXIS.

B. Correlation

1. Testing was done at 3 sites:

   a. Core Laboratory for Clinical Studies, Washington University School of Medicine, St. Louis, MO = WU

   b. Department of Pathology, Oregon Health and Science University, Portland, OR = OHSU

   c. Clinical Laboratory, Southwest Washington Medical Center, Vancouver, WA = SWMC

2. Testing at WU included CDC-standardized CHOL and TRIG methods (CHOL and TRIG reagents from Bayer) on a Hitachi 917 analyzer. HDL was tested using HDL-C plus reagents from Roche on the Hitachi 917 analyzer. Piccolo testing was also done at this site.

3. Testing at OSHU included CHOL, HDL, and TRIG methods on a Beckman LX-20 analyzer.

4. Testing at SWMC included CHOL and TRIG methods on an Ortho Vitros® 950 analyzer and CHOL, HDL, and TRIG methods on a Roche Integra® analyzer.

5. Aliquots of a total of 37 serum samples were shipped from WU to the two other sites.

6. Testing was done on each sample in duplicate. Both duplicates were plotted providing 74 points. One sample had a TRIG of >400 mg/dL which is above the TRIG limit of the Piccolo for HDL. The software would suppress any HDL value for this sample and indicate LIP. This specimen was omitted from the HDL correlation analysis.
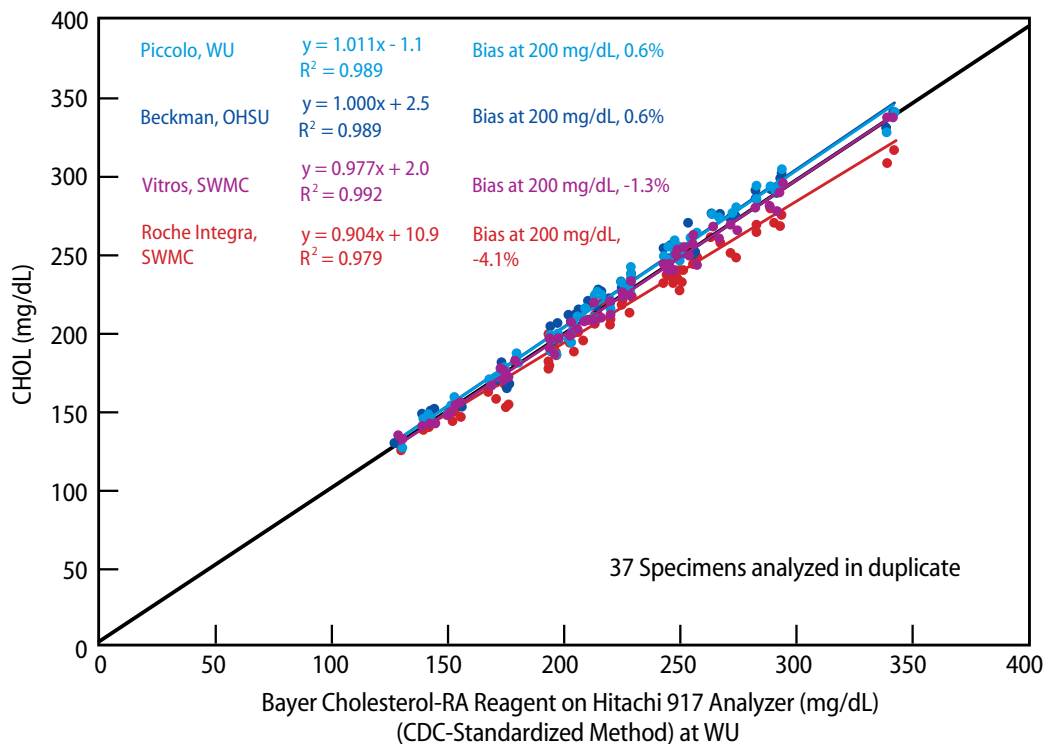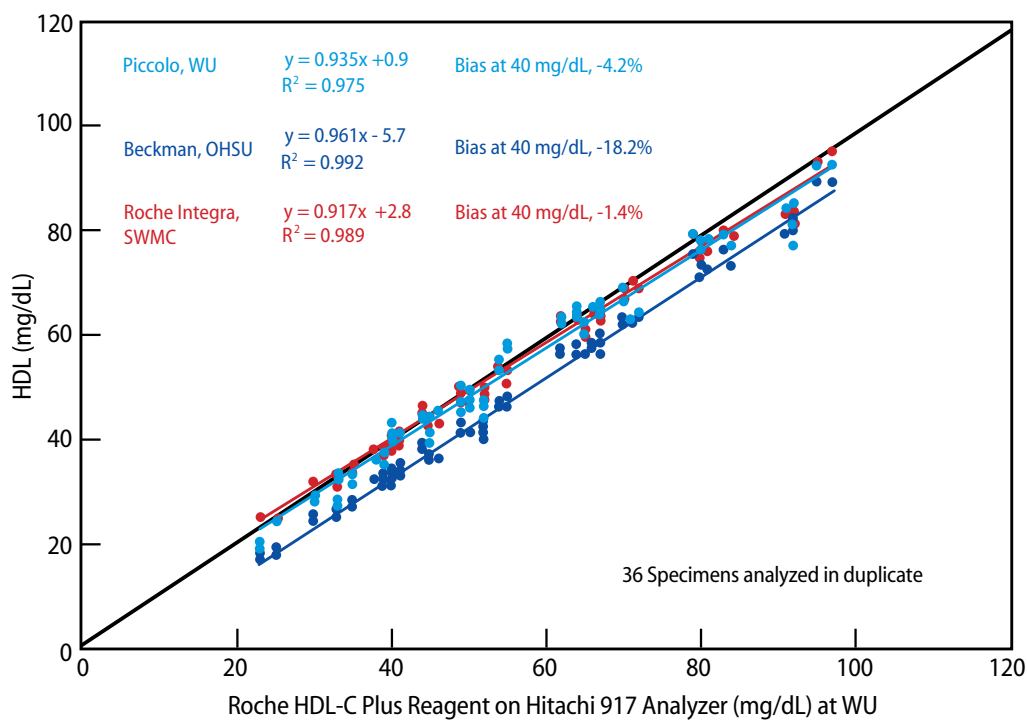
Figure 4. Total Cholesterol (CHOL) Comparisons

Piccolo, WU — $y = 1.011x - 1.1$, $R^2 = 0.989$ — Bias at 200 mg/dL, 0.6%

Beckman, OHSU — $y = 1.000x + 2.5$, $R^2 = 0.989$ — Bias at 200 mg/dL, 0.6%

Vitros, SWMC — $y = 0.977x + 2.0$, $R^2 = 0.992$ — Bias at 200 mg/dL, -1.3%

Roche Integra, SWMC — $y = 0.904x + 10.9$, $R^2 = 0.979$ — Bias at 200 mg/dL, -4.1%

CHOL (mg/dL)

37 Specimens analyzed in duplicate

Bayer Cholesterol-RA Reagent on Hitachi 917 Analyzer (mg/dL)
(CDC-Standardized Method) at WU

Figure 5. High-Density Lipoprotein Cholesterol (HDL) Comparisons

Piccolo, WU — $y = 0.935x + 0.9$, $R^2 = 0.975$ — Bias at 40 mg/dL, -4.2%

Beckman, OHSU — $y = 0.961x - 5.7$, $R^2 = 0.992$ — Bias at 40 mg/dL, -18.2%

Roche Integra, SWMC — $y = 0.917x + 2.8$, $R^2 = 0.989$ — Bias at 40 mg/dL, -1.4%

HDL (mg/dL)

36 Specimens analyzed in duplicate

Roche HDL-C Plus Reagent on Hitachi 917 Analyzer (mg/dL) at WU

Figure 6. Total Triglycerides (TRIG) Comparisons

Piccolo, WU — y = 0.960x +3.7, R^2 = 0.997 — Bias at 200 mg/dL, -2.2%

Beckman, OHSU — y = 0.923x - 4.6, R^2 = 0.998 — Bias at 200 mg/dL, -10.1%

Vitros, SWMC — y = 0.910x - 0.6, R^2 = 0.996 — Bias at 200 mg/dL, -9.3%

Roche Integra, SWMC — y = 0.925x - 1.1, R^2 = 0.998 — Bias at 200 mg/dL, -8.1%

37 Specimens analyzed in duplicate

Bayer Triglycerides RA Reagent on Hitachi 917 Analyzer (mg/dL)
(CDC-Standardized Method) at WU
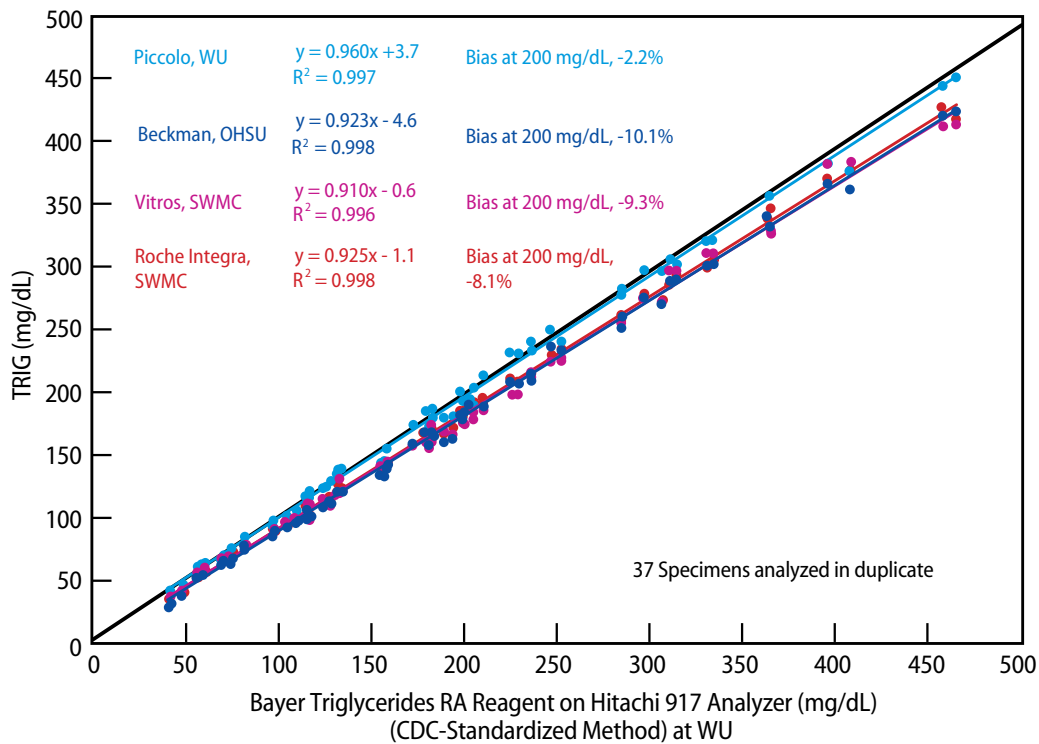
Figure 7. Total Triglycerides Comparisons vs Net Triglycerides

Piccolo, WU — y = 0.981x +9.5, R^2 = 0.998 — Bias at 200 mg/dL, 2.9%

Bechman, OHSU — y = 0.9429x +1.0, R^2 = 0.997 — Bias at 200 mg/dL, -5.2%

Vitros, SWMC — y = 0.9297x +5.0, R^2 = 0.995 — Bias at 200 mg/dL, -4.5%

Roche Integra, SWMC — y = 0.9448x +4.5, R^2 = 0.998 — Bias at 200 mg/dL, -3.3%

37 Specimens analyzed in duplicate

Bayer Triglycerides RA Reagent on Hitachi 917 Analyzer (mg/dL)
(CDC-Standardized Method)
TRIG-GLYCEROL BLANK, at WU
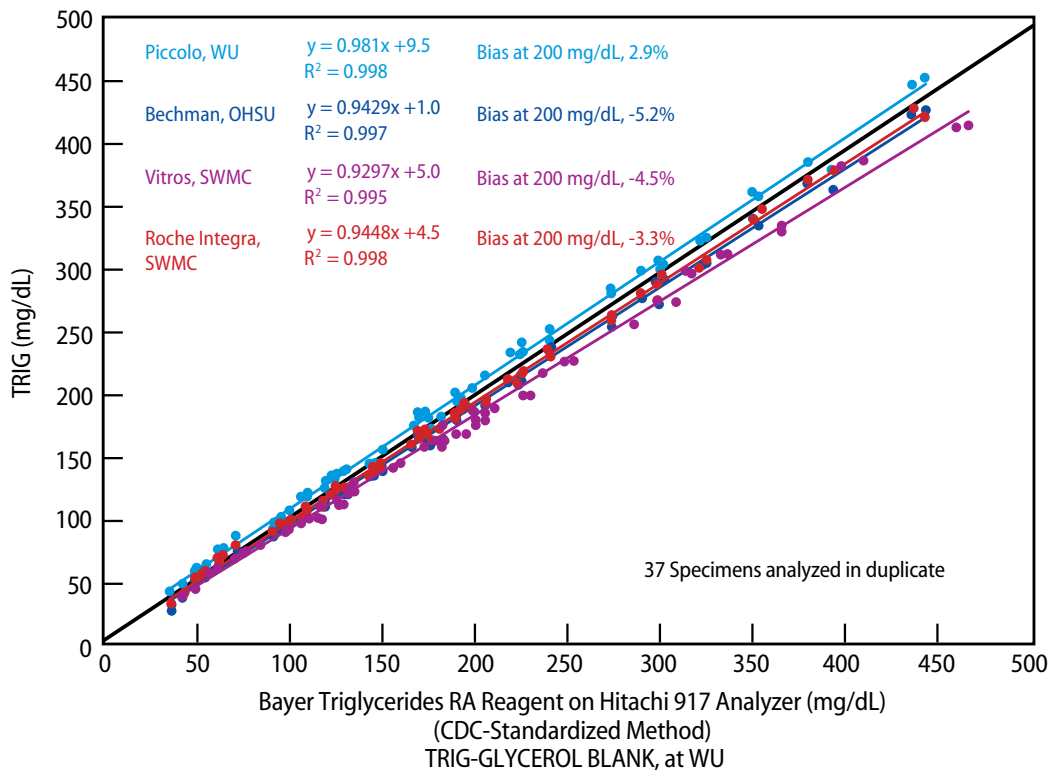
Results

Table 1. Precision

Analyte	Sample Size	Within-Run	Total
Total Cholesterol (CHOL)			
Serum 1	N = 160		
Mean (mg/dL)		223.7	223.7
SD		3.0	5.7
%CV		1.3	2.6
Serum 2	N = 160		
Mean (mg/dL)		202.2	202.2
SD		3.1	4.4
%CV		1.5	2.2
HDL			
Serum 1	N = 160		
Mean (mg/dL)		55.3	55.3
SD		1.4	1.9
%CV		2.6	3.5
Serum 2	N = 160		
Mean (mg/dL)		38.0	38.0
SD		1.3	1.6
%CV		3.5	4.3
Triglycerides (TRIG)			
Serum 1	N = 160		
Mean (mg/dL)		206.8	206.8
SD		4.7	5.5
%CV		2.3	2.6
Serum 2	N = 160		
Mean (mg/dL)		163.7	163.7
SD		1.8	2.4
%CV		1.1	1.5

These data indicate that all three assays meet the NCEP precision criteria.

Conclusions

- The new Piccolo Lipid Panel Disc allows reliable, accurate, simultaneous, and quantitative measurement of CHOL, HDL, and TRIG with automatic calculation of LDL, VLDL, and TC/H.

- Operation is appropriate for POC applications with push button, walk away convenience.

- The lipid panel reagents demonstrate acceptable precision and accuracy.

- Results for all analytes were in good agreement with CDC-standardized methods in a specialty lipid laboratory and in clinically acceptable agreement with conventional methods in routine clinical laboratories.

- The excellent accuracy of the methods for CHOL and HDL was established by meeting the requirements of the Cholesterol Reference Laboratory Network for certification of accuracy. (Note: CRMLN certification of triglyceride methods is not yet available through labs in the USA.)

- Potentially, Piccolo Lipid Panel Discs could be configured to include additional assays such as those for liver function and glucose. These could present a significant improvement in patient care allowing diagnosis and/or monitoring of therapy in a single visit in a POC setting.

- In summary, this study demonstrates the reliability of the newly developed Piccolo Lipid Panel and suitability for use in POC applications.