

Customer and Technical Service: 800-822-2947

CLIA Waived: Use lithium heparin whole blood, only
Moderate Complexity: Use lithium heparin whole blood,
lithium heparin plasma, or serum

April 2008

PN: 400-7144 Rev.: J

© 2002, Abaxis, Inc., Union City, CA 94587

1. Intended Use

The Piccolo[®] Lipid Panel Reagent Disc, used with the Piccolo Blood Chemistry Analyzer or the Piccolo xpress[™] Chemistry Analyzer, is intended for the *in vitro* quantitative determination of total cholesterol (CHOL), high-density lipoprotein cholesterol (HDL), and triglycerides (TRIG) in a clinical laboratory setting. From these determinations low-density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL), and a total cholesterol/high-density lipoprotein cholesterol ratio (TC/H) are calculated by the analyzer.

This test is waived under CLIA '88 regulations. If a laboratory modifies the test system instructions, then the test is considered high complexity and subject to all CLIA requirements. For CLIA waived labs, only lithium heparin whole blood may be tested. For use in moderate complexity labs, lithium heparinized whole blood, lithium heparinized plasma, or serum may be used.

A CLIA Certificate of Waiver is needed to perform CLIA waived testing. A Certificate of Waiver can be obtained from the Centers for Medicare & Medicaid Services (CMS). Please contact Abaxis Technical Service at (800) 822-2947 for assistance in obtaining one.

2. Summary and Explanation of Tests

Clinical Significance

Measurement of the serum lipids and lipoproteins is useful in characterizing an individual's risk of developing cardiovascular diseases (CVD) and in monitoring therapeutic interventions.¹ Consensus-based guidelines for measurement and cut-points for interpretation have been provided by the National Cholesterol Education Program.^{2,3,4}

The circulating lipids are carried on lipoproteins. The LDL fraction, the major lipoprotein contributor to the development of atherosclerosis and for which treatment has been conclusively demonstrated to be effective, carries most of the circulating cholesterol in the blood. Total serum cholesterol has been measured for many years to quantify the total amount of lipoproteins as a convenient means of assessing CVD risk. However, some of the cholesterol is carried on HDL particles, which are anti-atherogenic or inversely associated with risk of developing CVDs. Thus, quantitation of the major individual cholesterol-carrying lipoproteins, LDL and HDL, provides a better assessment of overall risk.

Triglycerides, the body's major fuel, are carried into the blood stream on large lipoproteins called chylomicrons (CM). VLDL particles also carry triglycerides, primarily synthesized in the liver from excess fatty acids. In the circulation triglycerides are hydrolyzed and their fatty acids transported into peripheral cells leaving remnant particles, precursors to LDL. After an overnight fast, chylomicrons have generally been cleared from the circulation. Higher levels of triglycerides measured in a fasting specimen indicate impaired clearance or over-production, which may increase risk of developing CVD, making their measurement useful in characterizing metabolic disorders and overall risk.

The US National Heart, Lung and Blood Institute organized the National Cholesterol Education Program, which convened expert panels to develop clinical guidelines for classification and treatment of high cholesterol. The most recent recommendations, the Adult Treatment Panel III guidelines,^{2,3,4} base treatment decisions primarily on the LDL levels, calculated as part of the lipid panel after measurement of total cholesterol, HDL, and triglycerides. LDL cut-points of 100, 130, 160, and 190 mg/dL define optimal, near optimal, borderline high, high and very high risk categories. An HDL value below 40 mg/dL is low, considered to be a risk factor by the ATP III, modifying the LDL treatment goal. An HDL value above 60 mg/dL is defined as high, considered desirable and a negative risk factor, subtracting from the total number of risk factors in selecting the appropriate treatment goal for LDL. For triglycerides, cut-points of 150, 200, and 500 mg/dL define normal, borderline-high, high, and very-high levels.

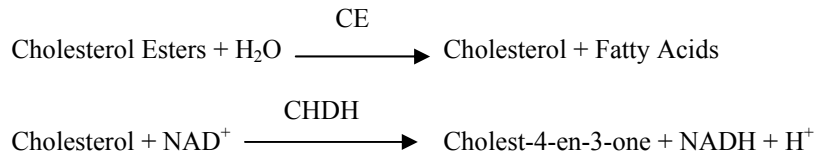
The Piccolo Total Cholesterol and HDL assays have met the requirements for certification of accuracy and precision by the Cholesterol Reference Method Laboratory Network (CRMLN), which is coordinated by the Centers for Disease Control. The certification process evaluates the accuracy of method calibration and precision, helping to assure reliable classification of patients based on the NCEP cut-points.

As with any diagnostic test procedure, all other test procedures including the clinical status of the patient, should be considered prior to final diagnosis.

3. Principles of Procedures

Total Cholesterol (CHOL)

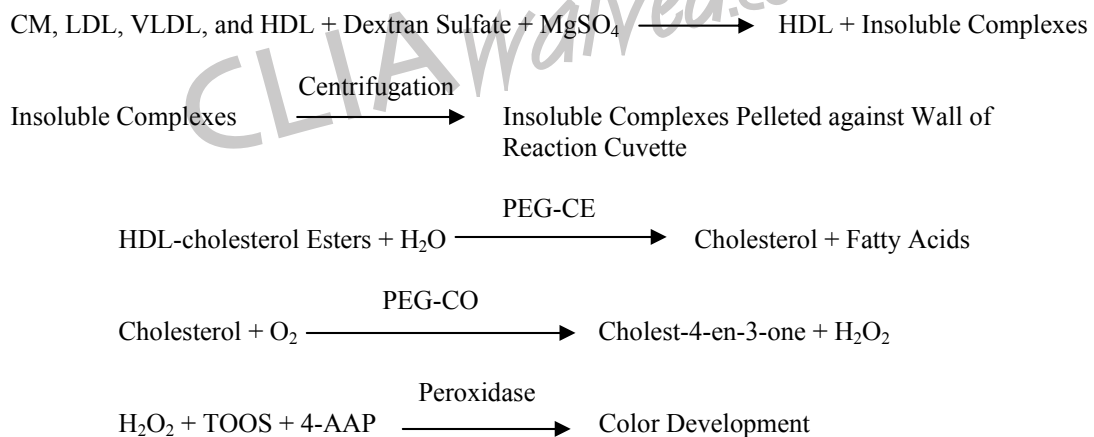
The Abaxis CHOL assay is an enzymatic end-point method that uses cholesterol esterase (CE) and cholesterol dehydrogenase (CHDH).⁵



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 Abaxis HDL assay is a precipitation method that utilizes polyethylene glycol-modified cholesterol esterase (PEG-CE) and cholesterol oxidase (PEG-CO) for additional specificity.⁶ The reaction mechanism follows:

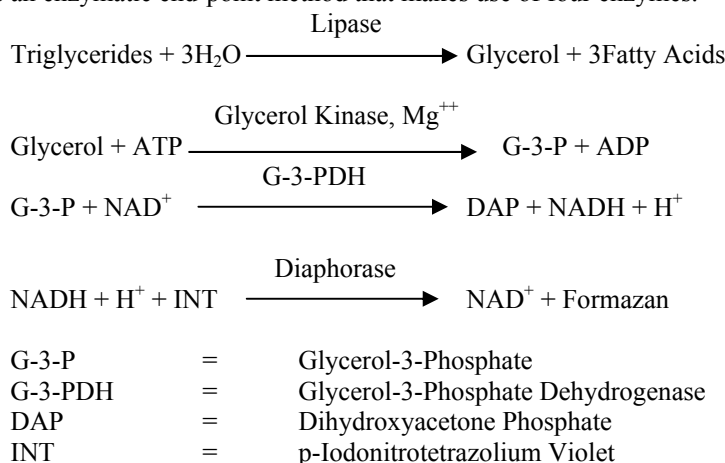


TOOS = N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline, sodium salt, dihydrate
4-AAP = 4-Aminoantipyrine

The precipitating agents dextran sulfate and magnesium sulfate (MgSO₄) 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 (H₂O₂). 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)

The Abaxis TRIG assay is an enzymatic end-point method that makes use of four enzymes.^{7,8} The reaction mechanism follows:



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 glycerol phosphate 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 Analyzer or Piccolo xpress Analyzer automatically calculates the concentration of LDL in each sample using the directly determined values for total cholesterol, HDL, and triglycerides and the standard Friedewald equation.⁹ This equation is not valid for triglyceride concentrations above 400 mg/dL, non-fasting patients, and patients with Type III hyperlipoproteinemia (dysbetalipoproteinemia).^{9,10} 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. On the print card, the calculated value for LDL is followed by a "c" to indicate that it is calculated.

VLDL (Calculated)

The Piccolo Analyzer or the Piccolo xpress Analyzer automatically calculates the concentration of VLDL in each sample using the standard triglycerides/5 (if units in mg/dL) equation.⁹ This equation is not valid for triglyceride concentrations above 400 mg/dL, non-fasting patients, and patients with Type III hyperlipoproteinemia (dysbetalipoproteinemia).^{9,10} Of course, no VLDL value is calculated if no triglyceride value is available. On the print card, the calculated value for VLDL is followed by a "c" to indicate that it is calculated.

Total Cholesterol/HDL Ratio (Calculated)

The Piccolo Analyzer or the Piccolo xpress Analyzer 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. On the print card, the calculated value for TC/H is followed by a "c" to indicate that it is calculated.

4. Principle of Operation

Refer to the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual, for the Principles and Limitations of the Procedure. A detailed description of the Piccolo analyzer and reagent disc has been described by Schembri et al.¹¹

5. Description of Reagents

Reagents

Each Piccolo Lipid Panel Reagent Disc contains dry test-specific reagent beads (described below). A dry sample blank reagent bead (comprised of buffer, surfactants, excipients, and preservatives) is included in each disc for use in calculating concentrations of HDL. Dedicated blank beads are also included in the disc to calculate concentrations of CHOL and TRIG. Each disc also contains a diluent consisting of a surfactant and preservatives.

Table 1: Reagents

Component	Quantity/Disc	
4-Aminoantipyrine	6.7	µg
Adenosine 5'-triphosphate, disodium salt	9.2	µg
Ascorbate oxidase	0.042	U
Cholesterol dehydrogenase	0.080	U
Cholesterol esterase (Genzyme-N)	0.27	U
Cholesterol esterase (Genzyme-P)	0.0080	U
Dextran sulfate	8.4	µg
Diaphorase	0.25	U
N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline, sodium salt, dihydrate (TOOS)	79	µg
Glycerol kinase	0.084	U
Glycerol-3-phosphate dehydrogenase	0.21	U
Iodonitrotetrazolium chloride (INT)	8.4	µg
Lipase	16.8	U
Magnesium chloride, hexahydrate	8.6	µg
Magnesium sulfate, heptahydrate	197	µg
Nicotinamide adenine dinucleotide, monosodium salt (NAD)	455	µg
PEG-cholesterol esterase	0.013	U
PEG-cholesterol oxidase	0.089	U
Peroxidase	0.27	U
Buffers, surfactants, excipients, and preservatives		

Warnings and Precautions

- For *In vitro* Diagnostic Use
- The diluent container in the reagent disc is automatically opened when the analyzer drawer closes. A disc with an opened diluent container cannot be re-used. Ensure that the sample or control has been placed into the disc before closing the drawer.
- Used reagent discs contain human body fluids. Follow good laboratory safety practices when handling and disposing of used discs.¹² See the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual for instructions on cleaning biohazardous spills.
- The reagent discs are plastic and may crack or chip if dropped. Never use a dropped disc as it may spray biohazardous material throughout the interior of the analyzer.
- Reagent beads may contain acids or caustic substances. The operator does not come into contact with the reagent beads when following the recommended procedures. In the event that the beads are handled (e.g., cleaning up after dropping and cracking a reagent disc), avoid ingestion, skin contact, or inhalation of the reagent beads.

Instructions for Reagent Handling

Reagent discs may be used directly from the refrigerator without warming. A disc not used within 20 minutes of opening the pouch should be discarded. Do not allow discs sealed in their foil pouches to remain at room temperature longer than 48 hours prior to use. Open the sealed foil pouch, remove the disc, and use according to the instructions provided in the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual.

Storage

Store reagent discs in their sealed pouches at 2-8°C (36-46°F). Do not expose opened or unopened discs to direct sunlight or temperatures above 32°C (90°F). Reagent discs may be used until the expiration date printed on the box label and on each pouch. The expiration date is also encoded in the bar code printed on the bar code ring. An error message will appear on the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Display if the reagents have expired.

Indications of Reagent Disc Instability/Deterioration

A torn or otherwise damaged pouch may allow moisture to reach the unused disc and adversely affect reagent performance. Do not use a disc from a damaged pouch.

6. Instrument

See the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual for complete information on use of the analyzer.

7. Sample Collection and Preparation

Sample collection techniques are described in the "Sample Collection" section of the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual.

- According to the ATP III,^{2,3,4} fasting samples (eight to 12 hours) should be used to determine CHOL, HDL, TRIG, and LDL. Hence, it is highly recommended that fasting samples be used with the Lipid Panel Disc. Should the patient not be fasting, the TRIG and calculated LDL values are not valid.
- Use only lithium heparin (green stopper) evacuated specimen collection tubes with or without gel separators for whole blood or plasma samples. Use no-additive (red stopper) evacuated specimen collection tubes or serum separator tubes (red or red/black stopper) for serum samples.
- The minimum required sample size is ~100 µL of heparinized whole blood, heparinized plasma, serum or control material. The reagent disc sample chamber can contain up to 120 µL of sample.
- Whole blood samples obtained by venipuncture must be homogeneous before transferring a sample to the reagent disc. Gently invert the collection tube several times just prior to sample transfer. Do not shake the collection tube; shaking may cause hemolysis.
- Whole blood venipuncture samples should be run within 60 minutes of collection.^{13,14} The sample may be separated into plasma or serum and stored in capped sample tubes at 2-8°C (36-46°F) if the sample cannot be run within 60 minutes.
- Start the test within 10 minutes of transferring the sample into the reagent disc.

8. Procedure

Materials Provided

- One Piccolo Lipid Panel Reagent Disc PN: 400-1025 (a box of discs PN: 400-0025)

Materials Required but not Provided

- Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer
- A sample transfer pipette (fixed volume approximately 100 µL) and tips are provided with each Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer and may be reordered from Abaxis.
- Commercially available control reagents recommended by Abaxis (contact Abaxis Technical Service for approved control materials and expected values).
- Timer

Test Parameters

The Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer operates at ambient temperatures between 15°C and 32°C (59-90°F). The analysis time for each Piccolo Lipid Panel Reagent Disc is less than 15 minutes. The analyzer maintains the reagent disc at a temperature of 37°C (98.6°F) over the measurement interval.

Test Procedure

The complete sample collection and step-by-step operating procedures are detailed in the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual.

Calibration

The Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer is calibrated by the manufacturer before shipment. The bar code printed on the bar code ring provides the analyzer with disc-specific calibration data. See the Piccolo Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual.

Quality Control

See Section 2.4 of the Piccolo Operator's Manual or Section 6 (Calibration and Quality Control) of the Piccolo xpress Operator's Manual. Performance of the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer can be verified by running controls. For a list of approved quality control materials with acceptance ranges, please contact Abaxis Technical Support. Other human serum or plasma-based controls may not be compatible. Quality control materials should be stored as per the package-insert included with the controls.

If control results are out of range, repeat one time. If still out of range, call Technical Support. Do not report results if controls are outside their labeled limits. See the Piccolo or Piccolo xpress Operator's Manual for a detailed discussion on running, recording, interpreting, and plotting control results.

Waived Laboratories: Abaxis recommends control testing as follows:

- at least every 30 days
- whenever the laboratory conditions have changed significantly, e.g. Piccolo moved to a new location or changes in temperature control
- when training or retraining of personnel is indicated
- with each new lot (CLIA waived tests in waived status labs)

Non-Waived Laboratories: Abaxis recommends control testing to follow federal, state, and local guidelines.

9. Results

The Piccolo Blood Chemistry Analyzer or Piccolo xpress Chemistry Analyzer automatically tests, calculates, and prints the analyte concentrations in the sample. For those results that are calculated and not directly determined, LDL, VLDL, and TC/H, the results are followed by a "c" to indicate that they are calculated. Details of the endpoint and rate reaction calculations are found in the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual.

Interpretation of results is detailed in the Operator's Manual. Results are printed onto result cards supplied by Abaxis. The result cards have an adhesive backing for easy placement in the patient's files.

10. Limitations of Procedure

General procedural limitations are discussed in the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual.

- The only anticoagulant **recommended for use** with the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer is **lithium heparin**. Do not use sodium heparin.
- Samples with hematocrits in excess of 62% packed red cell volume may give inaccurate results. Samples with high hematocrits may be reported as hemolyzed. These samples may be spun down to get plasma and then re-run in a new reagent disc.

- **Any result for a particular test that exceeds the assay range should be analyzed by another approved test method or sent to a referral laboratory. Do not dilute the sample and run it again on the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer.**

Warning: Extensive testing of the Piccolo Blood Chemistry System or the Piccolo xpress Chemistry System has shown that, in very rare instances, sample dispensed into the reagent disc may not flow smoothly into the sample chamber. Due to the uneven flow, an inadequate quantity of sample may be analyzed and several results may fall outside the expected ranges. The sample may be re-run using a new reagent disc.

Interference

Substances were tested as interferents with the analytes. Human serum pools were prepared. The concentration at which each potential interferent was tested was based on the testing levels in NCCLS EP7-A.¹⁵

Effects of Endogenous Substances

- Physiological interferents (hemolysis, icterus and lipemia) cause changes in the reported concentrations of some analytes. The sample indices are printed on the bottom of each result card to inform the operator about the levels of interferents present in each sample.
- The Piccolo Blood Chemistry System or the Piccolo xpress Chemistry System suppresses any results that are affected by >10% interference from hemolysis, lipemia or icterus. “HEM”, “LIP”, or “ICT” respectively, is printed on the result card in place of the result.
- For maximum levels of endogenous substances contact Abaxis Technical Service.

Effects of Therapeutic Substances

Sixteen therapeutic substances were selected as potential interferents for the total cholesterol, HDL, and triglycerides assays based on recommendations by Young.¹⁶ Significant interference is defined as a >10% shift in the result for a normal range specimen. Human serum pools were supplemented with a known concentration of the drugs or chemicals and then analyzed.

Table 2: Therapeutic Substances Evaluated

	Physiologic or Therapeutic Range^{15,16} (mg/dL)	Highest Concentration Tested (mg/dL)
Acetaminophen	2 -10	100
Acetoacetate, Lithium	0.05-3.6	102
Acetylsalicylic Acid	1-2	50
Ascorbic Acid		3
Digoxin	0.8-1.5	5
Glutathione		30
Heparin, Lithium		4.4 (800 U/dL)
β-Hydroxybutyrate	0.21-2.81	1,000
Ibuprofen	0.5-4.2	50
Isoniazide	0.1-0.7	4
Lactate, Lithium	6-12	84
Lidocaine	0.5-0.6	1
Methicillin, Sodium		100
Phenytoin	1-2	3
Salicylic Acid		50
Theophylline	1-2	20

None of the assays demonstrated significant interference at the concentrations of exogenous substances tested.

Table 3: Concentration of Analytes in Serum Pools (2 Levels) Used for Exogenous Substance Interference Studies

Analyte	Concentration
Cholesterol (CHOL)	163 and 197 mg/dL
HDL	39 and 52 mg/dL
Triglycerides (TRIG)	125 and 183 mg/dL

11. Cutpoints

Consensus based cutpoints for the lipid/lipoprotein analytes have been established by the National Cholesterol Education Program as follows:^{2,3,4}

Table 4: Medical Decision Values^{2,3,4}

	Interpretation	Cutpoints mg/dL	Cutpoints mmol/L
Total Cholesterol (CHOL)	Desirable	< 200	< 5.17
	Borderline High	200–239	5.17–6.18
	High	≥ 240	6.20
HDL	Low HDL - Risk Factor	< 40	< 1.03
	High HDL - Negative Risk Factor (Desirable)	≥ 60	≥ 1.55
Triglycerides (TRIG)	Normal	< 150	< 1.70
	Borderline High	150–199	1.70–2.25
	High	200–499	2.26–5.64
	Very High	≥ 500	≥ 5.65
LDL*	Optimal	< 100	< 2.58
	Near Optimal	100–129	2.58–3.33
	Borderline High	130–159	3.36–4.11
	High	160–189	4.13–4.88
	Very High	≥ 190	≥ 4.91
VLDL (CALC) **	Normal	< 30	
	High	≥ 30	
		Male	Female
Total Chol/HDL Ratio	Low Risk	≤ 5	≤ 4.5
	High Risk	> 5	> 4.5

* The Piccolo or Piccolo xpresscalculates the LDL concentration using the Friedewald equation.⁹

** For further information see: NCEP, ATP III Report 2002, Section II. Rationale for Intervention, 3. Other Lipid Risk Factors, Page II-8.²

Total Cholesterol / HDL Ratios (TC/H)

The total cholesterol to HDL ratio (TC/H) is calculated as a convenience to users. A TC/H ratio of ≤ 5 is generally considered desirable for men. Because women usually have higher HDL values than men, some recommend a cutpoint of 4.5 for women.¹⁷ This ratio has been advocated by some as a simple and convenient means of expressing CVD risk in a single number, incorporating total cholesterol as a marker for atherogenic lipoproteins in the numerator and the anti-atherogenic HDL cholesterol in the denominator.¹ User's should be aware that even though the TC/H ratio is a powerful predictor of CVD risk as shown by numerous epidemiology studies,¹ the NCEP does not recommend its use in managing patients. The NCEP clinical guidelines base treatment decisions on the individual lipoproteins (Table 5) and consider use of the ratio as a possible diversion from the priority, the individual lipoprotein measurements.^{2,3,4}

12. Performance Characteristics

Linearity

The chemistry for each analyte is linear over the dynamic range listed below when the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer is operated according to the recommended procedure (refer to the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual). This evaluation made use of NCCLS EP6-P2.¹⁸

Table 5: Piccolo Dynamic Ranges

Analyte	Common Units	SI Units
CHOL	20–520 mg/dL	0.52–13.5 mmol/L
HDL	15–100 mg/dL	0.39–2.59 mmol/L
TRIG	20–500 mg/dL	0.23–5.65 mmol/L

If the analyte concentration is above the measuring range (dynamic range), but less than the system range, the print card will indicate a ">" sign at the upper limit and an asterisk after the number, e.g. CHOL >520* mg/dL. If lower than the dynamic range, a "<" will be printed with an asterisk, e.g. CHOL <20* mg/dL. For values that are grossly beyond the measurement range (system range), "~~~~" will be printed instead of a result. Any time "~~~~" appears on a print card, collect a new sample and rerun the test. If results for the second sample are suppressed again, please call Abaxis Technical Service.

Sensitivity

The lower limit of the reportable (dynamic) range for each analyte is: cholesterol 20 mg/dL (0.52 mmol/L); HDL 15 mg/dL (0.39 mmol/L), and triglycerides 20 mg/dL (0.23 mmol/L).

Precision

Precision studies were conducted using NCCLS EP5-A guidelines¹⁹ with modifications based on NCCLS EP18-P²⁰ for unit-use devices. Results for within-run and total precision were determined using two serum samples. The studies made use of data from four analyzers, two lots of discs, and two different sites across 10 days for both samples. The results are summarized below.

Table 6: Precision

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

This data indicate that all three assays meet the NCEP precision criteria.^{2,3,4}

Correlation

Serum samples were collected and assayed on the Piccolo Blood Chemistry Analyzer and by comparative methods. All test results were generated at a field site. The samples were chosen in order to provide a distribution of values using NCCLS EP9-A2 guideline as a target for each analyte.²¹ Representative correlation statistics are shown in Table 8.

Table 7: Correlation of Piccolo Blood Chemistry Analyzer with Comparative Methods

Assay	Correlation Coefficient (r)	Slope	Intercept	SEE	N	Sample Range (mg/dL)	Comparative Method
Cholesterol (CHOL)	0.997	1.079	-17.1	4.5	174	115-342	Bayer Cholesterol Assay on Hitachi 917
HDL	0.965	0.851	8.3	3.9	166	23-97	Roche HDL-C plus on Hitachi 917
Triglycerides (TRIG)	0.999	0.983	8.2	4.4	172	38-487	Bayer Triglyceride Assay on Hitachi 917

Table 8: Calculated Recovery of the Abaxis Lipid Panel Assays

Assay	Predicate Device Concentration mg/dL	Calculated Piccolo Recovery from Linear Regression Data Above mg/dL	Bias mg/dL	% Bias
Cholesterol (CHOL)	200	199	-1	-0.5
	240	242	2	0.8
HDL	40	42	2	5.0
	60	59	-1	-1.7
Triglycerides (TRIG)	150	156	6	4.0
	200	205	5	2.5

Accuracy – Cholesterol Reference Method Laboratory Network (CRMLN) Certification

Accuracy of the Piccolo assays for total cholesterol and HDL cholesterol was established by completing the certification process of the CRMLN for these analytes in serum. A key part of the CRMLN certification is linear regression analysis of the Piccolo assays versus the reference methods. The accuracy of the Total Cholesterol Assay compared to Abell-Kendall reference method is indicated by the correlation coefficient (R^2) of 0.996, slope of 0.972, and intercept of 7.2 mg/dL. An among-run (n=10) CV for the Piccolo Total Cholesterol Assay was determined to be 0.8%.

For the Piccolo HDL Assay compared to the HDL reference method, precipitation followed by Abell-Kendall cholesterol assay, the correlation coefficient (R^2) was 0.986, slope of 0.968, and intercept of 2.1 mg/dL. An among-run (n=20) CV for the Piccolo HDL Assay was determined to be 1.9%.

The observed analytical performance met the requirements for CRMLN certification for total cholesterol and HDL for serum. CRMLN certification for triglyceride assays is not yet available in the United States. However, the correlation study described above for triglycerides was performed in a laboratory that was standardized for triglycerides through the CDC-NHLBI Lipid Standardization Program.

Results of Untrained User Study

An “untrained user” study was conducted in which participants were given only the test instructions and asked to perform testing of 3 lipid panels with blinded randomized samples. The samples consisted of serum pools prepared at three levels for each of the three analytes, total cholesterol, HDL cholesterol, and triglycerides. The participants were not given any training on the use of the test. A total of 63 participants were enrolled from 3 sites, representing a diverse demographic (educational, age, gender, etc) population.

Tables below present the summary of the performance for each analyte.

Total Cholesterol

	Level 1	Level 2	Level 3
N	63	63	63
Mean	144.2 mg/dL	198.4 mg/dL	245.1 mg/dL
%CV	2.9%	2.3%	1.3%
Observed Range	122 - 154	186 - 222	237 – 255
Percent of Results in the Range ± 11.1%*	98.4% (62/63) 95%CI: 91.5% to 100%	100% (63/63) 95% CI: 94.3% to 100%	100% (63/63) 95% CI: 94.3% to 100%

*This percent is based on the premise that one cannot distinguish properly between normal and abnormal values when errors are greater than one-quarter of the normal range. The range of (140 mg/dL -220 mg/dL) was considered.

HDL Cholesterol

	Level 1	Level 2	Level 3
N	63	63	63
Mean	29.4 mg/dL	44.4 mg/dL	58.9 mg/dL
%CV	3.3%	3.2%	2.0%
Observed Range	28 - 32	42 - 48	57 - 62
Percent of Results in the Range ± 15.0%	100% (63/63) 95%CI: 94.3% to 100%	100% (63/63) 95% CI: 94.3% to 100%	100% (63/63) 95% CI: 94.3% to 100%

Triglycerides

	Level 1	Level 2	Level 3
N	63	63	63
Mean	83.4 mg/dL	152.7 mg/dL	205.6 mg/dL
%CV	3.0%	1.5%	0.9%
Observed Range	77 - 96	148 - 164	201 - 210
Percent of Results in the Range ± 15.0%	100% (63/63) 95%CI: 94.3% to 100%	100% (63/63) 95% CI: 94.3% to 100%	100% (63/63) 95% CI: 94.3% to 100%

13. Bibliography

1. Castelli, WP, et al. Lipids and risk of coronary heart disease. The Framingham Study. *Ann Epidemiol* 1992; 2:23-28.
2. National Cholesterol Education Program Expert Panel. Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III). NIH Publication. Bethesda, MD: National Heart, Lung, and Blood Institute, 2002.
3. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001; 285:2486-97.
4. Warnick, GR, et al. Impact of the third cholesterol report from the Adult Treatment Panel of the National Cholesterol Education Program on the Clinical Laboratory. *Clin Chem* 2002; 48:11-17.
5. Kayamori, Y, et al. Endpoint colorimetric method for assaying total cholesterol in serum with cholesterol dehydrogenase. *Clin Chem* 1999; 45:2158-2163.
6. Warnick G, Nauck M, Rifai N. Evolution of methods for measurement of high-density lipoprotein cholesterol: from ultracentrifugation to homogeneous assays. *Clin Chem* 2001; 47:1579-96.
7. Klotzsch SG, McNamara JR. Triglyceride measurements: a review of methods and interferences. *Clin Chem* 1990; 36:1605-1613.
8. Cole TG, Klotzsch SG, McNamara JR. Measurement of Triglyceride Concentration. In *Handbook of Lipoprotein Testing*, 2nd ed, Rifai N, Warnick GR, Dominiczak MH, eds. Washington, DC: AACC Press. 2000: 207-219.
9. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18:499-502.
10. Bachorik PS. Measurement of low-density-lipoprotein cholesterol. In *Handbook of Lipoprotein Testing*, 2nd ed, Rifai N, Warnick GR, Dominiczak MH, eds. Washington, DC: AACC Press. 2000: 245-263.
11. Schembri CT, et al. Centrifugation and capillarity integrated into a multiple analyte whole blood analyser. *J Automatic Chem* 1995; 17:99-104. (journal's name changed in 2000 to *J Automated Methods & Management in Chemistry*)

13. Bibliography (continued)

12. Clinical and Laboratory Standards Institute (formerly, National Committee for Clinical Laboratory Standards, NCCLS). Clinical laboratory waste management; approved guideline – second edition. NCCLS Document GP5-A2. Wayne, PA: NCCLS, 2002.
13. Clinical and Laboratory Standards Institute (formerly, National Committee for Clinical Laboratory Standards, NCCLS). Procedure for the collection of diagnostic specimens by venipuncture; approved guideline – fourth edition. NCCLS Document H3-A4. Wayne, PA: NCCLS, 1998.
14. Clinical and Laboratory Standards Institute (formerly, National Committee for Clinical Laboratory Standards, NCCLS). Procedures for the handling and processing of blood specimens; approved guideline – second edition. NCCLS Document H18-A2. Wayne, PA: NCCLS, 1999.
15. Clinical and Laboratory Standards Institute (formerly, National Committee for Clinical Laboratory Standards, NCCLS). Interference testing in clinical chemistry; proposed guideline. NCCLS Document EP7-A. Wayne, PA: NCCLS, 2002.
16. Young, DS. Effects of drugs on clinical laboratory tests, 3rd ed. Washington, DC: AACCC Press. 1990 and 1991 Supplement.
17. Kroll MH, et al. Standardization of Lipoprotein Reporting. Am J Clin Pathol 2000; 114:696-702.
18. Clinical and Laboratory Standards Institute (formerly, National Committee for Clinical Laboratory Standards, NCCLS). Evaluation of Linearity of Quantitative Analytical methods; proposed guideline – second edition. NCCLS Document EP6-P2. Wayne, PA: NCCLS, 2001.
19. Clinical and Laboratory Standards Institute (formerly, National Committee for Clinical Laboratory Standards, NCCLS). Evaluation of precision performance of clinical chemistry devices; approved guideline. NCCLS Document EP5-A. Wayne, PA: NCCLS, 1999.
20. Clinical and Laboratory Standards Institute (formerly, National Committee for Clinical Laboratory Standards, NCCLS). Quality management for unit-use testing; proposed guideline. NCCLS Document EP18-P. Wayne, PA: NCCLS, 1999.
21. Clinical and Laboratory Standards Institute (formerly, National Committee for Clinical Laboratory Standards, NCCLS). Method comparison and bias estimation using patient samples; approved guideline – second edition. NCCLS Document EP9-A2. Wayne, PA: NCCLS, 2002.

CLIAwaived.com™

Information Provided By: **CLIAwaived.com**
San Diego, CA 92121
tel 858-481-5031
toll free 888-882-7739
www.cliawaived.com