# Piccolo® Lipid Panel Plus

CE

**For In Vitro Diagnostic Use and For Professional Use Only**
**Customer and Technical Service: 800-822-2947**
**Customers outside the US should contact their local Abaxis representative for customer service**

**Applicable to US customers only**
**CLIA Waived: Use lithium heparin whole blood, only**
**Moderate Complexity: Use lithium heparin whole blood, lithium heparin plasma, or serum**

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## 1. Intended Use

The Piccolo® Lipid Panel Plus, 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), triglycerides (TRIG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and glucose (GLU) in capillary (fingerstick) lithium heparinized whole blood, venous lithium heparinized whole blood, lithium heparinized plasma, or serum in a clinical laboratory setting or point-of-care location. From the CHOL, HDL and TRIG determinations, low-density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL), non-HDL cholesterol, and a total cholesterol/high-density lipoprotein cholesterol ratio (TC/H) are calculated by the analyzer.

Lipid measurements are used in the diagnosis and treatment of lipid and lipoprotein disorders, atherosclerosis, various liver and renal diseases, diabetes mellitus, and other diseases involving lipid metabolism or various endocrine disorders.

**For US Customers Only**

The tests on this panel are waived under CLIA '88 regulations. If a laboratory modifies the test system instructions, then the tests are considered high complexity and subject to all CLIA requirements. For CLIA waived labs, only lithium heparin whole blood (venous or capillary) 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 (NCEP).[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 ATPIII, 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. Additionally, the calculated non-HDL cholesterol optimally should be < 130 mg/dL, with increased risk for cardiovascular disease associated with concentrations of 130 – 189 mg/dL, and high risk of CVD associated with values > 189 mg/dL.

The Piccolo Lipid Panel Plus and the Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer comprise an *in vitro* diagnostic system that aids the physician in diagnosing and monitoring the following disorders:

| | |
|---|---|
| Alanine Aminotransferase: | Liver diseases; including viral hepatitis and cirrhosis |
| Aspartate Aminotransferase: | Liver disease including hepatitis and viral jaundice; shock |
| Glucose: | Carbohydrate metabolism disorders, including adult and juvenile diabetes mellitus and hypoglycemia |

A**s** 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]

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

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

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:

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

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

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

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

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

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 ($MgSO_4$) 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)**

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

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

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol Kinase, } Mg^{++}} \text{G-3-P} + \text{ADP}$$

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

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

G-3-P = Glycerol-3-Phosphate
G-3-PDH = Glycerol-3-Phosphate Dehydrogenase
DAP = Dihydroxyacetone Phosphate
INT = p-Iodonitrotetrazolium Violet

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.

**Alanine Aminotransferase (ALT)**

Alanine aminotransferase (ALT) has been measured by three methods. Two of these methods—the colorimetric dinitrophenylhydrazine coupling technique[9,10] and the fluorescent enzymatic assay—are rarely used.[11] An enzymatic method based on the work of Wróblewski and LaDue[12] is the most common technique for determining ALT concentrations in serum. A modified Wróblewski and LaDue procedure has been proposed as the recommended procedure of the International Federation of Clinical Chemistry (IFCC).[13]

The method developed for use on the Piccolo analyzer or Piccolo Xpress analyzer is a modification of the IFCC-recommended procedure. In this reaction, ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate to form L-glutamate and pyruvate. Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to $NAD^+$, as illustrated in the following reaction scheme.

$$\text{L-Alanine} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{ALT}} \text{L-Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{Lactate} + NAD^+$$

The rate of change of the absorbance difference between 340 nm and 405 nm is due to the conversion of NADH to $NAD^+$ and is directly proportional to the amount of ALT present in the sample.

**Aspartate Aminotransferase (AST)**

The aspartate aminotransferase (AST) test is based on the Karmen rate method[14] as modified by Bergmeyer.[15] The current International Federation of Clinical Chemistry (IFCC) reference method utilizes the Karmen/Bergmeyer technique of coupling malate dehydrogenase (MDH) and reduced nicotinamide dinucleotide (NADH) in the detection of AST in serum.[15,16] Lactate dehydrogenase (LDH) is added to the reaction to decrease interference caused by endogenous pyruvate.

AST catalyzes the reaction of L-aspartate and α-ketoglutarate into oxaloacetate and L-glutamate. Oxaloacetate is converted to malate and NADH is oxidized to $NAD^+$ by the catalyst MDH.

$$\text{L-aspartate} + \alpha\text{-ketoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{L-glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}^+$$

The rate of absorbance change at 340 nm/405 nm caused by the conversion of NADH to $NAD^+$ is directly proportional to the amount of AST present in the sample.

**Glucose (GLU)**
Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu[17] and Somogyi-Nelson[18,19]). The lack of specificity in copper-reduction techniques led to the development of quantitative procedures using the enzymes hexokinase and glucose oxidase. The glucose test incorporated into the Piccolo Lipid Panel Plus reagent disc is a modified version of the hexokinase method, which has been proposed as the basis of the glucose reference method.[20]

The reaction of glucose with adenosine triphosphate (ATP), catalyzed by hexokinase (HK), produces glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G-6-PDH) catalyzes the reaction of G-6-P into 6-phosphogluconate and the reduction of nicotinamide adenine dinucleotide ($NAD^+$) to NADH.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-Phosphate} + \text{ADP}$$

$$\text{G-6-P} + \text{NAD}^+ \xrightarrow{\text{G-6-PDH}} \text{6-Phosphogluconate} + \text{NADH} + \text{H}^+$$

The absorbance is measured bichromatically at 340 nm and 850 nm. The production of NADH is directly proportional to the amount of glucose present in the sample.

**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.[21] This equation is not valid for triglyceride concentrations above 400 mg/dL, non-fasting patients, and patients with Type III hyperlipoproteinemia (dysbetalipoproteinemia).[21,22] 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 or tape, the calculated value for LDL is followed by a "C" to indicate that it is calculated.

**VLDL (Calculated)**
The Piccolo analyzer or Piccolo Xpress analyzer automatically calculates the concentration of VLDL in each sample using the standard triglycerides/5 (if units in mg/dL) equation.[21] This equation is not valid for triglyceride concentrations above 400 mg/dL, non-fasting patients, and patients with Type III hyperlipoproteinemia (dysbetalipoproteinemia).[21,22] Of course, no VLDL value is calculated if no triglyceride value is available. On the print card or tape, 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 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 or tape, the calculated value for TC/H is followed by a "C" to indicate that it is calculated.

**Non-HDL (Calculated) – only available on the Xpress**

The Piccolo Xpress analyzer automatically calculates the non-HDL cholesterol (abbreviated as nHDLc) for each sample. The nHDLc is calculated by subtracting HDL cholesterol from total cholesterol (CHOL). On the result tape, the calculated value for nHDLc is followed by a "C" to indicate that it is calculated. If the directly measured total cholesterol (CHOL) or HDL value is missing, nHDLc is not calculated.

## 4. Principle of Operation

Refer to the Piccolo blood chemistry analyzer or 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.[23]

## 5. Description of Reagents

**Reagents**
Each Piccolo Lipid Panel Plus reagent disc contains dry test-specific reagent beads (described below). A dry sample blank reagent bead (comprised of buffer, surfactants, and excipients) is included in each disc for use in calculating concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose (GLU), and high-density lipoprotein cholesterol (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 | 21.2 µg |
| L-Alanine | 492 µg |
| L-Aspartic acid | 426 µg |
| Ascorbate oxidase | 0.042 U |
| Cholesterol dehydrogenase | 0.27 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 |
| Glucose-6-phosphate dehydrogenase | 0.046 U |
| Glycerol kinase | 0.084 U |
| Glycerol-3-phosphate dehydrogenase | 0.21 U |
| Hexokinase | 0.059 U |
| Iodonitrotetrazolium chloride (INT) | 8.4 µg |
| α-Ketoglutarate, disodium salt | 37 µg |
| α-Ketoglutaric acid | 30 µg |
| Lactate dehydrogenase | 0.070 U |
| Lipase | 16.8 U |
| Magnesium acetate, tetrahydrate | 6.8 µg |
| Magnesium chloride, hexahydrate | 8.6 µg |
| Magnesium sulfate, heptahydrate | 197 µg |
| Malate dehydrogenase | 0.013 U |
| Nicotinamide adenine dinucleotide, free acid | 19.7 µg |
| Nicotinamide adenine dinucleotide, monosodium salt | 455 µg |
| Nicotinamide adenine dinucleotide, reduced | 9.6 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.[24] See the Piccolo blood chemistry analyzer or 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 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 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 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.

- The minimum required sample size is ~100 µL for all sample types and controls. The reagent disc sample chamber can contain up to 120 µL of sample.

- Whole blood venipuncture samples should be run within 60 minutes of collection.[25,26] **Glucose** concentrations are affected by the length of time since the patient has eaten and by the type of sample collected from the patient. To accurately determine glucose results, samples should be obtained from a patient who has been fasting for at least 12 hours. The glucose concentration decreases approximately 5-12 mg/dL in 1 hour in uncentrifuged samples stored at room temperature.[27]

- Refrigerating whole blood samples can cause significant changes in concentrations of **aspartate aminotransferase** and **glucose**.[28] 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.

Venous Sampling Notes:

- 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.

- 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.

Capillary Sampling Notes:

- Proper and adequate capillary sampling techniques are required for reliable results.

- For capillary blood collection, the patient's hands should be washed thoroughly with soap (without glycerin or glycerol) and warm water. Assure complete drying.

- For capillary sampling, gentle squeezing along the finger up to the puncture site is acceptable, but do not milk the finger to get the blood to flow, which can lead to excess hemolysis.

## 8. Procedure

**Materials Provided**
- One Piccolo Lipid Panel Plus PN: 400-1030 (a box of discs PN: 400-0030)

**Materials Required but not Provided**
- Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer

- A sample transfer pipette (fixed volume approximately 100 µL) and tips are provided with each Piccolo blood chemistry analyzer or 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 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 Plus 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 Piccolo Xpress chemistry analyzer Operator's Manual.

**Calibration**
The Piccolo blood chemistry analyzer or 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 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 Piccolo Xpress chemistry analyzer Operator's Manual.

Interpretation of results is detailed in the Operator's Manual. Results are printed onto result cards or tapes supplied by Abaxis. The result cards or tapes 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 Piccolo Xpress chemistry analyzer Operator's Manual**.**

- The only anticoagulant **recommended for use** with the Piccolo blood chemistry analyzer or 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 Piccolo Xpress chemistry analyzer.**

  **Warning:** Extensive testing of the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer 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 CLSI EP7-A.[29]

**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 or tape to inform the operator about the levels of interferents present in each sample.

- The Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer 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 or tape in place of the result.

- For maximum levels of endogenous substances contact Abaxis Technical Service.

**Effects of Therapeutic Substances**

Nineteen therapeutic substances were selected as potential interferents for the Abaxis test methods based on recommendations by Young.[30] 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[29,30] (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 |
| α-Ketoglutarate | | 5 |
| Lactate, Lithium | 6-12 | 84 |
| Lidocaine | 0.5-0.6 | 1 |
| Methicillin, Sodium | | 100 |
| Oxaloacetate | | 132 |
| Phenytoin | 1-2 | 3 |
| Pyruvate | 0.3-0.9 | 44 |
| Salicylic Acid | | 50 |
| Theophylline | 1-2 | 20 |

**Table 3: Substances With Significant Interference >10%**

| | **Physiologic/ Therapeutic Range (mg/dL)** | **Concentration with > 10% Interference (mg/dL)** | **% Interference** |
|---|---|---|---|
| **Alanine Aminotransferase (ALT)** | | | |
| Oxaloacetate | | 132 | 700% increase |
| **Aspartate Aminotransferase (AST)** | | | |
| Oxaloacetate | | 132 | 95% decrease |
| **Glucose (GLU)** | | | |
| Oxaloacetate | | 132 | 14% decrease |
| Pyruvate | 0.3-0.9 | 44 | 16% decrease |

Oxaloacetate and pyruvate were titrated to determine the concentration that resulted in less than a 10% interference. For oxaloacetate the limit is 6.6 mg/dL, 33 mg/dL, and 13.2 mg/dL respectively for ALT, AST and GLU. For pyruvate the limit is 11 mg/dL for GLU.

## 11. Cutpoints/Expected Values

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 | |
| nHDLc (CALC)** | Optimal | < 130 | < 3.37 |
| | Increased Risk | 130–189 | 3.37–4.90 |
| | High Risk | > 189 | > 4.90 |
| | | Male | Female |
| Total Chol/HDL Ratio | Low Risk | ≤ 5 | ≤ 4.5 |
| | High Risk | > 5 | > 4.5 |

* The Piccolo or Piccolo Xpress calculates the LDL concentration using the Friedewald equation.[21]

** For further information see: NCEP, ATP III Report 2002, Section II. Rationale for Intervention, 3. Other Lipid Risk Factors, Page II-9.[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.[31] 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 it's use in managing patients. The NCEP clinical guidelines base treatment decisions on the individual lipoproteins (Table 4) and consider use of the ratio as a possible diversion from the priority, the individual lipoprotein measurements.[2,3,4]

**Non-HDL Cholesterol (nHDLc)**

NCEP, ATP III Report 2002, reported the clinical utility of the calculated nHDLc. Studies have demonstrated that non-HDL cholesterol shows a stronger correlation with coronary mortality when compared with LDL cholesterol. Moreover, non-HDL cholesterol is highly correlated with total apolipoprotein B (apoB), the primary apolipoprotein associated with all atherogenic lipoproteins."[2]

**Expected Values**
Samples from a total of 125 adult males and females, analyzed on the Piccolo blood chemistry analyzer, were used to determine the reference ranges. These ranges are provided as a guideline only. It is recommended that your office or institution establish normal ranges for your particular patient population.

**Table 5: Piccolo Reference Intervals**

| Analyte | Common Units | SI Units |
|---|---|---|
| **Alanine Aminotransferase (ALT)** | 10-47 U/L | 10-47 U/L |
| **Aspartate Aminotransferase (AST)** | 11-38 U/L | 11-38 U/L |
| **Glucose (GLU)** | 73-118 mg/dL | 4.05-6.55 mmol/L |

## 12. Performance Characteristics

**Linearity**
The chemistry for each analyte is linear over the dynamic range listed below when the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer is operated according to the recommended procedure (refer to the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer Operator's Manual). This evaluation made use of CLSI EP6-P2.[32]

**Table 6: 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 |
| **ALT** | 5-1000 U/L | 5-1000 U/L |
| **AST** | 5-1000 U/L | 5-1000 U/L |
| **GLU** | 10-700 mg/dL | 0.56-38.9 mmol/L |

If the analyte concentration is above the measuring range (dynamic range), but less than the system range, the print card or tape 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 or tape, 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), triglycerides 20 mg/dL (0.23 mmol/L), alanine aminotransferase 5 U/L, aspartate aminotransferase 5 U/L, and glucose 10 mg/dL (0.56 mmol/L).

**Precision**
Precision studies were conducted using CLSI EP5-A guidelines[33] with modifications based on CLSI EP18-P[34] for unit-use devices. Results for within-run and total precision were determined using two samples. Representative precision statistics are shown in Table 7.

**Table 7: Precision**

| Analyte | Sample Size | Within-Run | Total |
|---|---|---|---|
| **CHOL (mg/dL)** | | | |
| Serum 1 | N = 160 | | |
| Mean | | 223.7 | 223.7 |
| SD | | 3.0 | 5.7 |
| %CV | | 1.3 | 2.6 |
| Serum 2 | N = 160 | | |
| Mean | | 202.2 | 202.2 |
| SD | | 3.1 | 4.4 |
| %CV | | 1.5 | 2.2 |
| **HDL (mg/dL)** | | | |
| Serum 1 | N = 160 | | |
| Mean | | 55.3 | 55.3 |
| SD | | 1.4 | 1.9 |
| %CV | | 2.6 | 3.5 |
| Serum 2 | N = 160 | | |
| Mean | | 38.0 | 38.0 |
| SD | | 1.3 | 1.6 |
| %CV | | 3.5 | 4.3 |
| **TRIG (mg/dL)** | | | |
| Serum 1 | N = 160 | | |
| Mean | | 206.8 | 206.8 |
| SD | | 4.7 | 5.5 |
| %CV | | 2.3 | 2.6 |
| Serum 2 | N = 160 | | |
| Mean | | 163.7 | 163.7 |
| SD | | 1.8 | 2.4 |
| %CV | | 1.1 | 1.5 |
| **ALT (U/L)** | | | |
| Control 1 | N = 80 | | |
| Mean | | 21 | 21 |
| SD | | 2.76 | 2.79 |
| %CV | | 13.4 | 13.5 |
| Control 2 | N = 80 | | |
| Mean | | 52 | 52 |
| SD | | 2.70 | 3.25 |
| %CV | | 5.2 | 6.2 |
| **AST (U/L)** | | | |
| Control 1 | N = 80 | | |
| Mean | | 46 | 46 |
| SD | | 1.58 | 1.59 |
| %CV | | 3.4 | 3.5 |
| Control 2 | N = 80 | | |
| Mean | | 147 | 145 |
| SD | | 1.70 | 1.83 |
| %CV | | 1.2 | 1.3 |
| **Glucose (mg/dL)** | | | |
| Control 1 | N = 80 | | |
| Mean | | 66 | 66 |
| SD | | 0.76 | 1.03 |
| %CV | | 1.1 | 1.6 |
| Control 2 | N = 80 | | |
| Mean | | 278 | 278 |
| SD | | 2.47 | 3.84 |
| %CV | | 0.9 | 1.4 |

This data indicate that CHOL, HDL and TRIG assays meet the NCEP precision criteria.[2,3,4]

**Correlation – Venous Sampling**
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 CLSI EP9-A2 guideline as a target for each analyte.[35] Representative correlation statistics are shown in Table 8.

**Table 8: Correlation of Piccolo Blood Chemistry Analyzer with Comparative Methods**

| Assay | Correlation Coefficient (r) | Slope | Intercept | SEE | N | Sample Range | Comparative Method |
|---|---|---|---|---|---|---|---|
| **Cholesterol (mg/dL)** | 0.997 | 1.079 | -17.1 | 4.5 | 174 | 115-342 | Bayer Cholesterol Assay on Hitachi 917 |
| **HDL (mg/dL)** | 0.965 | 0.851 | 8.3 | 3.9 | 166 | 23 - 97 | Roche HDL-C plus on Hitachi 917 |
| **Triglycerides (mg/dL)** | 0.999 | 0.983 | 8.2 | 4.4 | 172 | 38–487 | Bayer Triglyceride Assay on Hitachi 917 |
| **Alanine Aminotransferase (U/L)** | 0.981<br>0.985 | 0.905<br>0.946 | 1.3<br>-2.5 | 3.21<br>2.84 | 86<br>67 | 10-174<br>10-174 | Paramax®<br>Technicon |
| **Aspartate Aminotransferase (U/L)** | 0.93<br>1.0 | 0.87<br>0.97 | 5.3<br>3.0 | 2.76<br>1.9 | 159<br>46 | 13-111<br>13-252 | Paramax<br>DAX™ |
| **Glucose (mg/dL)** | 0.987<br>0.997 | 1.009<br>0.943 | -2.8<br>1.2 | 3.89<br>4.69 | 251<br>91 | 72-422<br>56-646 | Paramax<br>Beckman |

**Table 9: Calculated Recovery of the Abaxis Lipid Panel Assays – Venous Sampling**

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

**Correlation – Capillary Sampling**
Heparinized capillary whole blood samples were collected and tested in singlicate on the Piccolo Xpress chemistry analyzer. Matched venous plasma samples from the same subjects were tested in duplicate by Roche test methods. The capillary samples were tested in three non-laboratory settings and the data combined. The samples were chosen in order to provide a distribution of values using CLSI EP9-A2 guideline as a target for each analyte.[21]

Representative correlation statistics are shown in Table 9.

**Table 10: Correlation of Piccolo Xpress Chemistry Analyzer with Comparative Methods for Lipid Tests – Capillary Sampling**

| Assay | Correlation Coefficient (r) | Slope | Intercept | SEE | N | Sample Range (mg/dL) | Comparative Method |
|---|---|---|---|---|---|---|---|
| **Total Cholesterol (CHOL)** | 0.995 | 0.97 | 1.2 | 5.6 | 639 | 21 - 412 | Roche Total Cholesterol on Cobas 6000 |
| **HDL** | 0.981 | 0.99 | -1.6 | 2.7 | 559 | 21 - 93 | Roche HDL-Cholsterol Plus 3rd Generation on Cobas 6000 |
| **Triglycerides (TRIG)** | 0.996 | 0.96 | 4.1 | 7.9 | 588 | 36 - 496 | Roche Triglycerides on Cobas 6000 |

**Table 11: Calculated Recovery of the Abaxis Lipid Panel Assays – Capillary Sampling**

| Assay | Roche Test Concentration mg/dL | Calculated Piccolo Recovery from Linear Regression Data Above mg/dL | Bias mg/dL | % Bias |
|---|---|---|---|---|
| **Total Cholesterol (CHOL)** | 200 | 194 | -6 | - 3.0 |
| | 240 | 233 | -7 | - 3.3 |
| **HDL** | 40 | 38 | -2 | -5.0 |
| | 60 | 58 | -2 | -3.3 |
| **Triglycerides (TRIG)** | 150 | 148 | -2 | - 1.3 |
| | 200 | 196 | -4 | - 2.0 |

**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 traceability of the Abaxis TRIG test to a reference method has been established through correlation with the Cobas Triglycerides Test that has been standardized against the ID/MS method.

**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 discs with blinded randomized samples. The samples consisted of serum pools prepared at three levels for each of the six analytes, total cholesterol, HDL cholesterol, triglycerides, ALT, AST, and glucose. The participants were not given any

training on the use of the test. A total of approximately 60 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% |

**Alanine Aminotransferase (ALT)**

| | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| N | 62 | 62 | 62 |
| Mean | 45.4 U/L | 98.9 U/L | 184.3 U/L |
| %CV | 3.7% | 1.7% | 1.5% |
| Observed Range | 42–53 | 96–103 | 175–191 |
| Percent of Results in the Range ± 15.0% | 98.4%<br>61/62<br>95%CI: 91.3% to 100% | 100%<br>62/62<br>95%CI: 94.2% to 100% | 100%<br>62/62<br>95%CI: 94.2% to 100% |

**Aspartate Aminotransferase (AST)**

| | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| N | 62 | 62 | 62 |
| Mean | 56.0 | 120.4 | 276.3 |
| %CV | 2.4% | 1.1% | 1.0% |
| Observed Range | 54–60 | 117–124 | 266–285 |
| Percent of Results in the Range ± 15.0% | 100%<br>62/62<br>95%CI: 94.2% to 100% | 100%<br>62/62<br>95%CI: 94.2% to 100% | 100%<br>62/62<br>95%CI: 94.2% to 100% |

**Glucose (GLU)**

| | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| N | 62 | 62 | 62 |
| Mean | 95.2 | 130.3 | 365.8 |
| %CV | 1.1% | 1.0% | 0.8% |
| Observed Range | 93–98 | 125–133 | 351–373 |
| Percent of Results in the Range ± 10.4%** | 100%<br>62/62<br>95%CI: 94.2% to 100% | 100%<br>62/62<br>95%CI: 94.2% to 100% | 100%<br>62/62<br>95%CI: 94.2% to 100% |

** The range of (65 mg/dL - 99 mg/dL) was considered.

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