

Piccolo[®] Liver Panel Plus



For In Vitro Diagnostic Use and Healthcare Professional Use Only

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AB-PiccoloTechSupport@zoetis.com

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[®] Liver Panel Plus, used with the Piccolo Xpress[®] chemistry analyzer, utilizes dry and liquid reagents to provide in vitro quantitative determinations of alanine aminotransferase, albumin, alkaline phosphatase, amylase, aspartate aminotransferase, gamma glutamyltransferase, total bilirubin, and total protein in heparinized whole blood, heparinized plasma, or serum in a clinical laboratory setting or point-of-care location/ near patient testing.

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 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).

2. Summary and Explanation of Tests

The Piccolo Liver Panel Plus or the Piccolo Xpress chemistry analyzer comprise an *in vitro* diagnostic system that aids the physician in diagnosing the following disorders in adults:

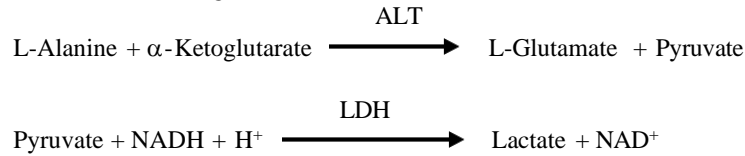
Alanine aminotransferase:	Liver diseases, for example viral hepatitis and cirrhosis; heart diseases.
Albumin:	Liver and kidney diseases.
Alkaline phosphatase:	General Liver and metabolic disease, for example, bone, parathyroid, and intestinal diseases.
Amylase:	Pancreatitis.
Aspartate aminotransferase:	Liver disease, for example, hepatitis and viral jaundice, shock liver.
Gamma glutamyltransferase:	Liver diseases, for example, alcoholic cirrhosis and primary and secondary liver tumors.
Total bilirubin:	Liver disorders, for example, hepatitis and biliary obstruction; jaundice.
Total protein:	Metabolic disorders, for example, nutritional disorders, Liver, kidney, bone marrow diseases

3. Principles of Method:

Alanine Aminotransferase (ALT)

Alanine aminotransferase (ALT) has been measured by three methods. Two of these methods—the colorimetric dinitrophenylhydrazine coupling technique^{1,2} and the fluorescent enzymatic assay—are rarely used.³ An enzymatic method based on the work of Wróblewski and LaDue⁴ 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).⁵

The method developed for use on the Piccolo Xpress chemistry analyzer is the same as that recommended by the IFCC but run at a higher temperature. 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.

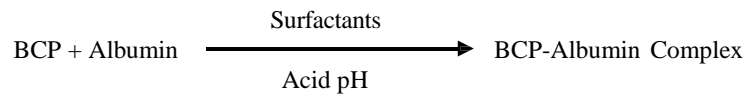


The rate of change of the absorbance difference between 340 nm and 405 nm is due to the conversion of NADH to NAD^+ and is directly proportional to the amount of ALT present in the sample.

Albumin (ALB)

Early methods used to measure albumin include fractionation techniques^{6,7,8} and tryptophan content of globulins.^{9,10} These methods are unwieldy to perform and do not have a high specificity. Two immunochemical techniques are considered as reference methods, but are expensive and time consuming.¹¹ Dye binding techniques are the most frequently used methods for measuring albumin. Bromocresol green (BCG) is the most commonly used of the dye binding methods but may over-estimate albumin concentration, especially at the low end of the normal range.¹² Bromocresol purple (BCP) is the most specific of the dyes in use.^{13,14}

Bromocresol purple, when bound with albumin, changes from a yellow to blue color. The absorbance maximum changes with the color shift.

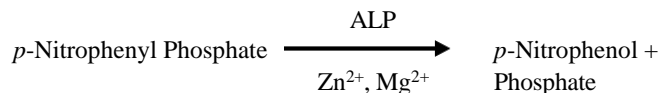


Bound albumin is proportional to the concentration of albumin in the sample. This is an endpoint reaction that is measured as the difference in absorbance between 600 nm and 550 nm.

Alkaline Phosphatase (ALP)

Techniques to measure alkaline phosphatase were first developed over 60 years ago. Several of these end-point or two-point spectrophotometric methods^{15,16} are now considered obsolete or too cumbersome. The use of *p*-nitrophenyl phosphate (*p*-NPP) increased the speed of the reaction.^{17,18} The reliability of this technique was greatly increased by the use of a metal-ion buffer to maintain the concentration of magnesium and zinc ions in the reaction.¹⁹ The American Association for Clinical Chemistry (AACC) reference method²⁰ uses *p*-NPP as a substrate and a metal-ion buffer.

The Piccolo procedure is modified from the AACC²⁰ and IFCC²¹ methods. Alkaline phosphatase hydrolyzes *p*-NPP in a metal-ion buffer and forms *p*-nitrophenol and phosphate.

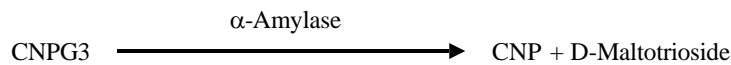


The amount of ALP in the sample is proportional to the rate of increase in absorbance difference between 405 nm and 500 nm.

Amylase (AMY)

About 200 different tests have been developed to measure amylase. Most procedures use a buffered polysaccharide solution but employ different detection techniques. Viscosimetric methods are lacking in precision and accuracy²², while turbidimetric and iodometric methods are difficult to standardize.^{23,24} Commonly used are saccharogenic and chromolytic methods. The “classic” amylase measurement technique is a saccharogenic method²⁵, but is difficult and time-consuming.²⁶ Chromolytic methods using *p*-nitrophenylglycosides as substrates have been recently developed.²⁷ These assays have a higher specificity for pancreatic amylase than for salivary amylase and are easily monitored.²⁷

In the Piccolo method, the substrate, 2-chloro-*p*-nitrophenyl- α -D-maltotrioxide (CNPG3), reacts with α -amylase in the patient sample, releasing 2-chloro-*p*-nitrophenol (CNP). The release of CNP creates a change in color.

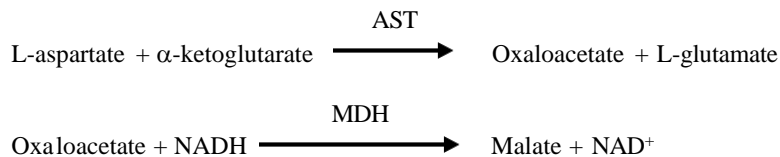


The reaction is measured bichromatically at 405 nm and 500 nm. The change in absorbance due to the formation of CNP is directly proportional to α -amylase activity in the sample.

Aspartate Aminotransferase (AST)

The aspartate aminotransferase (AST) test is based on the Karmen rate method²⁸ as modified by Bergmeyer.²⁹ 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.^{29,30} 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.

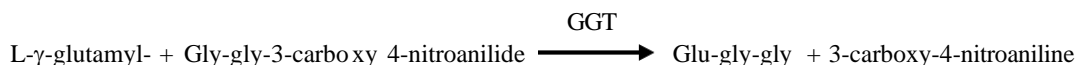


The rate of absorbance change at 340 nm/405 nm caused by the conversion of NADH to NAD^+ is directly proportional to the amount of AST present in the sample.

Gamma Glutamyltransferase (GGT)

The first quantitative methods developed to measure gamma glutamyltransferase (GGT) involved a second reaction to form an azo dye that combined with a chromophore.^{39,40} The change to L- γ -glutamyl-*p*-nitroanilide as the substrate in the reaction eliminated the dye-formation step.⁴¹ Due to the poor solubility and stability of L- γ -glutamyl-*p*-nitroanilide, this procedure was modified to use the substrate L- γ -glutamyl-3-carboxy-4-nitroanilide.⁴² The International Federation of Clinical Chemistry (IFCC) recommended GGT method is based on the latter substrate, with glycylglycine as the other substrate.⁴³

Abaxis has modified the IFCC method to react at 37°C. The addition of sample containing gamma glutamyltransferase to the substrates L- γ -glutamyl-3-carboxy-4-nitroanilide and glycylglycine (gly-gly) causes the formation of L- γ -glutamyl-glycylglycine (glu-gly-gly) and 3-carboxy-4-nitroaniline.

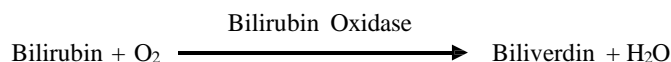


The absorbance of this rate reaction is measured at 405 nm. The production of 3-carboxy-4-nitroaniline is directly proportional to the GGT activity in the sample.

Total Bilirubin (TBIL)

Total bilirubin levels have been typically measured by tests that employ diazotized sulfanilic acid.^{32,44} A newer, more specific method has been developed using the enzyme bilirubin oxidase.^{34,35,36} In addition to using the more specific total bilirubin test method, photodegradation of the analyte is minimized in the Piccolo System because the sample can be tested immediately after collection.

In the enzyme procedure, bilirubin is oxidized by bilirubin oxidase into biliverdin. The final reaction is the conversion of biliverdin into various purple compounds.

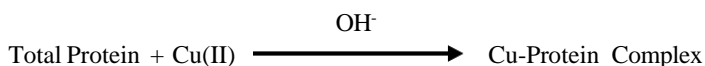


Bilirubin is quantitated as the difference in absorbance between 467 nm and 550 nm. The initial absorbance of this endpoint reaction is determined from the bilirubin blank cuvette and the final absorbance is obtained from the bilirubin test cuvette. The amount of bilirubin in the sample is proportional to the difference between the initial and final absorbance measurements.

Total Protein (TP)

The total protein method is a modification of the biuret reaction, noted for its precision, accuracy, and specificity.⁴⁵ Originally developed by Riegler⁴⁶ and modified by Weichselbaum⁴⁷, Dumas, et al⁴⁸ proposed a biuret reaction as a candidate total protein reference method.

In the biuret reaction, the protein solution is treated with cupric [Cu(II)] ions in a strong alkaline medium. Sodium potassium tartrate and potassium iodide are added to prevent the precipitation of copper hydroxide and the auto reduction of copper, respectively.⁴⁷ The Cu(II) ions react with peptide bonds between the carbonyl oxygen and amide nitrogen atoms to form a colored Cu-Protein complex.



The amount of total protein present in the sample is directly proportional to the absorbance of the Cu-protein complex. The total protein test is an endpoint reaction and the absorbance is measured as the difference in absorbance between 550 nm and 850 nm.

4. Principles of Operation

Refer to the Piccolo Xpress chemistry analyzer Operator's Manual, for the Principles and Limitations of the Procedure.

5. Description of Reagents

Reagents

Each Piccolo Liver Panel Plus reagent disc contains dry test-specific reagent beads (described below). A dry sample blank reagent (comprised of buffer, surfactants, excipients, and preservatives) is included in each disc for use in calculating concentrations of alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), amylase (AMY), aspartate aminotransferase (AST), and gamma glutamyl transferase (GGT). Dedicated sample blanks are included in the disc for total bilirubin, and total protein. Each reagent disc also contains a liquid diluent consisting of surfactants, excipients, and preservatives.

Table 1: Reagents

L-alanine	874 µg
L-aspartic acid	426 µg
Beckman Bilirubin Enzyme Reagent	0.1 U
Bromocresol purple, sodium salt	2 µg
2-Chloro-4-nitrophenyl- α -maltotrioxide (CNPG3)	40 µg
Cupric sulfate	134 µg
L-glutamic acid γ -(3-carboxy-4-nitroanilide)	30 µg
Glycylglycine	317 µg
α -ketoglutaric acid	86 µg
Lactate dehydrogenase (LDH)	0.11 U
Malate dehydrogenase (MDH)	0.01 U
β -nicotinamide adenine dinucleotide, reduced (NADH)	12 µg
<i>p</i> -NPP, disodium salt	48 µg
Potassium iodide	56 µg
Sodium potassium tartrate	686 µg
Zinc sulfate	2 µg
Excipients and preservatives	
4-Aminoantipyrine	5.4 µg
Bilirubin oxidase	0.1 U
3,5-Dichloro-2-hydroxybenzenesulfonic acid, Sodium salt (DHBSA)	34.8 µg
Ethylenediaminetetraacetic acid (EDTA), disodium salt	57 µg
Ethylenediaminetetraacetic acid (EDTA), Tetrasodium salt	201 µg
L-Glutamic acid γ - (3-carboxy-4-nitroanilide), ammonium salt	32 µg
alpha-Ketoglutaric acid, disodium salt	37 µg

Peroxidase	0 U
Uricase	0 U
Magnesium chloride, hexahydrate	2 µg

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 infection control practices when handling and disposing of used discs.⁴⁹ See 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 bio-hazards 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.
- Reagent beads and diluent contain sodium azide which may react with lead and copper plumbing to form highly explosive metal azides. Reagents will not come into contact with lead and copper plumbing when following recommended procedures. However, if the reagents do come into contact with such plumbing, flush with a large volume of water to prevent azide buildup.
- Materials incorporated into the device may consist of substances derived from other microbial and animal-sourced material.
- This mixture does not contain substances assessed to be vPvB / PBT according to Regulation (EC) No 1907/2006, Annex XIII. This product contains components considered to have endocrine-disrupting properties for environment, according to REACH Article 57(f), Regulation (EU) 2018/605 or Regulation (EU) 2017/2100. Refer to the Safety Data Sheet (SDS) for specific details.

Instructions for Reagent Handling

Reagent discs may be used directly from the refrigerator without warming. Do not allow discs to remain at room temperature longer than 48 hours prior to use. Open the sealed foil pouch and remove the disc, being careful not to touch the bar code ring located on the top of the disc. Use according to the instructions provided in the Piccolo Xpress chemistry analyzer Operator's Manual. A disc not used within 20 minutes of opening the pouch should be discarded.

Storage

Store reagent discs in their sealed pouches at 2-8 °C (36-46 °F). To use reagent discs, remove the discs in their sealed foil pouches from the refrigerator. Discs in sealed pouches can be left at room temperature and then placed back in the refrigerator several times. Ensure that the total time discs are at room temperature at 20-25°C (68-77°F) does not exceed 48 hours. Open the pouch and remove the disc just prior to running the test.

Do not expose discs, in or out of the foil pouches, to direct sunlight or to temperatures above 32 °C (90 °F). A disc must be used within 20 minutes of opening the pouch; a disc in an opened pouch cannot be placed back in the refrigerator for use at a later time.

Indications of Reagent Disc Instability/Deterioration

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

6. Instrument

See the Piccolo Xpress chemistry analyzer Operator's Manual for complete information on using the analyzer.

7. Sample Collection and Preparation

Sample collection techniques are described in the “Sample Collection” section of the Piccolo Xpress chemistry analyzer Operator’s Manual.

- The minimum required sample size is ~100 µL of heparinized whole blood, heparinized plasma, serum, or serum control. 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 can cause hemolysis.
- Whole blood venipuncture samples should be run within 60 minutes of collection.⁵⁰ Refrigerating whole blood samples can cause significant changes in concentrations of **aspartate aminotransferase**.⁵¹ 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.
- Use only lithium heparin evacuated specimen collection tubes for whole blood or plasma samples. Use no-additive evacuated specimen collection tubes or serum separator tubes for serum samples.
- Start the test within 10 minutes of transferring the sample into the reagent disc.
- **Total Bilirubin** results may be adversely affected by photodegradation.⁶¹ Whole blood samples not run immediately should be stored in the dark for no longer than 60 minutes. If the sample cannot be analyzed within that period, it should be separated into plasma or serum and stored in a capped sample tube in the dark at 2-8°C (36-46°F).⁵²

Known Interfering Substances

- The only anticoagulant recommended for use in the Piccolo test protocol is lithium heparin. Abaxis has performed studies demonstrating that EDTA, fluoride, oxalate, and any anticoagulant containing ammonium ions will interfere with at least one chemistry contained in the Piccolo Liver Panel Plus.
- **Amylase** is secreted by several glands as well as by the pancreas. Only pancreatic amylase is of clinical interest.⁵³ Contamination of a sample with nonpancreatic amylase will cause artificially elevated results. Finger puncture samples are more prone to contamination than are venipuncture samples. If amylase results from a finger puncture sample are not consistent with the patient’s clinical symptoms, repeat the test using a venipuncture sample.
- Interference may be seen in the **total protein** test when analyzing samples with a triglyceride concentration > 4.52 mmol/L may show an increased total protein level. The Piccolo analyzer or the Piccolo Xpress chemistry analyzer suppresses any results that are affected by >10% interference from lipemia. “LIP” is printed on the result card in place of the result.

8. Procedure

Materials Required

- One Piccolo Liver Panel Plus PN: 400-1003 (a box of discs PN: 400-0003)

Materials Required but not Provided

- Piccolo Xpress chemistry analyzer
- Sample transfer pipettes (fixed volume approximately 100 µL) and tips are provided with each 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 Xpress chemistry analyzer operates at ambient temperatures between 15 °C and 32 °C (59-90 °F). The analysis time for each Piccolo Liver Panel Plus is <14 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 Xpress chemistry analyzer Operator's Manual.

Quality Control

See Section 6 (Calibration and Quality Control) of the Piccolo Xpress chemistry analyzer Operator's Manual. Performance of 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 Abaxis Technical Support. Do not report results if controls are outside their labeled limits. See the Piccolo Xpress chemistry analyzer Operator's Manual for a detailed discussion on running, recording, interpreting, and plotting control results.

Waived Laboratories (US only): 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 (US only): Abaxis recommends control testing to follow federal, state, and local guidelines.

And the non-waived ones will use the below:

- Performance of 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 Abaxis Technical Support. Do not report results if controls are outside their labeled limits. See the Piccolo Xpress chemistry analyzer Operator's Manual for a detailed discussion on running, recording, interpreting, and plotting control results.

Calibration

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 reagent disc-specific calibration data. See the Piccolo Xpress chemistry analyzer Operator's Manual.

The Piccolo Xpress chemistry analyzer is calibrated using internal calibrators or reference materials. The methods and materials used in the procedures for the calibration and control value assignments are traceable either to the comparative method and/or to the standards listed below:

- IFCC – International Federation of Clinical Chemistry
- NIST – National Institute of Standards and Technology
- CRMLN – Cholesterol Reference Method Laboratory Network
- SRM – Standard Reference Material
- CRM – Certified Reference Material

The calibration and value assignment processes are in compliance with EN ISO 17511, Metrological Traceability of Values Assigned to Calibrators and Control Materials.

The measured analytes in the Liver Panel Plus are traceable to the following reference materials or methods. The Piccolo Xpress Analysis System controls and calibration verification materials are validated for use only with the Piccolo Xpress Analysis System and assigned values may not be commutable with other methods. For specific analyte reference standard methods, refer to Section 3. Principles of Method.

Analyte	Calibration Standard	Method
ALT	IFCC	Colorimetric
ALB (BCP)	Correlation to Beckman LX-20 / DX-20	Dye Binding Bromocresol Purple
ALP	IFCC	Colorimetric
AMY	IFCC	Colorimetric
AST	IFCC	Colorimetric
GGT	IFCC	Colorimetric
TBIL	NIST SRM #916A	Enzymatic
TP	NIST SRM #909	Biuret (Copper II)

9. Results

The Piccolo Xpress chemistry analyzer automatically calculates and prints the analyte concentrations in the sample. Details of the endpoint and rate reaction calculations are found in the Piccolo Xpress chemistry analyzer Operator's Manual.

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

The reaction for each analyte occurs at 37 °C (98.6°F).

10. Limitations of Procedure

General procedural limitations are discussed in the Piccolo Xpress chemistry analyzer Operator's Manual.

- The only anticoagulant **recommended for use** with the Piccolo Xpress chemistry analyzer is **lithium heparin**. Do not use sodium heparin. Abaxis has performed studies demonstrating that EDTA, fluoride, oxalate, and any anticoagulant containing ammonium ions will interfere with at least one chemistry contained in the Piccolo Liver Panel Plus reagent disc.
- It is recommended that **albumin** tests be run using venous whole blood or serum rather than finger puncture whole blood. Finger puncture sampling techniques may cause more cellular trauma than venipuncture techniques.
- Samples with hematocrits in excess of 62% packed red cell volume (a volume fraction of 0.62) 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.
- **Amylase** is secreted by several glands as well as by the pancreas. Only pancreatic amylase is of clinical interest.⁵³ Contamination of a sample with nonpancreatic amylase will cause artificially elevated results. Finger puncture samples are more prone to contamination than are venipuncture samples. If amylase results from a finger puncture sample are not consistent with the patient's clinical symptoms, repeat the test using a venipuncture sample.
- **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 Xpress chemistry analyzer.**

Warning: Extensive testing of the 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.

Note: Operators shall report any serious incident that has occurred in relation to the device to the manufacturer.

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 printout to inform the operator about the levels of interferents present in each sample.
- The 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 printout in place of the result.
- For maximum levels of endogenous substances contact Abaxis Technical Service.
- Additionally, lactate at 25.5 mmol/L and lactate dehydrogenase at 10,000 U/L were found to have no effect on any of the assays on this disc.

Effects of Exogenous and Therapeutic Substances

The following compounds do not significantly interfere with the chemistries in the Piccolo reagent disc. Significant interference is defined as >10% shift in the result for a normal range specimen. Human serum pools were supplemented with known concentrations of the drugs or chemicals and then analyzed.

Therapeutic or Exogenous Substances	Concentration with No Substances (mg/dL)
Acetaminophen	100
Acetylsalicylic acid	50
Chloramphenicol	100
Cimetidine	16
Dextran	300
Erythromycin	10
Hydrochlorothiazide	7.5
Isoniazide	4
Ketoprofen	50
Lidocaine	1
Methicillin	100
Methotrexate	0.5
Metronidazole	5
Nafcillin	1
Oxacillin	1
Phenytoin	3
Rifampin	0.5
Salicylic acid	25

The following substances showed greater than 10% interference. Significant interference is defined as >10% shift in the result for a normal range specimen. Human serum pools were supplemented with known concentrations of the drugs or chemicals and then analyzed.

	Concentration with Significant Interference (mg/dL)
Alanine Aminotransferase (ALT)	
Ascorbic acid	20
Oxaloacetate	132
Albumin (ALB)	
Acetoacetate	102
Ampicillin	30
Caffeine	10
Calcium chloride	20
Cephalothin (Keflin)	400
Ibuprofen	50
α -Ketoglutarate	5
Nitrofurantoin	20
Proline	4
Sulfasalazine	10
Sulfanilamide	50
Theophylline	20

Alkaline Phosphatase (ALP)	
Theophylline	20
Total Bilirubin⁹ (TBIL)	
Dopamine	19
L-dopa	5

For additional information on potential chemical interferents, see the Bibliography.

11. Expected Values

Samples from a total of 193 adult males and females were used to determine the reference ranges for alanine aminotransferase, albumin, alkaline phosphatase, amylase, total bilirubin, and total protein. Samples from a total of 186 adult males and females were used to determine the reference ranges for aspartate aminotransferase. Samples from a total of 131 adult males and females were used to determine the reference ranges for gamma glutamyl transferase.

These ranges are provided as a guideline only. It is recommended that your office or institution establish normal ranges for the geographic area in which you are located.

Table 2: Piccolo References Intervals*

Analyte	Reference Range	
	Common Units	SI Units
Alanine Aminotransferase (ALT)	10-47 U/L	10-47 U/L
Albumin (ALB)	3.3-5.5 g/dL	33-55 g/L
Alkaline Phosphatase (ALP), Male	53-128 U/L	53-128 U/L
Alkaline Phosphatase (ALP), Female	42-141 U/L	42-141 U/L
Amylase (AMY)	14-97 U/L	14-97 U/L
Aspartate Aminotransferase (AST)	11-38 U/L	11-38 U/L
Gamma Glutamyltransferase (GGT)	5-65 U/L	5-65 U/L
Total Bilirubin (TBIL)	0.2-1.6 mg/dL	3.4-27.4 µmol/L
Total Protein (TP)	6.4-8.1 g/dL	64-81 g/L

*Data was generated using equivalence device Piccolo Blood Chemistry Analyzer

Amylase is secreted by several glands as well as by the pancreas. Only pancreatic amylase is of clinical interest.⁵³ Contamination of a sample with nonpancreatic amylase will cause artificially elevated results. Finger puncture samples are more prone to contamination than are venipuncture samples. If amylase results from a finger puncture sample are not consistent with the patient's clinical symptoms, repeat the test using a venipuncture sample.

12. Performance Characteristics

Linearity

The chemistry for each analyte is linear over the dynamic range listed below when the Piccolo Xpress chemistry analyzer is operated according to the recommended procedure (see Piccolo Xpress chemistry analyzer Operator's Manual).

Table 3: Piccolo Dynamic Ranges

Analyte	Dynamic Range	
	Common Units	SI Units
Alanine Aminotransferase (ALT)	5-2000 U/L	5-2000 U/L
Albumin (ALB)	1-6.5 g/dL	10-65 g/L
Alkaline Phosphatase (ALP)	5-2400 U/L	5-2400 U/L
Amylase (AMY)	5-4000 U/L	5-4000 U/L
Aspartate Aminotransferase (AST)	5-2000 U/L	5-2000 U/L
Gamma Glutamyltransferase (GGT)	5-3000 U/L	5-3000 U/L

Total Bilirubin(TBIL)	0.1-30 mg/dL	1.7-513 μ mol/L
Total Protein (TP)	2-14 g/dL	20-140 g/L

If the analyte concentration is above the measuring range (dynamic range), but less than the system range, the printout will indicate a ">" sign at the upper limit and an asterisk after the number, e.g. ALT >2000* U/L. If lower than the dynamic range, a "<" will be printed with an asterisk, e.g. ALT <5* U/L. For values that are grossly beyond the measurement range (system range), "~~~~" will be printed instead of a result. Any time "~~~~" appears on a printout, collect a new sample and rerun the test. If results for the second sample are suppressed again, please call Abaxis Customer Service.

Sensitivity (Limits of Detection)

The lower limit of detection for each analyte is: alanine aminotransferase 10 U/L; albumin 1 g/dL (10 g/L); alkaline phosphatase 5 U/L; amylase 5 U/L; aspartate aminotransferase 5 U/L; gamma glutamyl transferase 5 U/L; total bilirubin 0.1 mg/dL (1.7 µmol/L); and total protein 2 g/dL (20 g/L).

Precision

Precision studies were conducted using NCCLS EP5-T2 guidelines.⁶⁰ Results for within-run and total precision were determined by testing two levels of control material.

Table 4: Precision (N=80)

Analyte	Within-Run	Total
Alanine Aminotransferase (U/L)		
<u>Control Level 1</u>		
Mean	21	21
SD	2.76	2.79
%CV	13.4	13.5
<u>Control Level 2</u>		
Mean	52	52
SD	2.70	3.25
%CV	5.2	6.2
Albumin (g/L)		
<u>Control Level 1</u>		
Mean	56	56
SD	0.9	1.1
%CV	1.7	2.1
<u>Control Level 2</u>		
Mean	3.7	37
SD	0.7	1.1
%CV	2.0	2.9
Alkaline Phosphatase (U/L)		
<u>Control Level 1</u>		
Mean	39	39
SD	1.81	2.29
%CV	4.6	5.8
<u>Control Level 2</u>		
Mean	281	281
SD	4.08	8.75
%CV	1.5	3.1
Amylase (U/L)		
<u>Control Level 1</u>		
Mean	46	46
SD	2.40	2.63
%CV	5.2	5.7
<u>Control Level 2</u>		
Mean	300	300
SD	11.15	11.50
%CV	3.7	3.8

Table 4 continued: Precision (N=80)

Analyte	Within-Run	Total
Aspartate Aminotransferase (U/L)		
<u>Control Level 1</u>		
Mean	47	49
SD	0.98	1.84
%CV	2.07	3.9
<u>Control Level 2</u>		
Mean	145	147
SD	1.83	4.62
%CV	1.26	3.2
Gamma Glutamyltransferase (U/L)		
<u>Control Level 1</u>		
Mean	25	25
SD	0.59	0.74
%CV	2.34	2.94
<u>Control Level 2</u>		
Mean	106	106
SD	1.52	2.29
%CV	1.43	2.15
Total Bilirubin (µmol/L)		
<u>Control Level 1</u>		
Mean	13.7	13.7
SD	1.03	1.20
%CV	8.0	9.3
<u>Control Level 2</u>		
Mean	88.9	88.9
SD	1.54	2.57
%CV	1.7	2.8
Total Protein (g/L)		
<u>Control Level 1</u>		
Mean	68	68
SD	0.5	0.8
%CV	0.8	1.2
<u>Control Level 2</u>		
Mean	47	47
SD	0.9	0.9
%CV	2.0	2.0

Correlation

Heparinized whole blood and serum samples were collected from patients at two sites. The whole blood samples were analyzed at the field sites and the serum samples were analyzed by the Piccolo analyzer and by comparative methods. In some cases, high and low supplemented samples were used to cover the dynamic range. All samples were run in singlicate on the same day. Representative correlation statistics are shown in Table 5.

Table 5: Correlation with Comparative Method**

	Whole Blood	
	Lab 1	Lab 2
Alanine Aminotransferase (U/L)		
Correlation	0.98	0.99
Slope	0.91	0.946
Intercept	1.3	-2.5
SEE	3.21	2.84
N	86	67
Sample range	10-174	10-174
Comparative method	Paramax®	Technicon
Albumin (g/L)		
Correlation	0.85	0.90
Slope	1.0	0.88
Intercept	-0.3	-0.1
SEE	0.22	0.21
N	261	100
Sample range	11-53	15-50
Comparative method	Paramax®	Beckman
Alkaline Phosphatase (U/L)		
Correlation	0.99	0.93
Slope	0.97	1.14
Intercept	-5.9	-17.6
SEE	3.97	4.79
N	99	80
Sample range	27-368	26-150
Comparative method	Paramax®	Technicon
Amylase (U/L)		
Correlation	0.98	0.96
Slope	0.69	1.07
Intercept	-4.7	-4.1
SEE	3.11	3.47
N	99	80
Sample range	11-92	19-118
Comparative method	Paramax®	Technicon
Aspartate Aminotransferase (U/L)		
Correlation	0.93	1.0
Slope	0.87	0.97
Intercept	5.3	3.0
SEE	2.76	1.90
N	159	46
Sample range	13-111	13-252
Comparative method	Paramax®	DAX™
Gamma Glutamyltransferase (U/L)		
Correlation	1.0	1.0*
Slope	0.98	1.60*
Intercept	-0.4	3.1*
SEE	3.29	18.57*
N	135	49
Sample range	5-312	27-1848
Comparative method	Paramax®	Beckman Synchron® CX

Table 5 continued: Correlation with Comparative Method**

	Whole Blood	
	Lab 1	Lab 2
Total Bilirubin (µmol/L)		
Correlation	0.97	0.98
Slope	0.90	1.11
Intercept	0.0	-0.4
SEE	0.07	0.09
N	250	91
Sample range	3.4-63.3	1.7-109.5
Comparative method	Paramax®	Beckman
Total Protein (g/L)		
Correlation	0.85	0.87
Slope	0.93	0.94
Intercept	0.6	0.3
SEE	0.19	0.16
N	251	92
Sample range	57-92	65-92
Comparative method	Paramax®	Beckman

*Laboratory 2 ran only serum on the Piccolo analyzer for the gamma glutamyltransferase test correlation.

** Data was generated using equivalence device Piccolo Blood Chemistry Analyzer

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 eight analytes, ALT, albumin, ALP, AMY, AST, GGT, total bilirubin, and total protein. 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.

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	98.4% 61/62	100% 62/62	100% 62/62
± 15.0%*	95%CI: 91.3% to 100%	95%CI: 94.2% to 100%	95%CI: 94.2% 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 (10 U/L - 47 U/L) was considered.

Albumin (ALB)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	30 g/L	35 g/L	42 g/L
%CV	2.7%	2.5%	1.8%
Observed Range	29 – 32	33 – 37	40 – 44
Percent of Results in the Range	100% 62/62	100% 62/62	100% 62/62
± 12.5%	95%CI: 94.2% to 100%	95%CI: 94.2% to 100%	95%CI: 94.2% to 100%

Alkaline Phosphatase (ALP)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	94.5 U/L	171.5 U/L	337.5 U/L
%CV	5.2%	3.2%	2.4%
Observed Range	85 – 106	160-184	287 – 388
Percent of Results in the Range ± 15.0%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

Amylase (AMY)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	72.1 U/L	126.9 U/L	260.0 U/L
%CV	2.4%	2.1%	1.9%
Observed Range	67 – 75	120 – 133	248 – 273
Percent of Results in the Range ± 15.0%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 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% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

Gamma Glutamyltransferase (GGT)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	35.0 U/L	86.2 U/L	131.3 U/L
%CV	2.8%	1.5%	1.5%
Observed Range	33 – 38	83 – 90	123 – 135
Percent of Results in the Range ± 15.0%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

Total Bilirubin (TBIL)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	14.71 µmol/L	22.76 µmol/L	97.49 µmol/L
%CV	6.1%	2.6%	1.8%
Observed Range	13.68 – 17.10	39.34 – 44.47	92.36 – 100.91
Percent of Results in the Range ± 15.0%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

Total Protein (TP)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	48 g/L	57 g/L	71 g/L
%CV	2.0%	1.5%	1.5%
Observed Range	46 – 53	53 – 59	67 – 75
Percent of Results in the Range ± 5.9%	98.4% 61/62 95%CI: 91.3% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

13. Symbols



Use By



Catalog Number



Batch Code



In Vitro Diagnostic
Medical Device



Consult Instructions
for Use



Manufacturer



Do Not Reuse



X Number of Test
Devices In Kit



Manufacturing
Sequence



Serial Number



Authorized
Representative
In the European
Community



Temperature
Limitation



PN:
Part Number

Caution



denotes conformity to specified
European directives

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