

# Piccolo<sup>®</sup> Hepatic Function Panel





For In Vitro Diagnostic Use and For Professional Use Only


Customer and Technical Service: 1-800-822-2947

Customers outside the US: +49 6155 780 210

[AB-PiccoloTechSupport@zoetis.com](mailto:AB-PiccoloTechSupport@zoetis.com)

 Abaxis, Inc.  
3240 Whipple Rd.  
Union City, CA 94587  
USA

 Tao of Excellence GmbH  
Vorstadt 26,  
8200 Schaffhausen  
Switzerland

 ABAXIS Europe GmbH  
Bunsenstr. 9-11  
64347 Griesheim  
Germany

## 1. Intended Use

The Piccolo<sup>®</sup> Hepatic Function Panel, used with the Piccolo Xpress<sup>®</sup> chemistry analyzer, is intended to be used for the *in vitro* quantitative determinations of alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, direct bilirubin, 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.

## 2. Summary and Explanation of Tests

The Piccolo Hepatic Function Panel reagent disc and 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.
Aspartate aminotransferase:	Liver disease, for example, hepatitis and viral jaundice, shock liver.
Direct bilirubin:	Liver disorders, for example, hemolytic anemia, hepatitis and biliary obstruction.
Total bilirubin:	Liver disorders, for example, hepatitis and biliary obstruction; jaundice.
Total protein:	Metabolic disorders, for example, nutritional disorders, Liver, kidney, bone marrow diseases.

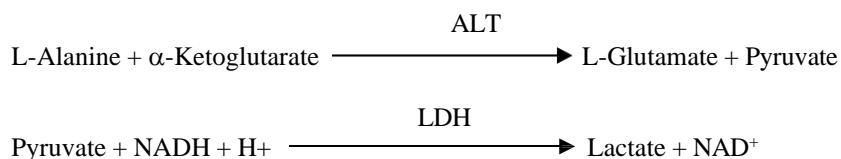
**As with any diagnostic test procedure, all other test procedures including the clinical status of the patient should be considered prior to final diagnosis.**

## 3. Principle of Method

### Alanine Aminotransferase (ALT)

Alanine aminotransferase (ALT) has been measured by three methods. Two of these methods—the colorimetric dinitrophenylhydrazine coupling technique<sup>1,2</sup> and the fluorescent enzymatic assay—are rarely used.<sup>3</sup> An enzymatic method based on the work of Wróblewski and LaDue<sup>4</sup> 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).<sup>5</sup>

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

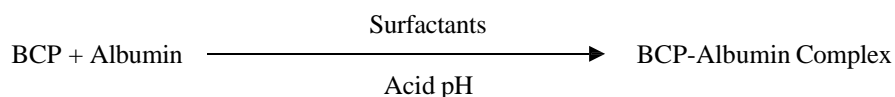


The rate of change of the absorbance difference between 340 nm and 405 nm is due to the conversion of NADH to NAD<sup>+</sup> and is directly proportional to the amount of ALT present in the sample.

### Albumin (ALB)

Early methods used to measure albumin include fractionation techniques<sup>6,7,8</sup> and tryptophan content of globulins.<sup>9,10</sup> 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.<sup>11</sup> 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.<sup>12</sup> Bromocresol purple (BCP) is the most specific of the dyes in use.<sup>13,14</sup>

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

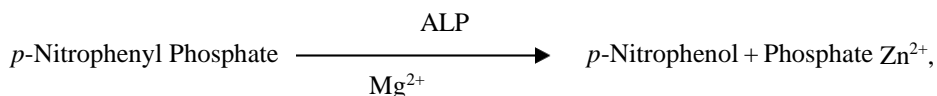


Bound albumin is proportional to the concentration of albumin in the sample. This is an endpoint reaction that is measured as the difference in absorbance between 600 nm and 550 nm.

### Alkaline Phosphatase (ALP)

Techniques to measure alkaline phosphatase were first developed over 60 years ago. Several of these endpoint or two-point spectrophotometric methods<sup>15,16</sup> are now considered obsolete or too cumbersome. The use of *p*-nitrophenyl phosphate (*p*-NPP) increased the speed of the reaction.<sup>17,18</sup> 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.<sup>19</sup> The American Association for Clinical Chemistry (AACC) reference method<sup>20</sup> uses *p*-NPP as a substrate and a metal-ion buffer.

The Piccolo procedure is modified from the AACC and IFCC<sup>21</sup> methods. Alkaline phosphatase hydrolyzes *p*-NPP in a metal-ion buffer and forms *p*-nitrophenol and phosphate.

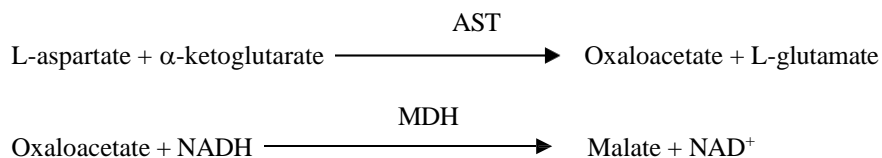


The amount of ALP in the sample is proportional to the rate of increase in absorbance difference between 405 nm and 500 nm.

### Aspartate Aminotransferase (AST)

The aspartate aminotransferase (AST) test is based on the Karmen rate method<sup>22</sup> as modified by Bergmeyer.<sup>23</sup> 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.<sup>23,24</sup> Lactate dehydrogenase (LDH) is added to the reaction to decrease interference caused by endogenous pyruvate.

AST catalyzes the reaction of L-aspartate and  $\alpha$ -ketoglutarate into oxaloacetate and L-glutamate. Oxaloacetate is converted to malate and NADH is oxidized to NAD<sup>+</sup> by the catalyst MDH.



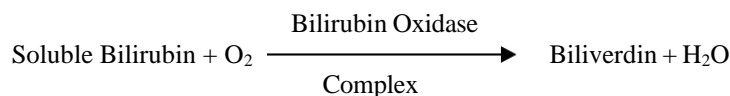
The rate of absorbance change at 340 nm / 405 nm caused by the conversion of NADH to NAD<sup>+</sup> is directly proportional to the amount of

AST present in the sample.

### Direct Bilirubin (DBIL)

Direct Bilirubin was first detected in human serum by Van Den Bergh and Müller when they observed that the pigment in human bile reacted with diazotized sulfanilic acid in the absence of alcohol.<sup>25</sup> Today, commonly used techniques to measure direct bilirubin are modifications of a method developed by Malloy and Evelyn<sup>26</sup> which relies on diazotized sulfanilic acid combining with bilirubin to form the chromophore azobilirubin. Some diazo methods produce unreliable results because unconjugated may be quantitated as direct bilirubin.<sup>27</sup> A more specific assay for total bilirubin was developed after the enzyme bilirubin oxidase was isolated from *Myrothecium verrucaria* MT-1.<sup>28-30</sup> This method is also specific for direct bilirubin when the reaction is run at a lower pH.<sup>31,32</sup>

In the enzymatic procedure, the soluble bilirubin complex (direct bilirubin) is oxidized by bilirubin oxidase into biliverdin.

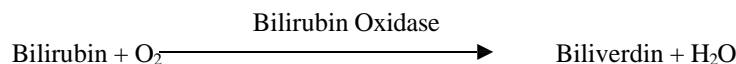


Direct Bilirubin is quantitated as the difference in absorbance between 467nm and 550nm. The initial absorbance of this end point reaction is determined from the direct bilirubin blank cuvette and the final absorbance is obtained from the direct bilirubin test cuvette. The amount of direct bilirubin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

### Total Bilirubin (TBIL)

Total bilirubin levels have been typically measured by tests that employ diazotized sulfanilic acid.<sup>26,33</sup> A newer, more specific method has been developed using the enzyme bilirubin oxidase.<sup>28-30</sup> In addition to using the more specific total bilirubin test method, photodegradation of the analyte is minimized on the Piccolo Analyzer because the sample can be tested immediately after collection.

In the enzyme procedure, bilirubin is oxidized by bilirubin oxidase into biliverdin.

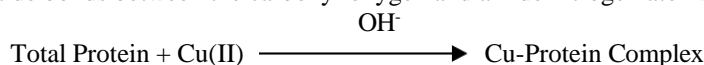


Bilirubin is quantitated as the difference in absorbance between 467 nm and 550 nm. The initial absorbance of this endpoint reaction is determined from the bilirubin blank cuvette and the final absorbance is obtained from the bilirubin test cuvette. The amount of bilirubin in the sample is proportional 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.<sup>34</sup> Originally developed by Riegler<sup>35</sup> and modified by Weichselbaum<sup>36</sup>, Doumas, et al.<sup>37</sup> 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.<sup>36</sup> 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. Principle 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 Hepatic Function Panel 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), aspartate aminotransferase (AST). Dedicated sample blanks are included in the disc for total bilirubin (TBIL), direct bilirubin (DBIL), and total protein (TP). Each reagent disc

also contains a diluent consisting of surfactants, excipients, and preservatives.

**Table 1: Reagents**

Component	Quantity/Disc
L-Alanine	874 µg
L-Aspartic acid	426 µg
4-Aminoantipyrine	5.4 µg
Bilirubin oxidase	0.2 U
Bromcresol purple	2 µg
3,5-Dichloro-2-hydroxybenzenesulfonic acid, Sodium salt (DHBSA)	34.8 µg
Ethylenediaminetetraacetic acid (EDTA), disodium salt	57 µg
Ethylenediaminetetraacetic acid (EDTA), Tetrasodium Salt	215 µg
Cupric sulfate	134 µg
□-Ketoglutaric acid	82 µg
alpha-Ketoglutaric acid, disodium salt	60 µg
Lactate dehydrogenase	0.11 U
Magnesium chloride, hexahydrate	2 µg
Malate dehydrogenase (MDH)	0.01 U
□-Nicotinamide adenine dinucleotide, reduced (NADH)	12 µg
p-NPP	56 µg
Potassium iodide	56 µg
Peroxidase	0 U
Sodium potassium tartrate	896 µg
Zinc sulfate	2 µg
Uricase	0 U
Buffers, Surfactants, excipients, and preservatives	

### Warnings & 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.<sup>38</sup> 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 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.
- 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.
- Materials incorporated into the device may consist of substances derived from other microbial and animal sourced materials.

### Instructions for Reagent Handling

Reagent discs may be used directly from the refrigerator without warming. Do not allow discs sealed in foil pouches to remain at room temperature at 20-25°C (68-77°F) longer than 48 hours prior to use. Open the sealed foil pouch, remove the disc, and use it 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). 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 included on the package. The expiration date is also encoded in the bar code printed on the bar code ring. An error message will appear on 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 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 may cause hemolysis.
- Whole blood samples should only be obtained via venipuncture, not from capillary blood.
- Whole blood venipuncture samples should be run within 60 minutes of collection.<sup>39</sup> Refrigerating whole blood samples can cause significant changes in concentrations of **aspartate aminotransferase**.<sup>40</sup> 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.
- **Total and direct bilirubin** results may be adversely affected by photodegradation.<sup>41</sup> 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).<sup>42</sup>
- 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.

## 8. Procedure

### Materials Required

- One Piccolo Hepatic Function Panel PN: 400-7137 (a box of discs PN: 400-0037)

### 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 (refer to the Piccolo Xpress chemistry analyzer Operator's Manual).
- 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 Hepatic Function Panel is less than 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.

### Calibration

The Piccolo Xpress chemistry analyzer is calibrated by the manufacturer before shipment. The bar code printed on the reagent disc bar code ring provides the analyzer with 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 Hepatic Function Panel 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
AST	IFCC	Colorimetric
DBIL	NIST SRM #916A	Enzymatic
TP	NIST SRM #909	Biuret (Copper II)
TBIL	NIST SRM #916A	Enzymatic

### Quality Control

See Section 6 (Calibration and Quality Control) of the Piccolo Xpress chemistry analyzer Operator's Manual. The 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 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.

## 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 Piccolo Xpress chemistry analyzer Operator's Manual. Results are printed onto results cards or paper rolls supplied by Abaxis. The result cards 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**. 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 Hepatic Function Panel. 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 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.**

**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 uneven flow, an inadequate quantity of sample may be analyzed and several results may fall outside the reference 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 testing levels in CLSI EP7-A.<sup>44</sup>

### Effects of Endogenous Substances

- Physiological interferents (hemolysis, lipemia, and icterus) cause changes in the reported concentrations of some analytes. The sample indices are printed on the bottom of each test 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, icterus, and lipemia. "HEM", "LIP", "ICT" respectively, is printed on the results printout in place of the result.
- For maximum levels of endogenous substances contact Abaxis Technical Support.

### Effects of Exogenous and Therapeutic Substances

- Significant interference is defined as a >10% shift in the results for a normal range specimen. Human pools were supplemented with a known concentration of the drugs or chemicals and then analyzed. **Please see Table 2 for a list of exogenous and therapeutic substances evaluated. Please see Table 3 for a list of analytes where interference was observed.**

**Table 2: Therapeutic Substances Evaluated**

	Highest Concentration Tested (mg/dL)
<b>Endogenous Substances</b>	
Acetaminophen	100
Acetylsalicylic acid	50
Chloramphenicol	100
Cimetidine	16
Erythromycin	10
Isoniazide	4
Ketoprofen	50
Methicillin	100
Methotrexate	0.5
Metronidazole	5
Nafcillin	1
Oxacillin	1
Phenytoin	3

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

	Concentration with No Significant Interference (mg/DL)
<b>Alanine Aminotransferase (ALT)</b>	
Ascorbic acid	20
Oxaloacetate	132
<b>Albumin (ALB)</b>	
Acetoacetate	102
Ampicillin	30
Caffeine	10
Calcium chloride	20
Cephalothin (Keflin)	400
Ibuprofen	50
$\alpha$ -Ketoglutarate	5
Nitrofurantoin	20
Proline	4
Sulfasalazine	10
Sulfanilamide	50
Theophylline	20
<b>Alkaline Phosphatase (ALP)</b>	
Theophylline	20
<b>Aspartate Aminotransferase (AST)</b>	
	None
<b>Direct Total Bilirubin (DBIL)</b>	
Ascorbic acid	2.5
Dopamine	15
<b>Total Bilirubin (TBIL)</b>	
Dopamine	19
L-dopa	5
Sulfasalazine	10
<b>Total Protein (TP)</b>	
	None

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



## 11. Expected Values

Samples from a total of 125 adult males and females were used to determine the reference intervals. These ranges are provided as a guideline only. ALP levels in growing children are highly variable.<sup>43</sup> It is recommended that your office or institution establish normal ranges for your particular patient population.

**Table 4: Piccolo References Intervals\***

Analyte	Reference Interval	
	Common Units	SI Units
Alanine Amino-transferase (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
Aspartate Amino-transferase (AST)	11-38 U/L	11-38 U/L
Direct Bilirubin (DBIL)	0-0.3 mg/dL	0-5.1 µmol/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.

## 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 (refer to the Piccolo Xpress chemistry analyzer Manual).

**Table 5: 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
Aspartate Amino-transferase (AST)	5-2000 U/L	5-2000 U/L
Direct Bilirubin (DBIL)	0.1-15 mg/dL	1.7-257 µmol/L
Total Bilirubin (TBIL)	0.1-30 mg/dL	1.7-513 µmol/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 5 U/L; albumin 1 g/dL (10 g/L); alkaline phosphatase 5 U/L; aspartate aminotransferase 5 U/L; direct bilirubin 0.1mg/dL (1.7 µmol/L); total bilirubin 0.1 mg/dL (1.7 µmol/L); and total protein 2 g/dL (20 g/L).

### Precision

For all assays precision studies were conducted using CLSI EP5-A guidelines.<sup>43</sup> Results for within-run and total precision were determined by testing two levels of control material. Controls were run in duplicate twice each day for 20 days over a four-week period. The results of the precision studies are shown in Table 6.

**Table 6: Precision (N=80)**

Analyte	Within-Run	Total
<b>Alanine Aminotransferase (U/L)</b>		
<u>Control Level 1</u>	21	21
Mean	2.76	2.79
SD	13.4	13.5
%CV		
<u>Control Level 2</u>		
Mean	52	52
SD	2.70	3.25
%CV	5.2	6.2
<b>Albumin (g/L)</b>		
<u>Control Level 1</u>		
Mean	56	56
SD	0.9	1.1
%CV	1.7	2.1
<u>Control Level 2</u>		
Mean	37	37
SD	0.7	1.1
%CV	2.0	2.9
<b>Alkaline Phosphatase (U/L)</b>		
<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
<b>Aspartate Aminotransferase (U/L)</b>		
<u>Control Level 1</u>		
Mean	47	49
SD	0.98	1.84
%CV	2.1	3.9
<u>Control Level 2</u>		
Mean	145	147
SD	1.83	4.62
%CV	1.3	3.2
<b>Direct Bilirubin (µmol/L)</b>		
<u>Control Level 1</u>		
Mean	6.84	6.84
SD	0.51	0.51
%CV	6.5	6.6
<u>Control Level 2</u>		
Mean	37.62	37.62
SD	1.71	2.05
%CV	4.8	5.6

**Table 6: Precision (N=80) (continued)**

Analyte	Within-Run	Total
<b>Total Bilirubin</b> (μmol/L)		
Mean	88.94	88.94
SD		
%CV		
<u>Control Level 2</u>		
Mean	88.94	88.94
SD	1.54	2.57
%CV	1.7	2.8
<b>Total Protein</b> (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 by the Piccolo Xpress chemistry analyzer at the field sites and the serum samples were analyzed by the Piccolo Xpress chemistry 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 7.

**Table 7: Correlation with Comparative Method\*\***

Analyte	Correlation Coefficient	Slope	Intercept	SEE	N	Sample Range	Comparative Method
<b>Alanine Aminotransferase</b>	0.981	0.905	1.3	3.21	86	10-174	Paramax®
(U/L)	0.985	0.946	-2.5	2.84	67	10-174	Technicon
<b>Albumin</b>	0.854	1.001	-0.3	0.22	261	11-53	Paramax®
(g/L)	0.896	0.877	-0.1	0.21	100	15-50	Beckman
<b>Alkaline Phosphatase</b>	0.988	0.970	-5.9	3.97	99	27-368	Paramax®
(U/L)	0.929	1.136	-17.6	4.79	80	26-150	Technicon
<b>AspartateAminotransferase</b>	0.93	0.87	5.3	2.76	159	13-111	Paramax®
(U/L)	1.0	0.97	3.0	1.90	46	13-252	DAX™
<b>Direct Bilirubin</b>	0.998	0.88	-0.1	0.08	263	0-218.93	Paramax®
(μmol/L)							
<b>Total Bilirubin</b>	0.974	0.901	0.0	0.07	250	3.42-63.28	Paramax®
(μmol/L)	0.980	1.113	-0.4	0.09	91	1.71-109.47	Beckman
<b>Total Protein</b>	0.849	0.932	0.6	0.19	251	57-92	Paramax®
(g/L)	0.873	0.935	0.3	0.16	92	65-92	Beckman

\*Serum samples from hospitalized patients provided a broader, and possibly more useful, sample range than did venous whole blood samples from outpatients.

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

## 12. Symbols



Use By

**REF**

Catalog Number

**LOT**

Batch Code

**IVD**

In Vitro Diagnostic  
Medical Device



Consult Instructions  
for Use



Manufacturer



Do Not Reuse



X Number of Test  
Devices in Kit

**BOX**

Manufacturing  
Sequence

**SN**

Serial Number

**EC REP**

Authorized  
Representative  
in the European  
Community



Temperature  
Limitation



Caution

PN:  
Part Number



denotes conformity to specified  
European directives

### 13. Bibliography

1. Tonhazy NE, White NG, Umbreit WW. A rapid method for the estimation of the glutamic-aspartic transaminase in tissues and its application to radiation sickness. *Arch Biochem* 1950; 28:36-42.
2. Reitman S and Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol*, 1957; 28: 56-63.
3. Murray RL. Alanine aminotransferase. *In: Clinical Chemistry: Theory, Analysis, and Correlation*, 2<sup>nd</sup> ed. Kaplan LA, Pesce AJ, Eds. St. Louis: The C.V. Mosby Company, 1989: 895-898.
4. Wróblewski F, LaDue JS. Serum glutamic-pyruvic transaminase in cardiac and hepatic disease. *Proc Soc Exp Biol Med*, 1956; 91: 569-571.
5. Bergmeyer HU, Horder M. IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase. *J Clin Chem Clin Biochem*, 1980; 18: 521-534.
6. Howe PE. The use of sodium sulfate as the globulin precipitant in the determination of proteins in blood. *J Biol Chem*, 1921; 49: 93-107.
7. Howe PE. The determination of proteins in blood — a micro method. *J Biol Chem*, 1921; 49: 109-113.
8. Wolfson WQ, et al. A rapid procedure for the estimation of total protein, true albumin, total globulin, alpha globulin, beta globulin and gamma globulin in 10 ml of serum. *Am J Clin Pathol*, 1948; 18: 723-730.
9. Saifer A, Gerstenfeld S, Vacsler F. Photometric microdetermination of total serum globulins by means of a tryptophan reaction. *Clin Chem*, 1961; 7: 626-636.
10. Saifer A, Marven T. The photometric microdetermination of serum total globulins with a tryptophan reaction: a modified procedure. *Clin Chem*, 1966; 12: 414-417.
11. Gendler SM. Albumin. *In: Clinical chemistry: Theory, Analysis, and Correlation*. LA Kaplan and Pesce AJ, Eds. St. Louis: The C.V. Mosby Company, 1989: 1029-1033.
12. Webster D, Bignell AHC, Attwood EC. An assessment of the suitability of bromocresol green for the determination of serum albumin. *Clin Chim Acta* 53, 1974; 101-108.
13. Louderback A, Mealey EH, Taylor NA. A new dye-binding technique using bromocresol purple for determination of albumin in serum. *Clin Chem* 14, 1968; 793-794. (Abstract)
14. Pinnell AE, Northam BE. New automated dye-binding method for serum albumin determination with bromocresol purple. *Clin Chem*, 1978; 24: 80-86.
15. King EJ, Armstrong AR. A convenient method for determining serum and bile phosphatase activity. *Can Med Assoc J*, 1931; 31: 376-381.
16. Kind PRN, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. *J Clin Pathol*, 1954; 7: 322-326.
17. Ohmori Y. Uber die Phosphomonoesterase. *Enzymologia*, 1937; 4: 217-231.
18. Fujita H. Umber die Mikrobestimmung der Blutphosphatase. *J Biochem, Japan*, 1939; 30: 69-87.
19. Petitclerc C et al. Mechanism of action of Mg<sup>2+</sup> and Zn<sup>2+</sup> on rat placental alkaline phosphatase. I. Studies on the soluble Zn<sup>2+</sup> and Mg<sup>2+</sup> alkaline phosphatase. *Can J Biochem*, 1975; 53: 1089-1100.
20. Tietz NW, et al. A reference method for measurement of alkaline phosphatase activity in human serum. *Clin Chem*, 1983; 29: 751-761.
21. Bowers GN, et al. IFCC methods for the measurement of catalytic concentration of enzymes. Part I. General considerations concerning the determination of the catalytic concentration of an enzyme in the blood serum or plasma of man. *Clin Chem Acta*, 1979; 98: 163F-174F.
22. Karmen A. A note on the spectrophotometric assay of glutamic-oxalacetic transaminase in human blood serum. *J Clin Invest*, 1955; 34: 131-133.
23. Bergmeyer et al. Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes. Part 2. IFCC method for aspartate aminotransferase. *Clin Chem*, 1977; 23: 887-899.
24. Bergmeyer et al. Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes. Part 2. Revised IFCC method for aspartate aminotransferase. *Clin Chem*, 1978; 24: 720-721.
25. Van Den Bergh AAH, Müller P. Uber eine Direkte und eine indirekte Diazoreaktion auf Bilirubin. *Biochem Z*, 1916; 77: 90-103.
26. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. *J Biol Chem*, 1937; 119: 481-490
27. Dumas BT, Wu T-W. The measurement of bilirubin fractions in serum. *Crit Rev Clin Lab Sci*, 1991; 28: 415-445.
28. Murao S and Tanaka N. A new enzyme “bilirubin oxidase” produced by *Myrothecium verrucaria* MT-1. *Agric Biol Chem*, 1981; 45: 2383-2384.
29. Osaki S, Anderson S. Enzymatic determination of bilirubin. *Clin Chem*, 1984; 30: 971. (Abstract)
30. Perry B, et al. Measurement of total bilirubin by use of bilirubin oxidase. *Clin Chem*, 1986; 32: 329-332.
31. Dumas BT, et al. Measurement of total bilirubin by use of bilirubin oxidase. *Clin Chem*, 1986; 32: 1349-1353.
32. Otsuji S et al. A new enzymatic approach for estimating total and direct bilirubin. *Clin Biochem*, 1988; 21: 81-110.
33. Meites S. Bilirubin, direct reacting and total, modified Malloy-Evelyn method. *In: Faulkner WR and Meites S, eds. Selected*

- methods of clinical chemistry. Washington, DC: American Association for Clinical Chemistry, 1982; 9: 119-124.
34. Koller A and Kaplan LA. Total serum protein. *In: Clinical chemistry: Theory, Analysis, and Correlation*, 2<sup>nd</sup> ed. Kaplan LA, Pesce AJ, Eds. St. Louis: The C.V. Mosby Company, 1989: 1057-1060.
  35. Reigler E. Eine kolorimetrische Bestimmungsmethode des Eiweisses. *Z Anal Chem*, 1914; 53: 242-245.
  36. Weichselbaum TE. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. *Am J Clin Path*, 1946; 16: 40-49.
  37. Dumas BT et al. A candidate reference method for determination of total protein in serum. I. Development and validation. *Clin Chem*, 1981; 27: 1642-1650.
  38. CLSI. Physician's office laboratory guidelines, tentative guideline, 2<sup>nd</sup> ed. CLSI document POL1-T2. Wayne, PA: CLSI, 1992
  39. CLSI. Procedures for the handling and processing of blood specimens; approved guideline, 2<sup>nd</sup> ed. CLSI document H18-T. Wayne, PA: CLSI, 1988.
  40. Rehak NN, Chiang BT. Storage of whole blood: effect of temperature on the measured concentration of analytes in serum. *Clin Chem*, 1988; 34: 2111-2114.
  41. Sherwin JE, Obernolte R. Bilirubin. *In: Clinical chemistry: theory, analysis, and correlation*, 2<sup>nd</sup> ed. Kaplan LA and Pesce AJ, Eds. St. Louis: The C.V. Mosby Company, 1989:1009-1015.
  42. Henry RJ, Cannon DC, Winkelman JW. *Clinical chemistry: Principles and techniques*, 2<sup>nd</sup> ed. New York: Harper and Row; 1974; 417- 421, 1058 -1059.
  43. CLSI. Evaluation of precision performance of clinical chemistry devices, 2<sup>nd</sup> ed.; approved guideline. CLSI document EP5-A. Wayne, PA: CLSI, 1999.
  44. Painter PC, Cope JY, Smith JL. Appendix. *In: Tietz Textbook of Clinical Chemistry*, 2<sup>nd</sup> ed. Burtis CA, Ashwood ER, Eds. Philadelphia. W.B. Saunders Company, 1994: 2161-2217