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Assessment of liver function tests on Piccolo Xpress point of care chemistry analyzer in a pediatric hospital

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ABSTRACT

Objectives: Point of care testing (POCT) contributes to diagnosis and monitoring with fast testing time and easily performed assays. We evaluated the Abaxis Piccolo Xpress point of care chemistry analyzer using the Liver Panel Plus discs for our pediatric patient population at Texas Children's Hospital.
Design and methods: Analytical performance was evaluated for precision and linearity using quality control materials and commercially available verification samples. Comparison studies were performed between Piccolo Xpress analyzer and Vitros 5600 analyzer using patient samples. Interference studies were carried out using nine different patient pool sera. Lipemia interference was removed using LipoClear for severely lipemic sample pools.
Results: Precision of all tests was excellent (CVs < 5% for all measured analytes except TBIL). All assays were linear and accurate within the allowable total error. Comparison studies showed that three analytes (amylase, GGT and TBIL) had statistically significant bias. Interference study results did not exceed the total allowable error for hemoglobin (< 150 mg/dL), bilirubin (< 15 mg/dL) and lipemia (< 400 mg/dL except ALT, GGT and TP). LipoClear treatment removed lipemia interference for all analytes except total protein.
Conclusions: The Piccolo Xpress chemistry analyzer showed an acceptable analytical performance for precision, linearity and interference from common substances. Increased bias for three analytes in comparison studies could be due to different methodologies.

1. Introduction

Point of care tests (POCT) are gaining attention and popularity due to their simplicity of use and rapid turnaround time of results [1]. The need for more specialized and efficient support systems for neonatal and pediatric patients, in remote health centers and for infectious diseases such as viral hemorrhagic fever mandated the need for simplified equipment for POC liver function tests [2]. Recently, the Piccolo Xpress POC chemistry analyzer has been launched which includes a Liver Panel [2,3]. We have procured this instrumentation in our pediatric facility to assist physicians taking care of patients with viral hemorrhagic fever. As clinical laboratorians, one of our major focuses is to validate such POC instruments against the CAP-certified clinical laboratory methods used in the main laboratory to ensure the appropriate transferability and accuracy of

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such POC results for diagnosis and management of patients.

The aim of this study was to evaluate the performance of the Abaxis Piccolo Xpress point of care chemistry analyzer Liver Panel Plus discs assays in the pediatric setting. In this report, we provide data on precision, linearity, comparison with the central laboratory analyzer and on interference studies using the Liver Panel discs on the Piccolo Xpress POC analyzer. Various performance characteristics (e.g. accuracy, precision, specimen stability, consistency etc.) of the Piccolo Xpress analyzer have been investigated in adult and neonatal intensive care unit populations [4–7]. Our study adds to the literature by providing additional comprehensive evaluation in the pediatric population and interference studies using the Liver Panel Plus discs.

2. Materials and methods

We evaluated the Piccolo Xpress chemistry analyzer for the Liver Panel assays for our pediatric patient population at Texas Children's Hospital using the NCCLS (National Committee for Clinical Laboratory Standards) criteria [8,9]. Evaluations were performed for precision, linearity, method comparison and interferences from common substances using two different lots of discs with minimal variability between lots. The panel includes the following eight tests: alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), amylase (AMY), aspartate aminotransferase (AST), Gamma glutamyltransferase (GGT), total bilirubin (TBIL) and total protein (TP). The Piccolo instrument uses dry and liquid reagents with various testing principles (e.g. dye-binding bromocresol purple technique for albumin assay, enzymatic bilirubin oxidase method for total bilirubin assay) using absorption detection to give quantitative results [3]. Testing of eight analytes is performed on a single-use Liver Panel disposable disc. Briefly working principle of instrument includes loading the sample into a disc up to the designated mark. Once the disc is placed into the Piccolo analyzer, sample and diluent are measured for the volume requirements. As first step, instrument spins down the sample to separate the plasma. Then, sample and diluent are pushed into the mixing chamber where they are mixed before diluted samples flow into the reaction cuvettes for reaction and measurement. Since it is a centrifugal device manufacturer states that lithium heparinized plasma or serum samples are also acceptable sample types. The instrument requires only 100 μL lithium heparinized whole blood, lithium heparinized plasma or serum for measurement.

We used randomly selected de-identified patient samples, under an IRB approved protocol, for the entire study. The pediatric patient samples received in the main laboratory at Texas Children's Hospital were used in this study.

2.1. Precision and linearity studies

Precision studies were performed using commercially available quality control materials (Liquid Assayed Chemistry Control from Bioresearch Technology Inc.). Intra-assay precision was conducted on two levels of control (Control Level 1 and Control Level 2) as 10 replicates. Inter-assay precision was determined using two levels of control as 10 replicates over a period of 32 days.

Linearity studies were performed using commercially available verification samples (Verification Samples from Bioresearch Technology Inc.) which are human liquid serum samples at three concentration levels. Each concentration level was run as 5 replicates and the average concentration obtained was compared to the assigned values.

2.2. Comparison studies

Comparison studies were performed using lithium heparinized whole blood samples. Twenty samples run on Piccolo Xpress chemistry analyzer in duplicates and simultaneously run on Vitros 5600 analyzer (main laboratory chemistry instrument). Whole blood samples were centrifuged at 4400*g* for 3 min and plasma separated from the cells before they were run on Vitros 5600 analyzer for comparison. All analyses were conducted within 1–2 h of collection.

2.3. Interference studies

Interference studies were performed for hemoglobin, icterus and lipemia using lithium heparin plasma sample pools. Total of nine plasma pools (three sample pools for each interferent) were used for the interference studies. Increasing concentrations of commercially available hemolysate (INT-01 Routine Interference ASSURANCE™ Interference Test Kit by Sun Diagnostics®, LLC) was added into each sample pool to obtain final concentrations of 75 mg/dL, 150 mg/dL and 300 mg/dL hemoglobin. These samples were then analyzed on Piccolo chemistry analyzer.

Icteric samples were prepared by addition of commercial conjugated bilirubin (INT-01 Routine Interference ASSURANCE™ Interference Test Kit by Sun Diagnostics®, LLC) into separate plasma sample pools ($n=3$) to yield final concentrations of 7.5 mg/dL and 15 mg/dL. All samples were prepared in dark room and protected from light exposure until analysis to prevent possible photodegradation of bilirubin. Then interference from icterus was determined by analyzing these samples within 60 min of preparation.

Aliquots of commercial triglycerides from Sun Diagnostics (INT-01 Routine Interference ASSURANCE™ Interference Test Kit by Sun Diagnostics®, LLC) were added into pooled plasma samples to obtain final concentrations of 400 mg/dL, 1000 mg/

Table 1
Results of intra-assay and inter-assay precision studies.

Test name	Unit	Mean		Intra-assay (%CV)		Mean		Inter-assay (%CV)	
		QC Level 1	QC Level 2	QC Level 1	QC Level 2	QC Level 1	QC Level 2	QC Level 1	QC Level 2
Albumin (ALB)	g/dL	2.9 ± 0.1	4.4 ± 0.0	2.4	0.0	2.9 ± 0.1	4.4 ± 0.0	1.6	0.0
Alanine aminotransferase (ALT)	U/L	48.9 ± 1.7	175.2 ± 1.0	3.4	0.6	48.0 ± 1.6	175.8 ± 4.6	3.3	2.6
Aspartate aminotransferase (AST)	U/L	80.1 ± 1.8	298.2 ± 0.8	2.2	0.3	80.4 ± 1.5	296.2 ± 4.3	1.9	1.4
Alkaline phosphatase (ALP)	U/L	87.7 ± 3.1	371.1 ± 5.6	3.6	1.5	89.0 ± 3.5	364.7 ± 7.8	4.0	2.1
Amylase (AMY)	U/L	68.7 ± 3.0	284.7 ± 3.5	4.3	1.2	67.6 ± 0.7	281.9 ± 3.0	1.0	1.1
Gamma glutamyltransferase (GGT)	U/L	50.1 ± 0.6	191.5 ± 1.2	1.1	0.6	50.0 ± 0.8	190.6 ± 1.4	1.6	0.8
Total bilirubin (TBIL)	mg/dL	1.2 ± 0.1	4.0 ± 0.0	7.8	0.0	1.3 ± 0.1	4.0 ± 0.0	5.3	0.8
Total protein (TP)	g/dL	4.8 ± 0.1	7.1 ± 0.1	1.4	0.7	4.8 ± 0.0	7.1 ± 0.1	0.9	1.0

dL and 2000 mg/dL. Lipemic samples were then analyzed on Piccolo instrument and lipemia interference was determined.

In order to remove lipid interference, severely lipemic samples (at 2000 mg/dL triglyceride level) were treated with LipoClear reagent (StatSpin®) and reanalyzed. Procedure included mixing of sample with LipoClear reagent (5:1 v/v) and letting stand at room temperature for 5 min. The mixture then centrifuged at 3300 rpm for 20 min and the lipid free supernatant was reanalyzed. Obtained results were multiplied by dilution factor of 1.2.

2.4. Analysis of data

Results of precision and linearity studies were evaluated using the EP Evaluator Data Innovation (EE 10) program. Statistical significance of comparison data were determined by GraphPad Prizm program.

3. Results

3.1. Precision and linearity studies

Results of intra-assay and inter-assay precision studies are detailed in Table 1. Precision was assessed using calculated percent coefficient of variation (%CV). All eight tests showed a good precision with %CV being less than 10% for both QC Level 1 and QC Level 2.

Intra-assay precision results for QC Level 1 were less than 5% for all eight tests except TBIL (7.8%). Results of QC Level 2 for intra-assay precision were lower ($\leq$ 1.5%). Inter-assay precision results of QC Level 1 ranged from 0.9 to 5.3% while results for QC Level 2 ranged from 0.0 to 2.6%.

Results of linearity studies are listed in Table 2. Evaluations were performed using the total allowable error (TEa) for the laboratory analytes according the CAP and CLIA guidelines as given in column 3 of Table 2. Results of all analytes were linear and accurate (except TBIL) within the total allowable error. Linearity and accuracy of the analytes were analyzed over a measured range consistent with assigned values shown in column 4–6 of Table 2. The maximum deviation for a mean recovery from 100% was less than 10% for verification material (VM) Level 1. Only ALP and TBIL had higher percent recovery of 14.1% and 13.3%, respectively. The percent recovery for the eight tests for verification material Level 2 were less than 5% while the recovery (%) was $\leq$ 5.5% for verification material Level 3.

Table 2
Results of linearity studies.

Test name	Unit	Total allowable error (TEa, %)	Assigned values			Recovery (%)		
			VM Level 1	VM Level 2	VM Level 3	VM Level 1	VM Level 2	VM Level 3
Albumin (ALB)	g/dL	10	2.0	4.1	5.7	105.0	104.9	103.5
Alanine aminotransferase (ALT)	U/L	20	18	678	1377	96.7	100.2	100.7
Aspartate aminotransferase (AST)	U/L	20	20	750	1399	108.0	104.8	103.7
Alkaline phosphatase (ALP)	U/L	30	27	945	1761	114.1	98.6	99.6
Amylase (AMY)	U/L	30	24	1303	2430	100.0	97.1	101.8
Gamma glutamyltransferase (GGT)	U/L	20	17	1330	2444	102.4	102.0	103.2
Total bilirubin (TBIL)	mg/dL	20	0.3	2.6	5.1	113.3	97.7	94.5
Total protein (TP)	g/dL	10	3.5	6.7	10.0	101.1	98.8	100.2

VM: Verification materials

Table 3
Results of comparison studies.

Test name	Concentration range	Slope	Intercept	Correlation coefficient (R^2)	*p*-value	% Bias
Albumin (ALB)	1.7–4.8	0.918	0.053	0.835	0.892	1.3
Alanine aminotransferase (ALT)	12–141	0.879	−1.394	0.981	0.507	−2.3
Aspartate aminotransferase (AST)	24–101	0.876	2.579	0.984	0.095	4.9
Alkaline phosphatase (ALP)	67–311	1.044	−10.294	0.971	0.164	−6.6
Amylase (AMY)	24–362	0.865	−8.658	0.987	0.012	−9.9
Gamma glutamyltransferase (GGT)	6–212	1.042	−6.445	0.998	0.000	−13.4
Total bilirubin (TBIL)	0.1–1.4	0.717	0.242	0.813	0.000	43.9
Total protein (TP)	5.2–8.6	0.923	0.246	0.975	0.328	3.5

Average of 20 patient samples were analyzed on Piccolo analyzer and Vitros 5600 analyzer.

3.2. Comparison studies

All eight analytes were analyzed on average of 20 patient samples in duplicate on Piccolo analyzer and average of the results was compared to results obtained on Vitros 5600 analyzer. Results of method comparison studies are detailed in Table 3. Comparison studies showed that AMY, TBIL and GGT had statistically significant bias ($p \leq 0.05$). AMY and GGT had negative bias (9.9% and 13.4%) while TBIL had positive bias of 43.9%. Results of ALB, ALP, ALT, AST and TP on Piccolo instrument were lower than the results measured on Vitros analyzer. However, these remaining five assays did not show statistically significant bias (bias ranging from 1.3% to 6.6%).

3.3. Interference studies

Potential interference from common interferents (hemolysis, bilirubin and lipemia) was assessed on eight analytes. Results of interference studies are listed in Tables 4a–4c and they are highlighted in bold when any of the results exceed the total allowable limits. Comparison of baseline values to results obtained from samples with increasing hemolysate concentrations (Table 4a). Only AST and GGT were affected by moderate hemolysis with percent change from baseline values were 22.2% and –24.4%, respectively. The remaining six analytes were not affected by hemolysis up to interferent concentrations of 300 mg/dL.

None of the analytes exceeded the total allowable limit (Table 4b) for icterus interference in the presence of bilirubin at concentrations of 7.5 mg/dL (moderately icteric) and 15 mg/dL (severely icteric).

ALB was not affected from mild, moderate and severe lipemia (Table 4c). Three analytes (ALP, AMY and TBIL) were not affected by mild and moderate lipemia, however severely lipemic samples for these analytes resulted as “LIP” that is printed by the Piccolo instrument when results are adversely affected by lipemia. ALT gave lower results for mildly and moderately lipemic samples. Both results exceeded the total allowable error limit of 20%. Severely lipemic samples were adversely affected by lipemia and results could not be determined by instrument. AST was not affected by mild lipemia, while 95.8% positive change from baseline was observed for moderate lipemia. The Piccolo instrument printed the result of “LIP” for severely lipemic sample for AST. Two analytes (GGT and TP) were affected by mild, moderate and severe lipemia. Percent changes from baseline were −34.1% and −14.9% for GGT and TP, respectively. Both results were outside the total allowable limits of 20% for GGT and 10% for TP.

Severely lipemic sample pools were treated with LipoClear reagent to remove lipemia interferences and seven analytes (ALB, ALT, AST, ALP, AMY, GGT and TBIL) recovered their baseline values after treatment (percent change from baseline ranging between 0.0% and −6.8%). Only TP showed a poor recovery upon LipoClear treatment with −13.5% change from its baseline value.

Table 4a
Results of hemolysis studies.

Test name	Total allowable error (TEa, %)	Normal hemolysis (75 mg/dL) (%)	Slight hemolysis (150 mg/dL) (%)	Moderate hemolysis (300 mg/dL) (%)
Albumin (ALB)	10	0.0	0.0	0.0
Alanine aminotransferase (ALT)	20	−1.2	−2.5	−16.0
Aspartate aminotransferase (AST)	20	5.6	12.6	**22.2**
Alkaline phosphatase (ALP)	30	0.0	2.0	3.7
Amylase (AMY)	30	0.7	1.5	3.6
Gamma glutamyltransferase (GGT)	20	−4.7	−7.1	**−24.4**
Total bilirubin (TBIL)	20	0.0	0.0	−20.0
Total protein (TP)	10	0.0	1.3	4.0

$n=3$ different pools of sample.
Data are provided as percent change from baseline.

Table 4b
Results of icterus studies.

Test name	Total allowable error (TEa, %)	Moderate icterus (7.5 mg/dL) (%)	Severe icterus (15 mg/dL) (%)
Albumin (ALB)	10	0.0	0.0
Alanine aminotransferase (ALT)	20	6.4	0.6
Aspartate aminotransferase (AST)	20	−5.9	−2.9
Alkaline phosphatase (ALP)	30	−1.7	−1.4
Amylase (AMY)	30	−9.9	−11.0
Gamma glutamyltransferase (GGT)	20	−3.8	−13.1
Total protein (TP)	10	−2.9	−7.1

$n=3$ different pools of sample.
Data are provided as percent change from baseline.

Table 4c
Results of lipemia studies.

Test name	Total allowable error (TEa, %)	Mild lipemia (400 g/dL) (%)	Moderate lipemia (1000 g/dL) (%)	Severe lipemia (2000 g/dL) (%)	Severe lipemia +LipoClear (%)
Albumin (ALB)	10	4.4	2.2	0.0	6.7
Alanine aminotransferase (ALT)	20	**−24.4**	> **−76.5**	nd	−4.2
Aspartate aminotransferase (AST)	20	12.8	**95.8**	lip	−5.1
Alkaline phosphatase (ALP)	30	10.0	15.8	lip	−3.8
Amylase (AMY)	30	11.0	−17.3	lip	−6.8
Gamma glutamyltransferase (GGT)	20	**−34.1**	nd	lip	−2.2
Total bilirubin (TBIL)	20	20.0	20.0	lip	0.0
Total protein (TP)	10	**−14.9**	nd	lip	**−13.5**

$n=3$ different pools of sample.
Data are provided as percent change from baseline.
lip: lipemic samples; nd: results cannot be determined.

4. Discussions

In recent years, wide variety of laboratory tests have been developed for point of care testing with increasing instrumentation technology [2,10]. Point of care testing is mainly preferred in bedside due to its fast testing time and ease of use as well as reduced costs [1,11]. Point of care analyzers are of specific interest in pediatric settings due to small amount of sample requirement for most of the testing [4]. The Piccolo Xpress point of care chemistry analyzer is a good alternative in this regard and provides CLIA (Clinical Laboratory Improvement Amendments) waived testing for eight liver panel analytes in a single disc. At TCH, we obtained the Piccolo analyzer for use in a biocontainment facility built for patients suspected of viral hemorrhagic fever. In this study we evaluated the analytical performance of the Piccolo POC chemistry analyzer using the Liver Panel Plus discs for pediatric patient population in Texas Children's Hospital.

The study included the evaluation of precision, linearity, comparison with Vitros and effect of three most common interferences (hemolysis, incteris and lipemia) on eight analytes on the Liver Panel discs of the Piccolo POC analyzer.

Our studies showed an excellent precision with % CV of $<5\%$ for both intra- and inter-assay precision for seven studied analytes, except TBIL. These results met the manufacturer's specifications by even exceeding their specifications for most of the assays. Other studies have previously reported on the precision of analytes from the Liver Panel discs using Piccolo Xpress chemistry analyzer [5–7]. Results of most of these studies were consistent that reported CVs were less than 15%. Thus, it appears that like the other few reports published, the precision of Liver Panel Plus analytes on the Piccolo are acceptable for use in a point of care setting.

The comparison study results were closely comparable for twenty samples which were tested on the Piccolo chemistry analyzer and the main laboratory Vitros 5600 analyzer. Five analytes had less than 7% negative bias which was statistically not significant. Only three analytes (AMY, TBIL and GGT) had statistically significant bias. Among these, AMY and GGT had bias of less than 14%, while TBIL was particularly concerning with bias of 43.9%. The Piccolo AMY and GGT methods [3] are very similar to the Vitros 5600 methods [12,13] but not exactly the same. In these methods, the substrate reacts with the AMY or GGT to release a chromophore that leads change in absorbance at certain wavelength. Formation of the chromophore is determined by activity of the AMY or GGT in the patient sample. Since these methods only share the main principles, the methodological differences in details and possible standardization differences could be the cause of discrepancy in method comparison results. On the other hand, the TBIL methods on these two instruments have major differences [3,14]. While the Piccolo system uses the bilirubin oxidase enzyme in its method, the Vitros TBIL assay uses multilayered slide technology that applies dyphylline and diazonium salt. These methodological differences could be the potential cause of the

positive proportional bias between the results. Similarly, previous studies showed discrepant results when samples were performed on the Piccolo instrument versus Architect c8000, Toshiba or Roche analyzers [5–7]. In addition, the larger bias on the TBIL was mainly due to the lower concentrations of TBIL that were tested for the method comparison studies. Due to potential impact of the bias on reference intervals, clinical laboratories need to develop local reference ranges or re-validate the Piccolo-specific reference ranges for analytes tested using this POCT analyzer.

The strength of our study is that it is a comprehensive evaluation of the 8 analytes in the Liver Panel that one can obtain using the Piccolo analyzer. To our knowledge, there is no other study in literature which evaluates interference from hemolysis, icterus and lipemia for liver panel analytes on Piccolo chemistry analyzer. Investigation of interferences from common substances is particularly an emerging need for pediatric setting because those cases could be encountered frequently in routine operations especially for interference from hemolysis and bilirubin in the pediatric patient population. Results of our study help other laboratories as well by providing valuable data to evaluate interference from common substances in their patient population.

Our interference study results were promising since the eight Liver Panel Plus analytes were not affected even by severe icterus (up to 15 mg/dL of bilirubin), while only two analytes (AST and GGT) were affected by severe hemolysis (300 mg/dL of hemoglobin). On the other hand, the only assay that was not affected from the lipemia interference (2000 mg/dL of triglycerides) was ALB for our pediatric population. ALP, AMY and TBIL assays showed good performance in mildly and moderately lipemic samples. However, lipemia showed greater effect on pediatric samples for ALT, GGT and TP assays. Even though, pediatric samples were affected by lipemic interferences, treatment with lipemia removal agent (LipoClear) resolved the problem for seven assays and brought all results close back to baseline level except for TP. Because all of our interference studies involved spiking the sample with the interferent, we were unable to do it in whole blood and thus, performed all interference studies using only plasma. It needs to be pointed out that while these interference studies were conducted with plasma pools, we believe the same to be true for whole blood analyses using these disks since the Piccolo Xpress is a centrifugal analyzer.

In summary, the present study provides evaluation of the eight liver tests using Liver Panel Plus discs on a point of care instrument, the Piccolo Xpress chemistry analyzer using pediatric samples. This comprehensive investigation included assessment of common performance characteristics e.g. intra-assay and inter-assay precision, linearity and comparison of 20 patient samples between the benchtop Piccolo analyzer and main laboratory Vitros 5600 analyzer. Additionally, an extended interference study was performed for three common interferents in our pediatric population. Therefore our study provides a practical guidance for accurate test reporting of liver analytes using Piccolo Xpress POC analyzer in a remote setting.

Declarations

No competing interests, funding. Liver Panel Discs were provided by Abaxis.

Guarantor

S. Devaraj.

Contributorship

Dr. S. Devaraj conceived and supervised the study, Dr. N. Akbas, G. Gonzalez and R. Edwards performed the study.

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