

Clinical Evaluation of an Algorithm for Short Sample Detection on a Multi-Analyte Panel Using a Point-of-Care Analyzer

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Abstract

The ABAXIS Piccolo[®] point-of-care analyzer is a compact and lightweight analyzer capable of measuring up to 14 analytes from a single 90 microliter sample. The ABAXIS Piccolo[®] point-of-care analyzer can run a variety of standard CMS panels, including the 14-analyte Comprehensive Metabolic Panel, the 8-analyte Basic Metabolic Panel and the 7-analyte Hepatic Function Panel. One drawback common in all analyzers is the issue of “short sample.” Although clinical analyzers typically aspirate and dilute samples very precisely, and phlebotomists normally take great care in sample collection, human and machine errors can occur, resulting in incorrect test results. Fibrin may form in the sample probe or distribution channels of an analyzer, or a sample may be diluted by IV fluid. The measurement of short samples and diluted samples is a source of medical errors. These types of samples are normally very difficult to detect in analyzers and can have devastating consequences in the diagnosis and monitoring of patients. Currently there are no reliable and simple methods to detect the presence of short or diluted samples. Current tools used to identify short or diluted samples include delta-checking methods and abnormal/ critical value limit checks.

We have developed a short sample algorithm for the Piccolo[®] point-of-care analyzer. This algorithm is based on statistical analysis of analyte distributions from over 16,000 patients and can detect short samples and sample dilutions corresponding to as low as 5% insufficient sample volume or over-dilution of sample. This algorithm utilizes the population distributions from the multiple analytes available on Piccolo[®] rotors and is more robust than short sample techniques based on single analyte distributions or analyte distributions within a single class of analytes, such as electrolytes. Data has been evaluated showing the use of this algorithm with four different Piccolo[®] rotors in a clinical setting as well as in a controlled setting where deliberate short samples and sample dilutions were evaluated.

Results for the Comprehensive Metabolic Panel Piccolo[®] rotor are shown in Table 1. These were simulated short samples from actual clinical samples. The last column shows the percent dilution required to trigger the short sample warning. In actual clinical testing, all verified short samples were detected with no false positives.

This algorithm can be used as a useful tool to help ensure the integrity of results, particularly in a point-of-care setting where extensive oversight of results may not be available.

Table 1

#	ALB	ALP	ALT	AST	BUN	CA	CL	
1	3.0	105	20	31	24	9.0	97	
2	3.1	207	11	21	22	8.3	105	
3	2.4	67	470	865	30	8.1	113	
4	4.0	256	89	78	13	9.5	101	
5	3.1	64	20	29	13	9.3	107	
6	4.0	90	14	21	11	9.2	100	
#	CRE	GLU	K	NA	TBIL	CO2	TP	Dilution
1	2.9	118	4.3	126	2.4	19	7	14%
2	1.1	96	2.2	139	0.9	19	6	6%
3	5.6	109	6.3	142	1.2	17	4	15%
4	0.6	85	3.9	132	4.3	20	8	11%
5	0.8	131	3.5	142	1.1	25	6	12%
6	0.9	91	4.0	136	1.2	24	7	15%

Introduction

Inaccurate results can occur in clinical diagnostic assays due to addition of insufficient sample to the reaction mixture, or improper dilution of sample. The result of this type of error is under recovery of analyte and is often referred to as a “short sample.” These types of errors are usually caught by inspection of data by technicians, delta check analysis, or other data checking algorithms. However, even today, the most sophisticated analyzers cannot reliably detect short samples.



The Piccolo[®] Point-of-Care Chemistry Analyzer performs analysis of test panels contained in reagent discs. The system performs the functions of separating the sample into the plasma and the cellular components, measures the volume of plasma needed for analysis, measures a volume of diluent, mixes them, and delivers this aliquot to the reaction cuvettes, where the reagents are present in freeze-dried form. The system allows a complete panel of tests to be performed on the same diluted sample.

We have developed an algorithm to detect short samples by evaluating results from numerous analytes. The algorithm is based on the population distribution from over 16,000 test panels containing these analytes. The algorithm makes use of the probability distribution for several analytes in a panel, not just one or two.

The challenge for developing such an algorithm is to find the cut off threshold that permits detection of true short samples and can differentiate these from samples where the patient's results are low, but correct.

While this algorithm was developed for use with the Piccolo[®] analyzer, it has broad applications to other automated systems where test panels are run.

Methods

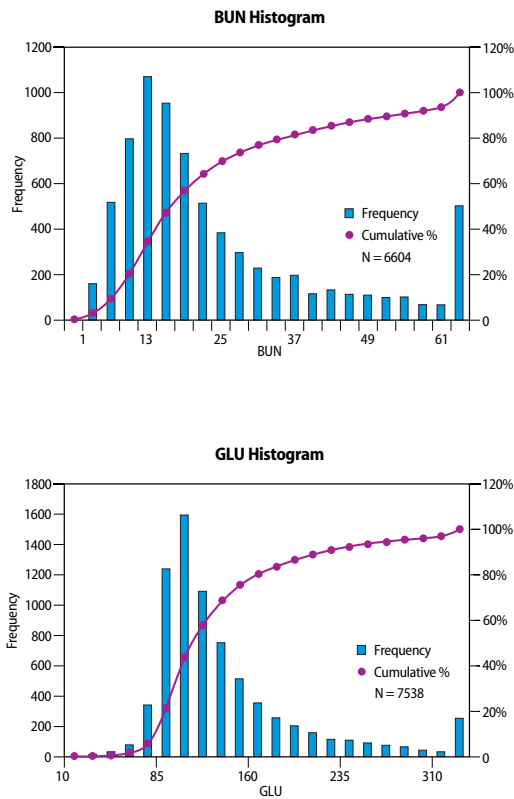
- Testing of a population of patients using an Olympus AU640 and the manufacturer's reagents and methods
- Results of over 16,000 test panels were cumulated over approximately a 60-day period
- ABAXIS Piccolo Point-of-Care Chemistry Analyzer and reagent discs
 - **General Chemistry 12** contains the following test methods: albumin, ALP, ALT, amylase, AST, t-Bili, BUN, calcium, total cholesterol, creatinine, glucose, & total protein
 - **MetLyte 8** contains: Na⁺, K⁺, Cl⁻, tCO₂, BUN, creatinine, glucose, & CK
 - **Comprehensive Metabolic Rotor** contains: Na⁺, K⁺, Cl⁻, tCO₂, albumin, ALP, ALT, AST, t-Bili, BUN, calcium, creatinine, glucose, & total protein
- Piccolo discs specifically manufactured to give a high incidence of “short samples”
- Diluted whole blood and serum samples

Procedure

- We established the “frequency distribution” of analytes for a large population hospital-based patient samples that encompassed a large spectrum of test values, and calculated the probability of finding a particular value.
- We examined the statistical characteristics of a panel of analytes and defined a mathematical function that will maximize the sensitivity of the algorithm to the presence of short samples.
- We assayed a large number of samples where the same test panel is measured and determine the cumulative distribution of the probability of a short sample.
- The cumulative distribution of a particular panel allows selection of the probability value to be used as the threshold for the flagging of results from short samples. By statistical analysis the number of false positives or false negatives can be determined.
- The measurement results from any sample can be used to simulate the effect of short samples on the probability by simply diluting that sample appropriately. By testing dilutions of samples with a significant range of analyte values, the decision threshold for flagging the results for any acceptable level of dilution can be fine-tuned.

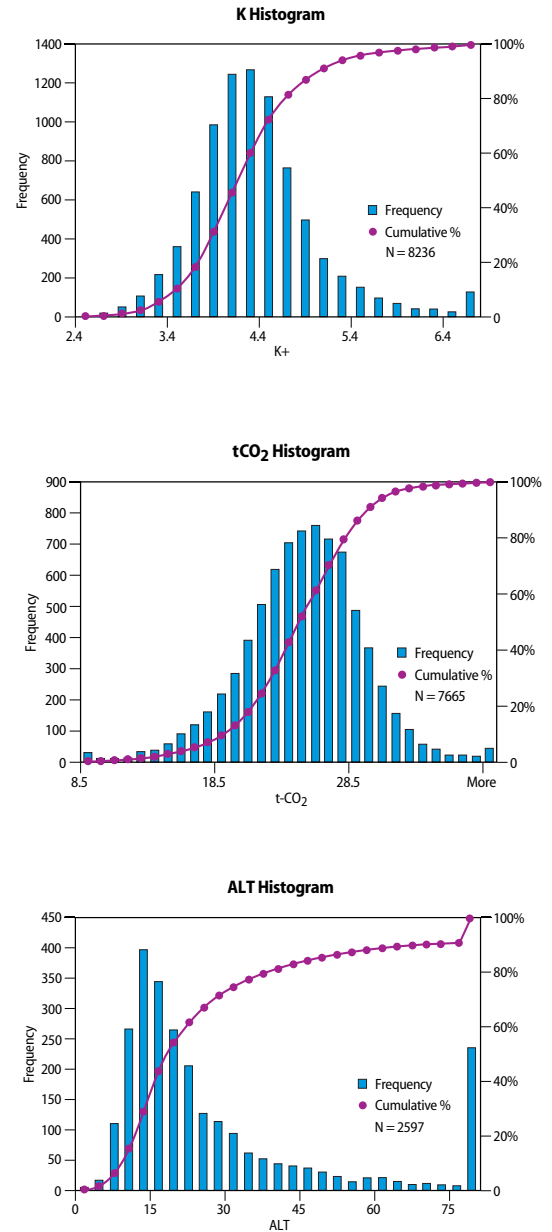
Results

Figure 1. Representative Frequency Distributions



Results (cont.)

Figure 1. Representative Frequency Distributions (cont.)



Results (cont.)

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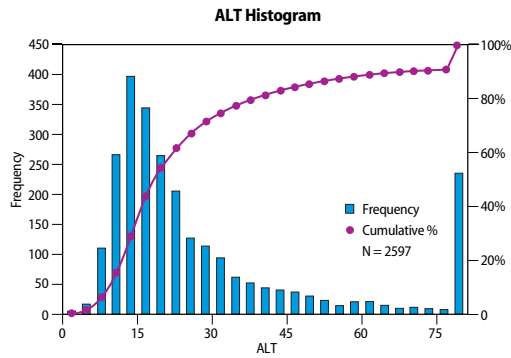


Figure 2. Short Sample Model–General Chemistry (12 Measurements)

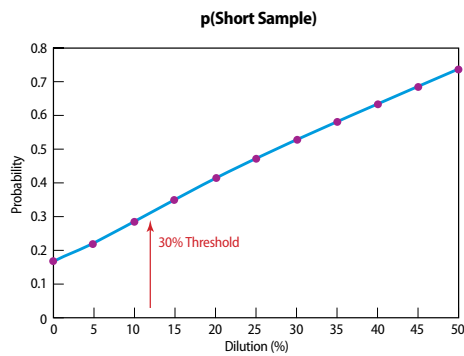
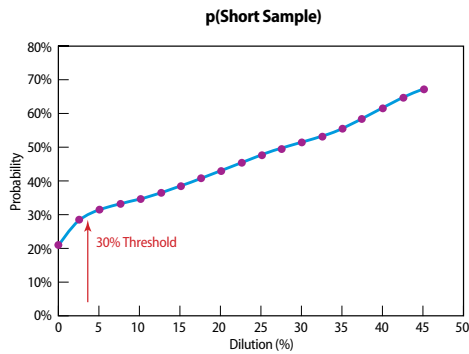


Figure 3. Short Sample Model–MetLyte 8 (8 Measurements)



Results (cont.)

Table 2. Testing the Model Using a Lot of Rotors Manufactured Specifically to Provide Short Samples – Whole Blood & Serum

MetLyte 8 [using GLU, BUN, Na⁺, K⁺, Cl⁻, & tCO₂ = 6 measurements for p(short sample) calculation]

1 Sample Tested n = 10 as Whole Blood & Serum

	GLU mg/dL	BUN mg/dL	CRE mg/dL	CK U/L	Na ⁺ mmol/L	K ⁺ mmol/L	Cl ⁻ mmol/L	tCO ₂ mmol/L	Dilution vs Avg Glucose	Threshold = 50% p(short sample)
WB	132	8	1.2	61	133	3.7	106	24	0.9	26%
WB	133	8	1.0	67	133	4.1	106	24	0.2	20%
WB	41	3	0.5	22	66	0.15	25	8	69.2	98%
WB	133	8	1.0	58	138	3.6	103	24	0.2	18%
WB	134	8	0.9	64	139	3.3	103	27	-0.6	22%
WB	127	8	0.8	62	130	3.2	97	23	4.7	52%
WB	133	8	1.0	59	138	3.7	105	25	0.2	14%
WB	133	9	0.8	65	139	3.7	105	25	0.2	11%
WB	133	8	1.1	64	140	3.5	104	24	0.2	19%
WB	135	9	1.2	61	136	3.8	105	24	-1.3	15%
Average without Short										
Sample	133	8	1.0	62	137	3.7	105	25		
Serum										
Serum	83	5	0.9	36	91	1.8	63	17	36.9	83%
Serum	131	8	0.8	64	131	3.6	106	24	0.5	31%
Serum	132	8	1.0	68	135	3.8	107	23	-0.3	21%
Serum	132	9	1.1	68	135	3.7	105	24	-0.3	19%
Serum	133	9	1.3	56	140	3.9	104	24	-1.0	9%
Serum	131	9	1.2	67	137	3.7	107	26	0.5	13%
Serum	132	9	1.0	63	138	3.7	104	26	-0.3	12%
Serum	130	9	1.0	63	135	3.8	105	24	1.2	17%
Serum	132	9	1.2	63	134	3.8	106	24	-0.3	20%
Serum	117	8	1.1	56	118	3.4	94	23	11.1	53%
Average without Short										
Sample	132	9	1.1	64	136	3.8	106	24		

Conclusions

- The probability of a patient sample having a particular analyte value was established using a large population of patient values.
- Using the probably as determined by analyte distribution statistics, a function has been derived that can describe the probability of a sample in a panel of tests to have a particular set of results. This function is:

$$p(\text{short sample}) = [N - \{p(A) + p(B) + \dots + p(X)\}] / N$$

p = probability

A, B, ... X = analyte measurements from A – X

N = number of analytes measured

- Analytes with narrow distributions are more useful to use in the prediction of short samples.
- Samples that are short by only 5% of their actual volume can be detected by this method. The sensitivity depends on factors such as the test panel that is being run and the analyte levels used in the calculations.
- Liver enzymes (such as AST, ALT, and ALP) and CK are not very helpful since they may have very elevated values—very skewed distributions toward high levels.
- At a p(short sample) threshold of 50%, no false positives (true results flagged as short samples) have been detected during testing of several hundred clinical specimens.
- These calculations may be extended to automated analyzers where test panels are being performed.
- Though not shown here, this same analysis can be used to detect “long samples.” Long samples would be the inaccurate results that can occur in assays due to addition of excess sample to the reaction mixture, or improper dilution of sample.