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Evaluation of a Portable Clinical Analyzer in Cynomolgus Macaques (*Macaca fasicularis*)

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The aim of this study was to evaluate the clinical performance of a portable analyzer for use in cynomolgus monkeys (*Macaca fasicularis*). During semiannual health screening, blood samples from 23 animals were analyzed by both the portable clinical analyzer and the institutional comparative pathology laboratory. Portable clinical analyzers have been evaluated for use in other species, but the suitability for macaques has not yet been determined. Results for glucose, urea (BUN), sodium, potassium, chloride, hematocrit, hemoglobin, and total CO_2 were compared by overall *t* test, paired *t* test, and Pearson correlation. Only glucose and BUN did not differ in the overall *t* test between methods. Only potassium values did not differ in the paired *t* test. Compared with those from the portable analyzer, laboratory values were lower for glucose, hematocrit, hemoglobin, and total CO_2 and higher for electrolytes and BUN. All values were within normal ranges for cynomolgus macaques which, in this study, were all apparently healthy, physiologically normal animals. We attributed differences between methods to sample type and handling and the physiologic changes in blood after collection. These results indicate that direct comparison of values obtained through different methods may not be valid, and normal ranges for point-of-care devices should be developed for each species.

Abbreviations: BUN, blood urea nitrogen; EDTA, ethylenediamine tetraacetic acid; HCT, hematocrit; HGB, hemoglobin

Portable clinical analyzers are commonly used in veterinary field and emergency medicine as well as in human surgery and pediatrics. The use of portable analyzers for critical point-of-care decisions in the field, and the subsequent submission of blood to a reference facility or commercial laboratory for follow-up requires the identification of any differences in values between the 2 methods. We compared results obtained for 7 commonly evaluated parameters from the blood of cynomolgus macaques (*Macaca fasicularis*) by use of a portable clinical analyzer with those from the in-house laboratory. Paired and unpaired values of glucose, blood urea nitrogen (BUN), sodium, potassium, chloride, total carbon dioxide (TCO_2), hematocrit (HCT), and hemoglobin (HGB) were compared because these were the tests used most commonly by both the technicians in the field and the University of Miami Comparative Pathology Laboratory, where routine and follow-up blood and chemistry analyses are done. We also compared results from both methods with published values for healthy macaques.

Portable clinical analyzers can provide valid results for a variety of human and animal medical applications. Physicians have used the portable analyzer in battlefield, helicopter, critical care, and emergency medicine.[2,3,5,7,8,14,16,17,19] Point-of-care analyzers have been used intraoperatively and in pediatric neonatal intensive care units because the blood volume required is only 65 µl.[14,15] Veterinary medical practitioners have used portable clinical analyzers in clinical, laboratory, and wildlife medicine.[11-13,21-23]

Many studies have compared results from portable, point-of-care analyzers with standard laboratory values in humans and other animals, with variable results. One study in a human critical care hospital led to cessation of portable clinical analyzer use, due to the large difference in blood gas values between portable clinical analyzer and laboratory results, whereas other studies found complete agreement in blood gases values between portable analyzer and laboratory results.[4,9,16,17,20] Similarly, some studies done in human laboratories found conflicting results when comparing blood chemistries and electrolytes between portable analyzers and conventional laboratories, while other studies did not.[3,9,10,15,17,18,20]

Various animal studies have examined the consistency between laboratory and portable clinical analyzer values, and the literature contains conflicting results. A recent study done with mouse blood showed that differences between portable clinical analyzer and laboratory values were statistically significant and that, for certain chemistries, the variability associated with the portable clinical analyzer was actually less than that for with laboratory methods.[22] The same study[22] also found that the variability of hematology values obtained with the portable analyzer actually made it unreliable for clinical use as compared with laboratory methods. The current study examines these questions in cynomolgus macaques.

Materials and Methods

Caged cynomolgus macaques were examined, and venous blood was drawn from 20 adult male and 3 adult female single-housed animals. All animals were on protocols that were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Miami, where the Division of Veterinary Resources is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. None of the animals was receiving any experimental or therapeutic chemical intervention. All animals were fed a standard monkey biscuit diet and lived under a 12:12-h light:dark cycle at 22 to 24 °C and 50% to 70% humidity. The animals ranged from 3 to 8 y of age and were free of antibodies to simian immunodeficiency virus, simian T lymphotropic virus, simian retroviruses, and *Cercopethicine herpesvirus 1* at the time of phlebotomy.

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Table 1. Summary of parameter means and ranges

Parameter	N	Method	Mean ± 1 standard deviation	Published mean	Range	Published range	Difference between methods (mean ± standard error)
Glucose, mg/dl	19	Analyzer	64 ± 8.9	63	51–87	42–111	4.6 ± 1.7
	23	Lab	59 ± 11.3		46–92		
BUN, mg/dl	18	Analyzer	16 ± 4.3	13	9–25	5–25	–0.8 ± 0.5
	23	Lab	17 ± 3.9		10–26		
Sodium, mmol/l	16	Analyzer	147 ± 1.86	147	142–149	135–154	–1.9 ± 0.9
	23	Lab	149 ± 2.4		144–153		
Potassium, mmol/l	16	Analyzer	3.3 ± 0.3	4.3	2.9–4.1	3.4–6.3	–0.2 ± 0.1
	23	Lab	3.6 ± 0.2		3.3–4.0		
Chloride, mmol/l	18	Analyzer	111 ± 3.0	107	107–118	97–113	–6.9 ± 0.8
	23	Lab	117 ± 2.4		113–121		
HCT, g/dl	18	Analyzer	42 ± 3.4	35.9	36–47	24–41[a]	5.6 ± 1.1
	23	Lab	36 ± 3.5		29–49		
HGB, g/dl	18	Analyzer	14.3 ± 1.1	11.7[a]	12.2–16.0	9.6–13.3[a]	2.5 ± 0.2
	23	Lab	11.7 ± 0.8		9.6–13.2		
TCO_2, mmol/l	19	Analyzer	26 ± 2.7	Not applicable	22–33	Not applicable	1.8 ± 2.6
	23	Lab	25 ± 2.4		20–28		

[a]Mean value for male animals is used for comparison.

The fasted macaques were sedated in their home cages with 15 mg/kg ketamine HCl (Ketaject, Phoenix Pharmaceuticals, St Joseph, MO) injected intramuscularly. Blood was obtained from the saphenous vein by use of a 25-gauge needle and 1-ml syringe (Monoject, Tyco Healthcare Group, Mansfield, MA) for immediate placement in the cartridge of the portable clinical analyzer (i-STAT, Abbott Point of Care, Abbott Laboratories, Abbott Park, IL) with no addition of heparin, to prevent dilution effect and changes in blood values secondary to exposure time outside of the cartridge.

For submission to the in-house laboratory, blood was obtained from the femoral or saphenous vein by use of a 22-gauge vacuum phlebotomy needle (Vacutainer Systems, Becton Dickinson, Franklin Lakes, NJ) and 10-ml serum-separator and 3-ml anticoagulant (ethylenediamine tetraacetic acid [EDTA]) vacuum phlebotomy tubes (Becton Dickinson). Clotted blood was spun at 3100 × *g* for 15 min. The serum was poured into a separate vial and sent to the laboratory in a chilled cooler. The EDTA tubes were immediately refrigerated and then placed in a chilled cooler for transport to the laboratory. All blood samples were analyzed within 4 h of phlebotomy.

The portable clinical analyzer we evaluated is maintained by installation of regular software updates. The machine is calibrated each time it is used, and the printer is kept loaded with paper. Only in-date cartridges (EC8+, Abbot Point of Care) were used for these analyses; these cartridges functioned as previously described (Larsen and colleagues).[1] Chemistry analyses in the Comparative Pathology Laboratory were run on a dry-slide analyzer (model 250, Ortho Vitros, Rochester, NY). Manual hematocrit determinations were made by drawing a small amount of sample from the EDTA tube into a Drummond plain 75-µl tube (VWR, Westchester, PA) and centrifuging it (Autocrit Ulta 3, Becton Dickinson Diagnostics, Franklin Lakes, NJ). The laboratory equipment is maintained and calibrated daily to accommodate commercial use.

Statistical analyses. All statistical analyses were done using SAS statistical software (version 9.1; SAS Institute, Cary, NC) on a personal computer (Dell, Round Rock, Texas). Tests for normality were addressed by use of the Shapiro-Wilkes test and comparison of mean, median, and mode. The mean, standard deviation, mean differences, standard deviation of the mean differences and ranges were determined for each analysis and method. A *t* test for difference in means between methods was done to compare overall range of values, as done in a recent study using mice.[22] Paired *t* tests for dependent samples addressed the mean differences and bias in the results of individual blood samples between methods. The threshold of significance was set for *t* values at $P = 0.05$. Ranges in values from the portable clinical analyzer and in-house laboratory were compared with published values for cynomolgus macaques.[6] Pearson correlations were calculated between portable clinical analyzer and laboratory values as another means to determine agreement between values. There were fewer paired samples than overall samples because some parameters were not read by using the portable clinical analyzer.

Results

Data regarding all parameters and both methods are summarized in Tables 1 and 2. Distributions for each parameter by each method were normal by having either a Shapiro-Wilkes constant of 0.85 or greater or agreement between the mean, median, and mode or both. Outliers in glucose and HCT measurements by laboratory methods and glucose, BUN, and potassium by the portable clinical analyzer skewed these distributions. The small sample size also contributed to skewing of the distributions. Total CO_2 values were not compared with species-specific published values. Unpaired *t* tests between methods showed no difference between means for only glucose ($t = 1.71$, $P = 0.095$) and BUN ($t = –0.94$, $P = 0.35$). Paired *t* tests showed no difference in sample values for only potassium ($t = –2.09$, $P = 0.054$) and BUN ($t = –1.44$, $P = 0.167$), whereas paired samples did not agree for glucose, sodium, chloride, HCT, HGB, and TCO_2 according to a significance level of $P = 0.05$ (Table 2).

Laboratory values tended to be lower for glucose, HCT, and TCO_2 and higher for BUN, sodium, potassium, and chloride.

Table 2. Summary of correlations and *t* tests

Parameter	Method	Standard deviation	Correlation between methods	Unpaired *t* value	No. of pairs	Paired *t* value
Glucose, mg/dl	Analyzer	8.9	$r = 0.80$ $P < 0.001$[a]	1.71 $P = 0.095$	19	2.7 $P = 0.015$
	Lab	11.3				
BUN, mg/dl	Analyzer	4.3	$r = 0.85$ $P < 0.0001$[a]	–0.94 $P = 0.35$	18	–1.44 $P = 0.167$
	Lab	3.9				
Sodium, mmol/l	Analyzer	1.9	$r = –0.09$ $P = 0.74$	–2.72 $P = 0.013$[a]	16	–2.23 $P = 0.041$[a]
	Lab	2.4				
Potassium, mmol/l	Analyzer	0.34	$r = 0.45$ $P = 0.08$	–2.91 $P = 0.008$[a]	16	–2.09 $P = 0.054$
	Lab	0.19				
Chloride, mmol/l	Analyzer	3.0	$r = 0.32$ $P = 0.19$	–7.94 $P < 0.0001$[a]	18	–9.18 $P < 0.0001$[a]
	Lab	2.4				
HCT, g/dl	Analyzer	3.4	$r = 0.09$ $P = 0.72$	5.22 $P < 0.0001$[a]	18	4.92 $P = 0.0001$[a]
	Lab	3.5				
HGB, g/dl	Analyzer	1.15	$r = 0.49$ $P = 0.04$[a]	8.42 $P < 0.0001$[a]	18	10.17 $P < 0.0001$[a]
	Lab	0.81				
TCO_2, mmol/l	Analyzer	2.7	$r = 0.67$ $P = 0.002$[a]	2.28 $P = 0.028$[a]	19	4.22 $P = 0.0005$[a]
	Lab	2.4				

[a]$P \leq 0.05$.

Standard deviations for means by method tended to be higher for the portable clinical analyzer as compared with laboratory values. When portable clinical analyzer and laboratory ranges were compared with reference ranges, the portable clinical analyzer range fell slightly low for sodium and potassium and slightly high for HCT (Table 1). All other laboratory and portable clinical analyzer values and ranges were consistent with known, clinically relevant values in cynomolgus macaques. Total CO_2 values were consistent with physiologic normal values found across species but did not agree between methods. Significant correlations between methods were not found for either BUN or glucose. Other significant correlations were found. Some of these correlations were low and were thought to be spurious and possibly due to outliers that also skewed the distributions of these parameters as well as to the small sample size (Table 2).

Discussion

Some of the differences in values seen in this study are consistent with previous findings of comparisons between portable analyzer and laboratory results that were done to confirm the usefulness of portable analyzer to assess the health status of animals and people. In the current study and in previous studies of elephant seals,[12] at a veterinary school clinic,[11] and in laboratory mice,[22] the BUN values were consistent with published reference values and differed only slightly between portable clinical analyzer and laboratory methods. One comparative study involving human samples showed a coefficient of variation of 9.4% for BUN; that difference was acceptable for the authors' purposes.[15] In the current study, BUN did not differ in either the overall or paired *t* tests, and BUN was the only parameter for which agreement by *t* test and a significant positive correlation were present. Therefore, in clinical cases, BUN data from both methods can be compared to follow the status of a patient.

Similarly, sodium values in this study were also lower by the portable clinical analyzer method than by the in-house laboratory, and by neither paired nor unpaired *t* tests was there agreement between methods. The cause may be secondary to electrolytes leaking from cells during clotting centrifugation in the laboratory samples, as noted in other similar comparative analyses.[12,22] The mean difference between methods was less than 2.0 mg/dl, so although the difference is statistically significant between methods, it is unlikely that a clinical treatment plan would be altered based on this difference. Because the difference is apparently consistent, adjustment can be made when evaluating results from one method versus another.

As in the study by Larsen and colleagues,[12] the present comparison also found that potassium values were lower in samples evaluated by the portable clinical analyzer. This finding is in contrast to comparative studies in mice and humans, for which the level of agreement was found to be acceptable.[7,15,17,22] Although the absolute numbers show that the potassium values were less and the correlation between methods was insignificant and low ($r = 0.45$, $P = 0.08$) in the present study, the paired *t* test did not show a significant difference ($t = –2.09$, $P = 0.054$) between methods. However, the overall unpaired *t* test did show a significant difference. The small difference that was present again may be due to the leaking of electrolyte from platelets during coagulation and from red blood cells both before and during centrifugation. The magnitude of the difference in potassium levels between methods is low enough that decisions in clinical treatment likely would not be affected (Table 1).

In the current study, chloride concentrations by both methods were within reference ranges but did not agree between methods by overall or paired *t* test. The difference was quite large and larger than that reported in other studies, where agreement between methods was found.[11,15,17,18] Laboratory values were significantly higher than portable clinical analyzer results, which is inconsistent with previous reports of chloride analysis by portable analyzers in other studies, where differences were attributed to the effects of sample type or protein binding on available chloride ion levels in the portable analyzer analysis.[12,22] The opposite effect was found in the current study. Perhaps, as with other ions, there is leaking from blood cells both before and during centrifugation for laboratory serum samples, causing the laboratory values to be higher in some samples. Rarely would the course of clinical treatment be based on chloride levels, so this difference alone probably would not alter the course of clinical treatment.

HCT and HGB values were within reference ranges for cynomolgus monkeys.[6] In this study, the portable clinical analyzer showed consistently higher values than did laboratory methods, and differences were significantly different by both overall and paired *t* tests (Table 2). As expected, HCT values did vary consistently with HGB values. Although not unexpected, the mean differences in values between methods were quite large (HCT, 5.6 ± 3.4 g/dl; HGB, 2.5 ± 0.2), and we thought them to be secondary to dilution in EDTA for the laboratory sample and to red blood cell loss during centrifugation. The portable clinical analyzer uses an automated cartridge and electrodes for immediate analysis, whereas the laboratory uses a simple technique of centrifugation and measurement. The results were consistent with studies done at a veterinary hospital[11] and in laboratory mice,[22] where differences again were considered to be secondary to sample type. In human studies, both complete agreement and no agreement have been found between portable clinical analyzer and laboratory values for HCT and HGB.[7,9,15,17] Differences in HCT were large enough to potentially affect a clinical treatment plan (Table 1). However, if a consistent difference could be established, then a factor could be applied that would allow suitable comparison with laboratory values when indicated.

In this study glucose values were significantly higher in portable clinical analyzer measurements than those from the laboratory. This pattern is consistent with what is seen in other species and humans and is a well-known phenomenon. Blood cells will use and metabolize glucose after a sample is taken, therefore effectively lowering blood glucose concentrations, especially in heparinized or EDTA-containing samples.[12,24,25] The longer a sample sits before processing, the lower the glucose concentration will be. Arguably then, the portable clinical analyzer may deliver a more realistic glucose value, because it is more representative of the immediate physiologic state of the animal and not a function of the length of time a sample has been sitting.

We did not compare TCO_2 values against reference values, because TCO_2 on a chemistry panel is a physiologic indicator of bicarbonate status and is consistent among mammals.[26] An elevated TCO_2 is an indication that blood gases should be considered in a diagnostic workup, and low values (less than 12 mEq/l) indicate noteworthy metabolic acidosis. Although the mean difference between the portable clinical analyzer and laboratory values in this study (1.8 ± 2.6 mmol/l) was low enough not to affect course of clinical treatment, the difference was significant ($P = 0.028$). This result may in part be due to the relatively low standard deviations for the values of both methods (2.7 and 2.4, Table 1). The portable clinical analyzer values tended to be higher, which may be due to diffusion of CO_2 from laboratory samples that are not immediately analyzed.[26] Interestingly, other studies in the literature addressed blood gases, but no study specifically evaluated TCO_2 as a chemistry analyte. TCO_2 behaves like both a blood gas and a serum chemistry analyte because of the biochemical nature of the molecule ($H + HCO_3 \Leftarrow H_2O + CO_2$ via carbonic anhydrase). Several studies involving emergency and critical care medicine have examined the differences between blood gas levels from the portable clinical analyzer and conventional laboratories. Studies done in dogs and exercising horses found blood gas values from the portable clinical analyzer to be valid and consistent with normal values.[21,23] Several studies in humans also found that the portable clinical analyzer is comparable to standard laboratory testing.[4,17,20] Other analyses in human medicine have found the portable clinical analyzer results to be so inconsistent that they are discounted completely.[9,16] It follows that values would be more consistent between methods because in both the portable clinical analyzer and the clinical laboratory, whole blood samples are processed immediately. So if there is a difference, it may be due to instrument calibration or operator error.

In conclusion, the portable, point-of-care analyzer we evaluated is a very useful tool for immediate monitoring of macaques and other animals in a field environment, provided that clinicians are aware of differences from laboratory values that may be due to the physiology of blood, serum, and cells and the sample type. Blood that does not clot or sit for a period of time and is processed immediately is likely to have lower values of electrolytes because they have not had sufficient time to leak from cells. Conversely, blood gas values from portable analyzers likely may be higher or in close agreement with those from the laboratory, because there should be limited opportunity for gases to diffuse from the sample. It is also important to recognize that placing blood in an EDTA tube may dilute the sample to a certain degree and that centrifugation may contribute to the loss of blood cells in the sample. Finally, blood that sits for any length of time is likely to have notably decreased glucose levels, so it is pertinent to follow blood-processing guidelines before submission of samples to a laboratory. For accurate use of portable clinical analyzers, normal ranges should be determined for each species at each clinic or institution. Paired samples from apparently healthy, clinically normal animals could be analyzed in the portable analyzer and also at a commercial laboratory. Then normal values and ranges could be generated for both modalities and compared to see how sample values vary with respect to method. Then comparisons could be made with follow-up results obtained from the laboratory with those done under field or emergency conditions with a portable clinical analyzer, by applying calculated adjustment factors. Finally, it is crucial to recognize that although differences between methods exist, the clinical relevance of those differences may or may not affect the clinical course of treatment.

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