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TECHNICAL REPORT

# Performance of a manually operated salad spinner centrifuge for serum separation in the healthy domestic horse (*Equus caballus*) and southern white rhinoceros (*Ceratotherium simum*)

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## Abstract

**Background:** Field veterinarians and researchers studying wild species, such as the southern white rhinoceros, often work in remote areas with limited access to standard laboratory equipment, hindering the ability to measure serum analytes.

**Objectives:** The first objective was to produce an inexpensive, manually operated centrifuge that could accept standard laboratory tubes by modifying a consumer-grade salad spinner with low-cost materials. The second objective was to compare biochemistry analysis results obtained from equine and southern white rhinoceros serum separated by traditional laboratory and manual salad spinner centrifugation.

**Methods:** We optimized the design and serum separation protocol using non-anticoagulated equine blood. Equine and rhinoceros serum samples were separated by manual salad spinner or traditional laboratory centrifugation. Measured analytes included sodium, potassium, chloride, urea nitrogen, creatinine, phosphorous, total calcium, magnesium, glucose, total protein, albumin, globulin, creatinine kinase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, bicarbonate, sorbitol dehydrogenase, and triglycerides. Results obtained from serum separated by each centrifugation technique were compared by Deming regression and Bland–Altman analyses.

**Results:** A tube adaptor insert modeled after a swing angle rotor and a two-step salad spinner centrifugation yielded serum comparable to traditional laboratory centrifugation. For the majority of analytes, no proportional or constant biases were detected between centrifugation methods. A positive proportional bias in the measurement of ALP in serum separated by manual centrifugation was detected in both equine and rhinoceros samples.

**Conclusions:** Manual centrifugation with a modified salad spinner yields diagnostic quality serum suitable for the measurement of most standard biochemistry analytes.

KEYWORDS
blood, clinical chemistry, field medicine, frugal science

## 1 | INTRODUCTION

Serum is the preferred sample for biochemistry analysis, which requires centrifugation of whole blood shortly after collection. Delayed or inadequate serum separation results in artifacts due to continued cellular metabolism (eg, hypoglycemia) and/or hemolysis. Hemolysis releases free hemoglobin that causes chromatic interferences in the measurement of multiple analytes and also releases cellular constituents that, depending on species, can change measured serum analytes, including potassium, lactate dehydrogenase, and others.[1–3]

Centrifugation protocols for serum separation vary by laboratory but are typically 1000–2500*g* for 10–15 minutes, although there is a paucity of studies investigating minimum requirements.[4,5] The laboratory equipment and steady electrical power supply required to obtain these forces are often unavailable in remote field settings, such as those encountered when studying or monitoring wild animal populations. Potential field alternatives include manually operated centrifuges (eg, Hettich; Tuttlingen, Germany), which can reach speeds up to 1298*g*; however, these require a sturdy surface (eg, bench, table, vehicle) to clamp onto, which is not available in all field situations. The Dremelfuge uses a high-speed battery-operated rotary tool (eg, Dremel) and fabricated rotor attachment that can accept microcentrifuge tubes.[6] Alternatively, battery-operated point-of-care analyzers that measure analytes in whole blood rather than serum have been used for biochemical testing in field settings; however, each machine must be validated for each species, which may not be practical.[7–14] Disadvantages of battery-operated devices include machine weight and bulk, which may preclude use in remote areas inaccessible to motorized vehicles, as well as the need for functional batteries. Temperature requirements for storage of reagent cartridges or rotors (2–8°C) and instrument operation (~15–30°C) may further limit their use in field conditions.[7,15,16] Lastly, the variety of analytes that portable instruments can measure is more restricted than what can be measured in serum at a diagnostic or research laboratory (eg, hormones, acute phase proteins, biomarkers).[7,8,10–14]

To increase accessibility to diagnostic medical testing in resource-poor conditions, scientists and engineers in the area of "frugal science" are developing inexpensive alternatives to traditional laboratory equipment. One approach has been to modify simple machines and toys, including salad spinners, fidget spinners, and egg beaters, to centrifuge low-volume samples, such as blood in microhematocrit tubes, to measure PCV.[6,17–20] To our knowledge, none of these simple, inexpensive, manually operated centrifuges have been investigated for centrifugation of volumes greater than 200 µL, nor have studies investigated whether manual centrifugation is sufficient for separation of diagnostic-quality serum suitable for biochemical analysis.

The southern white rhinoceros [*Ceratotherium simum*] is near-threatened, and other rhinoceros species (Black [*Diceros bicornis*], Javan [*Rhinoceros sondaicus*], Sumatran [*Dicerorhinus sumatrensis*]) are considered vulnerable or critically endangered.[21] There is an urgent need to protect the remaining wild individuals, which are often located in remote areas. In addition to physical and behavioral monitoring, the measurement of analytes in blood, urine, and feces provides crucial information regarding hematologic status, organ function, metabolism, nutritional status, presence of inflammation, and hormonal status.[22–31] Unfortunately, although we have hematologic data from both human-managed and wild rhinoceros species, there is a paucity of biochemistry data from wild rhinoceros populations, at least partially due to lack of access to traditional laboratory equipment in remote field settings.[9,13,24,25,32]

The objective of the study was to develop an inexpensive manual centrifugation method capable of separating serum that could be used for future field applications. We modified a consumer-grade salad spinner using inexpensive and readily accessible materials to securely hold standard laboratory tubes. We then determined whether serum separated by manual salad spinner centrifugation would yield biochemical results comparable to those obtained in serum separated by conventional laboratory centrifugation. Given the logistical considerations of performing these studies in wild populations, we elected to first conduct proof-of-principal studies in two human-managed species: the domestic horse, a readily accessible "model" equid, and the southern white rhinoceros.

## 2 | MATERIALS AND METHODS

Studies were conducted between October 2019 and March 2021. Signed owner consent for the use of discarded equine samples was obtained upon admission to the NCSU-VTH. Studies using southern white rhinoceros blood were approved by the North Carolina Zoo research committee and North Carolina State University Veterinary Institutional Animal Care and Use Committee (21-129-O).

### 2.1 | Animals

#### 2.1.1 | Horses

The study population was a convenience sample of 10 client-owned anesthetized domesticated horses undergoing planned procedures at North Carolina State University Veterinary Teaching Hospital (NCSU-VTH). Blood from three horses was used for the initial optimization of the technique. The final study population was seven horses. For each horse, 3 mL of anticoagulant-free whole blood was collected via an 18–22-gauge arterial catheter placed in the metatarsal, metacarpal, facial, or transverse facial artery for routine monitoring of blood gases. Leftover blood that would otherwise have been discarded was used for the study. Samples were excluded if there was insufficient volume or gross hemolysis. Horses were included regardless of underlying health status or the presence of laboratory abnormalities. Each sample was divided into two 2.7 mL tubes (S-Monovette, Clotting Activator/Serum, 66 × 11 mm tubes) and allowed to coagulate fully for 20–30 minutes prior to centrifugation.

### 2.1.2 | Rhinoceroses

The study population included seven human-managed female southern white rhinoceroses housed at the North Carolina Zoo in Asheboro, NC. All animals remained in their zoo enclosure for the duration of the study and were considered healthy based on habitus, physical exam, and normal CBC and biochemistry profile within the last year. Animals are trained for voluntary blood collection using positive reinforcement. A 20-gauge butterfly catheter placed in the right medial radial vein was used to collect 6–10 mL of blood into anticoagulant-free tubes (BD Vacutainer SST Tubes, Franklin Lakes, New Jersey). Exclusion criteria included insufficient volume or gross evidence of hemolysis. Each sample was divided into two aliquots (S-Monovette 2.7 mL, Clotting Activator/Serum, 66 × 11 mm tubes) and allowed to coagulate fully for 20–30 minutes prior to centrifugation.

## 2.2 | Manual and reference centrifugation

Manual centrifugation was performed using a consumer-grade 4.7-quart salad spinner (Andcolors), which consists of an outer bucket, an inner basket, and a lid that secures to the outer bucket (945 *g* total). Each turn of the hand crank attached to the lid rotates the basket five times. Modifications were designed to accept S-Monovette 2.7 mL, Clotting Activator/Serum, 66 × 11 mm tubes (SARSTEDT AG & Co. KG Sarstedtstraße 151 588, Nümbrecht, Germany) or 1.7 mL microcentrifuge tubes (Axygen Scientific, Union City, CA, USA). Several designs were tested and rejected during pilot testing. Rejected designs included (a) simple attachment of tubes to the outer basket with wire, which resulted in poor cell pellet formation, and (b) fabrication of a tube adaptor from modeling clay (Sargent Art, Hazleton, PA, USA), which was too heavy, resulting in operator fatigue and inadequate serum separation. The final successful design was to fabricate a lightweight angled tube attachment insert using egg carton material (StyrofoamTM) mounted on a 50 mm × 70 mm × 100 mm craft foam base (FloraCraft, Ludington, MI, USA). Holes large enough to accept the tubes were made in each compartment of a 2 × 2 egg carton. The bottoms of the tubes were not secured, similar to the tube configuration in a swing-out rotor. To increase the structural stability of the egg carton, two layers of newspaper and glue (Elmer's®, Westerville, OH, USA) were applied by papier-mâché technique. The egg carton was attached to the foam base (28 *g* total), which was attached to the base of the basket with 1/2″ laboratory tape (VWR Scientific). Photographs of the final design and line drawings of the components prepared using Manifold 8.0 software (Central, Hong Kong Island, Hong Kong) are in Figure 1.

We determined the maximum revolutions per minute (rpm) of the basket by two methods. First, we manually counted the average maximum rpm of the hand crank that could be achieved before the basket began to disengage from the hand crank or the salad spinner began to wobble. The hand crank rpm was then multiplied by the hand crank: basket ratio (1:5) to calculate the basket rpm. The manually calculated basket rpm was confirmed using a wireless cyclometer (CatEye Padrone, Boulder, CO, USA) with the complementary magnetic detector components affixed to the stationary lid and rotating basket. Relative centrifugal force (RCF) was calculated using the formula: $RCF = 1.118 \times 10^{-5} r_{cm} rpm^2$ where $r_{cm}$ was the radius from the center of the rotor to the bottom of the tube (4.75 cm), and rpm was the basket rpm.

The initial salad spinner centrifugation protocol was to centrifuge blood aliquoted into 2.7 mL tubes at maximum speed. The reference centrifugation protocol was to centrifuge blood aliquoted into a 2.7 mL tube from each animal for 10 min at 4000*g*. Equine samples were centrifuged in an Eppendorf 5810R centrifuge (Framingham, MA), while rhinoceros samples were centrifuged in a ThermoScientific Heraeus Clinifuge Centrifuge (Waltham, MA, USA).

Initial assessment of the adequacy of serum separation was first performed by gross examination. Separation was considered to be acceptable if there was a defined cell pellet and the serum supernatant was transparent and pale yellow. We next assessed for microscopic erythrocyte contamination by microscopic evaluation of a Diff-Quik-stained line smear prepared from the serum. Finally, we measured serum hemoglobin (Hgb) concentration on the ADVIA 120 hematology analyzer (Siemens Medical Solutions, Malvern, PA, USA).[4]

After centrifugation by salad spinner or reference centrifuge, serum was transferred to a new tube for analysis. Equine samples were analyzed within 30 minutes of separation. Rhinoceros serum samples were stored in a cooler with ice packs (approximately 5–8°C) for 2 hours during transport to the laboratory.

## 2.3 | Biochemistry analysis

Biochemistry analysis was conducted by the North Carolina State University Veterinary Teaching Hospital (NCSU-VTH) Clinical Pathology Laboratory on a Roche Cobas c501 Chemistry Analyzer (Roche, Switzerland). The full biochemical profile includes interference indices (hemolysis, icteric, lipemia), electrolytes (Na+, K+, Cl-), blood urea nitrogen (BUN), creatinine (Crea), phosphorous (P), total calcium (Ca), magnesium (Mg), glucose (Glu), total protein (TP), albumin (Alb), globulin (Glob), creatinine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin, and bicarbonate. Sorbitol dehydrogenase (SDH) and triglycerides (Trig) were measured in rhinoceros samples only.

## 2.4 | Statistical analysis

Data were analyzed by Deming regression analysis for the presence of constant and/or proportional bias, using y-intercept and slope values, respectively. Data were analyzed by Bland–Altman plots and analysis, with a 95% confidence interval of agreement. Analyses and

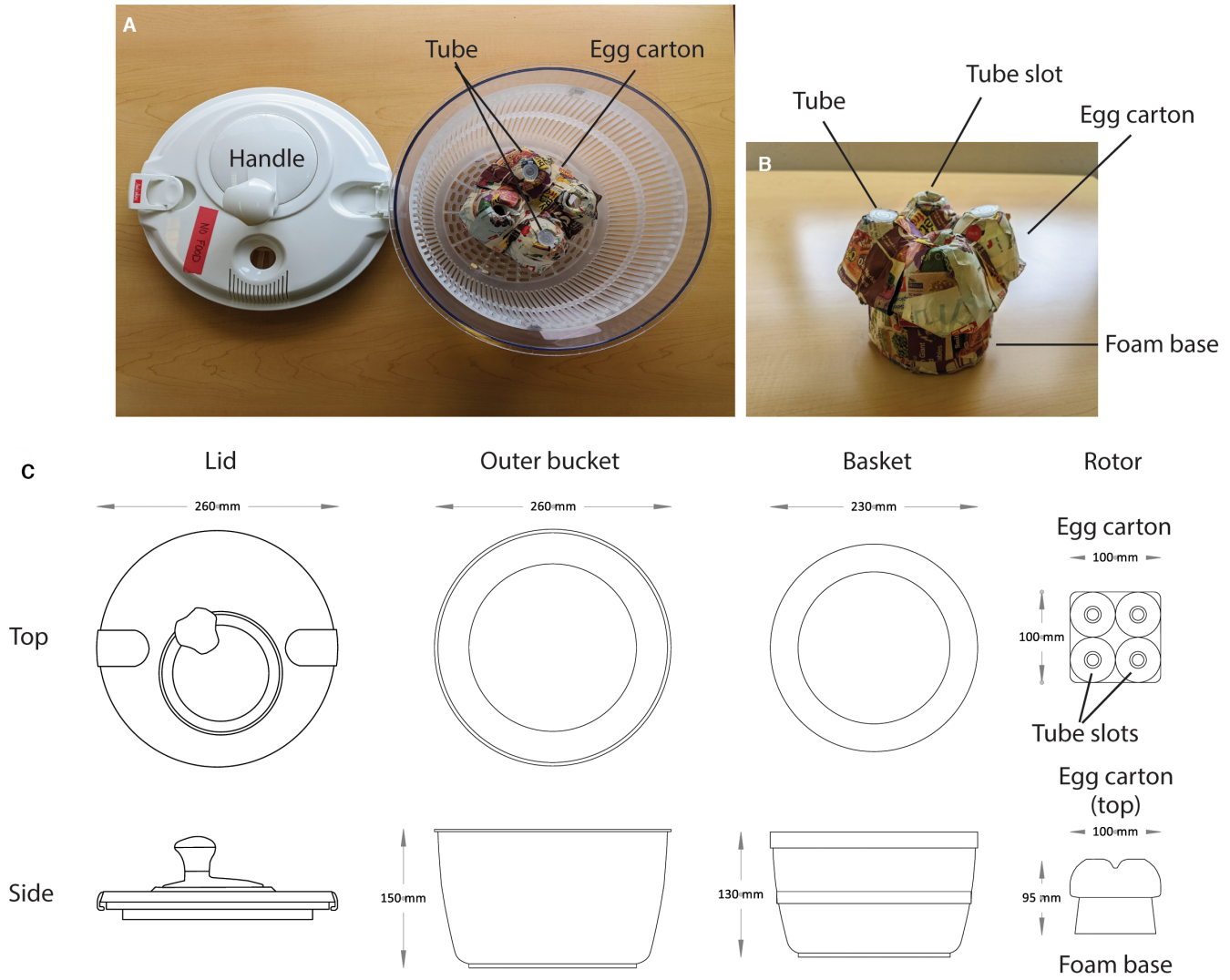


FIGURE 1 Salad spinner centrifuge. A, Top view of a bucket, rotating basket with attached foam rotor, and lid with crank handle. B, Egg carton and foam rotor, C, Schematic line drawings of components.

graphs were prepared using GraphPad Prism 8.1.1 software (San Diego, CA, USA).

## 3 | RESULTS

### 3.1 | Salad spinner centrifuge design and protocol

All three designs were initially tested by rotating the salad spinner basket with two 2.7 tubes containing 2 mL of equine blood at maximal user speed for 5 minutes. The maximum speed at which the handle could comfortably and sustainably be turned was 160 rpm, or approximately 2.7 revolutions per second, after which the crank no longer engaged with the basket, and the basket began to wobble. We confirmed the manufacturer-reported hand crank: basket ratio of 1:5 and calculated the maximum basket rpm to be 800 rpm. The average measured basket rpm by cyclometer at maximal user speed was 806 rpm ($n = 7$). Using 800 rpm, the calculated maximum average RCF for 1.7 mL tubes in the egg carton rotor was 32.2*g*.

Centrifugation using the egg carton swing angle rotor (Figure 1) yielded grossly clear pale-yellow serum with a distinct cell pellet at the bottom of the tube. To minimize microscopic cellular contamination, once the blood had fully clotted in its original collection tube, up to 1 mL of the supernatant was transferred to a 1.7 mL microcentrifuge tube prior to salad spinner centrifugation for 5 minutes at maximum speed. Microscopic evaluation of a Diff-Quik-stained line revealed low numbers of RBCs. To further reduce microscopic RBC contamination, we transferred the supernatant to a new 1.7 mL microcentrifuge tube prior to a second additional 5-minute centrifugation step. Serum isolated with this protocol yielded only rare erythrocytes, comparable to serum separated by the reference centrifugation method (performed by EG). The final protocol after spontaneous clot formation was: (a) transfer supernatant from the blood collection tube to a 1.7 mL microcentrifuge tube; (b) centrifuge for 5 minutes at maximum speed; (c) transfer serum to a second 1.7 mL

microcentrifuge tube; (d) centrifuge a second time for 5 minutes at maximum speed; (e) transfer serum to third 1.7 mL microcentrifuge tube.

### 3.2 | Interference indices and hemoglobin concentration in serum separated by salad spinner centrifugation vs traditional laboratory centrifugation

The study populations included seven horses and seven rhinoceroses, and no samples were excluded. The hemolysis index was significantly lower in equine serum separated by salad spinner centrifugation compared with traditional centrifugation (0.125 vs 4.25, $P=0.0172$). The icteric index was also slightly lower in equine serum separated by salad spinner compared with traditional centrifugation (2.875 vs 3.625, $P=0.0025$). In contrast, the lipemia index was significantly higher in serum separated by salad spinner compared with traditional centrifugation (71.88 vs 5.00, $P=0.0003$). In rhinoceros samples, there were no differences in hemolysis index or icteric index in serum samples separated by salad spinner or traditional centrifugation. As in horses, the lipemia index was significantly higher in serum separated by salad spinner compared with traditional centrifugation (13.14 vs 7.429, $P=0.0017$).

Hemoglobin concentrations of five equine serum samples separated by traditional laboratory centrifugation and salad spinner centrifugation were all 0.0 g/dL (performance range of ADVIA 120: 0.0–22.5 g/dL).[33]

### 3.3 | Comparison of biochemical analytes in serum separated by salad spinner vs traditional laboratory centrifugation (equine)

For most analytes, no systemic bias was detected by Deming regression analysis (Table 1). Compared with serum separated by traditional centrifugation, there was a slight negative proportional bias and negative constant bias for the hemolysis index in serum separated by salad spinner centrifugation (Table 1). Compared with serum separated by traditional centrifugation, we found a positive constant bias for ALP in serum separated by salad spinner centrifugation (Table 1 and Figure 2). Representative Deming regression and Bland–Altman plots comparing results obtained by salad spinner centrifugation and traditional laboratory centrifugation are shown in Figure 2. Descriptive statistics of all analytes are available in Table S1.

### 3.4 | Comparison of biochemical analytes in serum separated by salad spinner vs traditional laboratory centrifugation (rhinoceros)

Similar to results obtained in equine serum, no biases were detected in the measurement of the majority of analytes in rhinoceros serum separated by the two methods (Table 2). Compared with traditional centrifugation, there was a constant positive bias for ALP (Table 2 and Figure 3) in serum separated by salad spinner centrifugation. Representative Deming regression and Bland–Altman plots comparing results obtained by salad spinner centrifugation and traditional laboratory centrifugation are shown in Figure 3. Descriptive statistics of all rhinoceros analytes are available in Table S2.

## 4 | DISCUSSION

In this study, we demonstrate that a consumer-grade salad spinner can be modified using inexpensive materials to accept blood tubes and separate diagnostic-quality serum suitable for biochemical analysis from both domestic horses and southern white rhinoceroses. Manual centrifugation with the salad spinner separated grossly clear serum that contained only rare erythrocytes as observed by microscopic evaluation. Moreover, for nearly all biochemistry analytes, serum separated by salad spinner centrifugation yielded results that were comparable with those obtained from serum separated by traditional laboratory centrifugation. Importantly, we describe a manual centrifugation method capable of centrifuging a larger volume than previously reported for other inexpensive manually operated centrifuges.[17–20,34]

The manually operated salad spinner centrifuge has several advantages for field use. First, the final design modifications are simple and inexpensive to make and require no specialized engineering equipment or expertise. At the time of the study, the cost of the salad spinner was $24 USD, and similar models are currently available for under $30 USD. The remaining materials (Styrofoam, egg carton, glue, tape) are readily purchased or repurposed. At the time of writing, a manually operated salad spinner centrifuge similar to that described here could be constructed for less than $40 USD. Second, compared with other low-cost manually operated centrifuges,[17,19,20,34] the salad spinner can accept larger sample volumes (1.7 mL per tube). The simplicity of the rotor design and materials allow for customization to accommodate different tube sizes. Third, the salad spinner with the rotor is considerably lighter weight than manufactured manual centrifuges (<1 kg vs 1.5–3 kg), an important consideration for field projects with equipment weight limitations. Lastly, because the salad spinner can be operated on any flat surface, including the ground, there is no need for a sturdy structure to which the device must be attached, as required for the operation of manufactured clamp-style manual centrifuges.

Results for most analytes were comparable between serum separated by salad spinner centrifugation and serum separated by traditional laboratory centrifugation. The lipemia index was higher in both equine and rhinoceros samples separated by salad spinner compared with traditional methods, which we attribute to decreased centrifugal forces that can be obtained with manual salad spinner centrifugation. Clearance of lipids from clinical samples requires high or even ultracentrifugation. Depending on the assay, lipemia interferences in analyte measurement may be due to physical, chemical, and

**TABLE 1** Summary of Deming regression analysis comparing analyte measurements obtained from serum separated by salad spinner and traditional centrifugation of equine blood.

| | Slope | 95% CI | Y-intercept | 95% CI |
|---|---|---|---|---|
| Glu | 1.186 | 0.432–1.94 | −27.77 | −169.9 to 114.4 |
| BUN | 1.056 | 0.864–1.249 | −0.707 | −3.230 to 1.816 |
| Crea | 0.9649 | 0.830–1.1 | 0.070 | −0.128 to 0.267 |
| Phos | 0.96 | 0.763–1.157 | 0.160 | −0.556 to 0.876 |
| Ca | 0.8701 | 0.437–1.303 | 1.304 | −3.089 to 5.696 |
| Mg | 0.9409 | 0.633–1.249 | 0.057 | −0.351 to 0.465 |
| TP | 1.166 | −0.249 to 2.58 | −0.986 | −9.204 to 7.233 |
| Alb | 1 | 0.869–1.131 | 0 | −0.300 to 0.300 |
| Glob | 2.031 | 0.759–3.303 | −3.143 | −7.115 to 0.830 |
| Bili | 1.029 | 0.905–1.153 | −0.058 | −0.336 to 0.185 |
| AST | 1.072 | 0.956–1.187 | −14.33 | −50.57 to 21.91 |
| ALP* | 0.9434 | 0.83–1.057 | 10.68 | 0.2705–21.09 |
| GGT | 2.659 | −2.824 to 8.141 | −11.78 | −75.95 to 52.39 |
| CK | 1.018 | 0.851–1.186 | 5.938 | −55.73 to 67.6 |
| Na | 0.8706 | 0.691–1.051 | 17.9 | −6.696 to 42.49 |
| K | 0.9784 | 0.862–1.095 | 0.2689 | −0.1224 to 0.660 |
| Cl | 0.8136 | −0.226 to 1.854 | 18.6 | −18.52 to 122.7 |
| HCO3 | 0.7392 | 0.153–1.325 | 6.113 | −8.254 to 20.48 |
| Indices | | | | |
| ICT | 1.189 | 0.669–1.709 | −1.435 | −3.077 to 0.208 |
| Hemo*,** | 0.0830 | 0.019 to 0.147 | −0.2279 | −0.4251 to −0.031 |
| Lipe | 28.49 | −52.37 to 109.4 | −74.14 | −417 to 268.7 |

Abbreviations: Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Bili, total bilirubin; BUN, blood urea nitrogen; Ca, calcium (total); CK, creatinine kinase; Cl, chloride; Crea, creatinine; GGT, gamma-glutamyl transferase; Glob, globulin; Glu, glucose; HCO3, bicarbonate; Hemo, hemolysis; ICT, icteric; K, potassium; Lipe, lipemia; Mg, magnesium; Na, sodium; Phos, phosphorous; TP, total protein.

*Constant bias.; **Proportional bias.

spectrophotometric mechanisms, as well as volume displacement.[35] In the current study, the difference in lipemia indices in rhinoceros samples was minimal and unlikely to be clinically relevant. The lipemia index in equine samples separated by salad spinner centrifugation was higher than those separated by traditional centrifugation. The lipemia index in several equine samples separated by salad spinner was >60, with a maximum of 113. Most routinely measured analytes are not affected by this degree of lipemia; however, potential interference may occur with the measurement of bilirubin, ammonia, and GLDH.[36] Given these findings, it is advised that differences in species, lipid metabolism, and/or fasting status may render salad spinner centrifugation insufficient to clear lipemic samples, and subsequent centrifugation in a traditional laboratory may be warranted to avoid erroneous results. The statistically significant differences in hemolysis and icteric indices in equine samples separated by the two methods were minimal and not likely to be clinically relevant since all values were below the minimum threshold for interference. The positive biases for ALP in serum separated by salad spinner from equine and rhinoceros samples were small and also unlikely to be clinically relevant.

The investigation of low-cost alternatives to traditional laboratory equipment falls under a loose umbrella of "frugal science." Inexpensive centrifuges have been developed using existing household items, including the salad spinner, egg beater, or recycled computer DVD player.[17,18,34,37] Consumer-grade salad spinners have been used to centrifuge low-volume samples, including blood, in microhematocrit tubes to measure PCV and aid in the identification of Plasmodium organisms in people.[17] In veterinary medicine, a salad spinner was successfully modified to replicate traditional cytocentrifugation of body fluid samples, including cavitary effusions, cerebrospinal fluid, bronchoalveolar lavage, and urine (<200 μL).[18] An egg beater-based centrifuge was able to separate a small volume (<100 μL) of human plasma for semiquantitative measurement of cholesterol.[34] The electricity-powered computer DVD player centrifuge reached a maximum speed of 10 000 rpm (~6100*g*) but was only tested for its ability to separate copper sulfate from sodium carbonate (0.5 mL volume) at 5000 rpm (~3050*g*).[37]

Innovative, inexpensive centrifuges based on simple toys such as the whirligig or fidget spinner toys have also primarily been tested

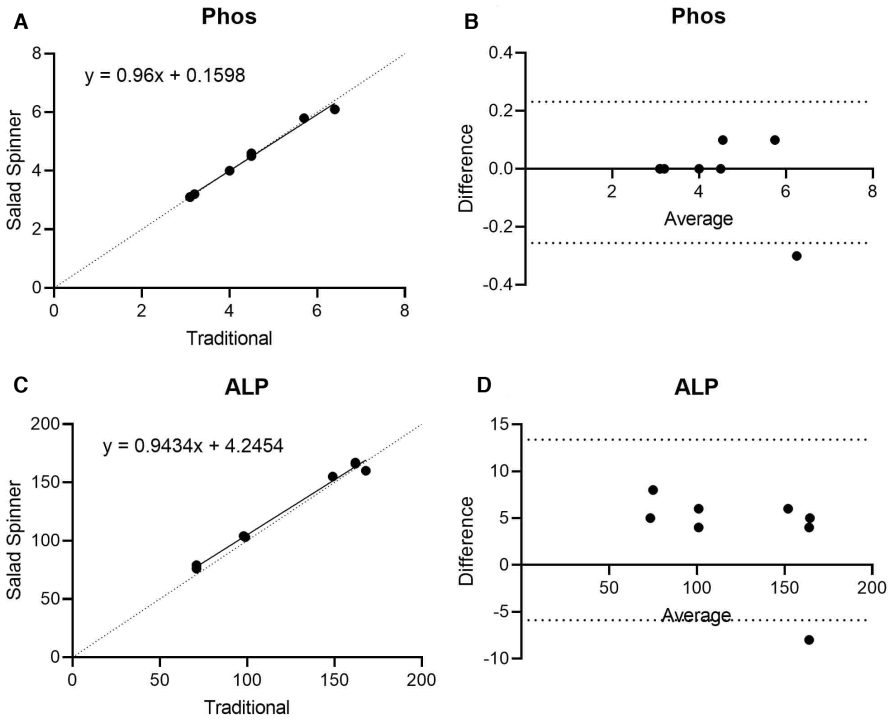


**FIGURE 2** Representative Deming and Bland–Altman plots comparing equine serum prepared by salad spinner and traditional laboratory centrifugation. A, Deming regression for phosphate (Phos). Each point represents one paired sample. The dotted line is the line of unity ($y = x$). The solid line indicates the best-fit line. B, Bland–Altman plot for phosphate. The difference between the two methods is on the *y*-axis, and the average of the two measures is on the *x*-axis. Each point represents one paired sample. Dotted lines indicate 95% confidence interval of agreement (C) As in (A) except for alkaline phosphatase (ALP). D, As in (B) except for ALP ($n = 7$).

for the centrifugation of small volumes, with the primary goal of diagnosing malaria in people in resource-poor conditions.[19,20,38] The fidget spinner centrifuge reached a rotational speed of 1200 rpm, but the maximum blood volume tested was only 15 µL.[20] The ultra-low-cost whirligig-inspired centrifuge constructed from paper was designed to hold microhematocrit tubes and reached rotational speeds up to 125 000 rpm (30 000*g*).[19]

3D printing, which produces 3-dimensional physical objects from computerized models using successive layering of polymer resins, has been used to fabricate splints, prosthetics, and medical models and has the potential to increase access to precision equipment at a fraction of the cost of traditional manufacturing.[39,40] For example, an open-source hand crank centrifuge fabricated entirely from 3D printed parts can reach a radial velocity >1750 rpm (approximately 550*g*), although it has not been tested with clinical samples.[41] A 3D-printed version of the paperfuge is capable of centrifuging larger volumes (up to 2 mL) up to 6000 rpm (2000*g*).[38] While 3D printing carries significant advantages (high degree of specification, increased waterproofing/cleanability), it requires access to a 3D printer, computer-assisted design software, and technical expertise.[42,43]

This study has several limitations. First, samples were collected from healthy animals, precluding comparison across the full range of analyte concentrations. Ex vivo sample manipulations, such as sample dilution or the addition of exogenous reagents, can be used to assess an analytical technique over a wider range of analyte concentrations than achievable with healthy specimens. Since the current study investigated centrifugation rather than a specific analytical method, post-centrifugation sample manipulations could only identify potential interfering matrix effects not detected by traditional interference indices (hemolysis, lipemia, icterus). Alternatively, sample manipulation prior to centrifugation could interfere with complete spontaneous clot formation, introducing potential sources of variability that would be difficult to discern from the effects of the centrifugation itself. Because we only evaluated manual centrifugation in healthy animals, it is possible that certain medical or metabolic conditions could alter serum separation, and additional studies in diseased populations may be warranted to identify potential pre-analytical factors that would impair serum separation by salad spinner centrifugation.

A second limitation is that the study includes only a small sample size for both species. A minimum of 40 samples is recommended for full-method comparison studies, and a larger sample size could reveal statistically significant differences not identified in the current study.[44] Third, we have only tested salad spinner centrifugation in blood collected from two mammalian species; thus, its suitability in other species, especially non-mammalian species, is unknown. Prior to use in other species, it would be prudent to perform similar comparison studies to ensure adequate serum separation. Fourth, the reported RCF should be considered an estimate of end-user RCF, as the end-user RCF will depend on the average maximal rpm, rotor radius, and tube size as chosen by

**TABLE 2** Summary of Deming regression analysis comparing analyte measurements obtained from serum separated by salad spinner and traditional centrifugation of southern white rhinoceros [*Ceratotherium simum*] blood.

| | Slope | 95% CI | Y-intercept | 95% CI |
|---|---|---|---|---|
| Glu | 0.9523 | 0.841–1.064 | 3.206 | −5.064 to 11.48 |
| BUN | 1 | 0.739–1.261 | −0.1429 | −4.319 to 4.034 |
| Crea | 1.113 | 0.844–1.382 | −0.08667 | −0.379 to 0.205 |
| Phos | 1.021 | 0.966–1.076 | −0.09674 | −0.353 to 0.160 |
| Ca | 1.054 | 0.448–1.66 | −0.6556 | −7.935 to 6.624 |
| Mg | 1.054 | 0.448–1.66 | −0.6556 | −7.935 to 6.624 |
| TP | 0.8144 | 0.273–1.356 | 1.42 | −2.756 to 5.596 |
| Alb | 0.9082 | 0.189–1.627 | 0.2728 | −1.974 to 2.519 |
| Glob | 0.9455 | 0.686–1.205 | 0.2814 | −0.927 to 1.49 |
| SDH | 0.9869 | 0.56–1.414 | 1.417 | −1.162 to 3.995 |
| AST | 1.008 | 0.946–1.071 | −0.1219 | −4.005 to 3.762 |
| ALP* | 0.953 | 0.878–1.028 | 8.251 | 2.23–14.27 |
| GGT | 1.027 | 0.787–1.268 | 0.2245 | −3.215 to 3.665 |
| CK | 1.003 | 0.977–1.03 | 0.4071 | −8.881 to 9.695 |
| NA | 0.7413 | 0.259–1.224 | 34.51 | −28.96 to 97.98 |
| K | 1.192 | 0.688–1.696 | −0.7901 | −3.058 to 1.478 |
| Cl | 0.9114 | 0.642–1.181 | 8.4 | −16.72 to 33.52 |
| HCO3 | 1.919 | −0.723 to 4.562 | −23.44 | −89.79 to 42.92 |
| Trig | 0.9766 | 0.887–1.066 | 1.661 | −1.895 to 5.217 |
| Indices | | | | |
| ICT | NC** | | | |
| Hemo | 5.293 | −6.24 to 16.83 | −27.61 | −108 to 52.8 |
| Lipe | 2.287 | −5.079 to 9.652 | −3.845 | −63.85 to 56.16 |

Abbreviations: Alb, albumin; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Ca, calcium (total); CK, creatinine kinase; Cl, chloride; Crea, creatinine; GGT, gamma-glutamyl transferase; Glob, globulin; Glu, glucose; HCO3, bicarbonate; Hemo, hemolysis; ICT, icteric; K, potassium; Lipe, lipemia; Mg, magnesium; Na, sodium; Phos, phosphorous; SDH, sorbitol dehydrogenase; TP, total protein; Trig, triglycerides.

*Constant bias.; **Not calculated due to all values = 0.

the end user. In our study, multiple users were able to obtain and maintain a rotational speed of approximately 800 rpm over the duration of a 5-minute centrifugation, but instantaneous speed (and therefore RCF) is likely to vary amongst users and over time. It is worth noting here that we initially attempted to measure basket rpm with laser tachometry, but found this approach to be unsuitable, which we attributed to light interference from the outer clear bucket. Instead, both manually counting the hand crank rpm to calculate the basket rpm and the magnet-based cyclometer yielded consistent and comparable results. Manual counting was simple and required no equipment. The wireless magnet-based cyclometer required careful attention to ensure that the components were aligned and did not move during use but yielded instantaneous results that corresponded to user-perceived rotational speed.

The RCF achieved by salad spinner centrifugation in this study is considerably less than traditional laboratory centrifugation techniques, although there is a surprising lack of data on minimum requirements.[4] Additional studies that investigate minimal requirements for serum separation may be warranted to unify laboratory techniques. Since the total duration of the centrifugation was 10 minutes for both the reference and manual method, we speculate that the duration of centrifugation may be sufficient to mitigate the impact of lower RCF for the separation of erythrocytes from serum. However, since lipid clearance typically requires higher RCF, it is unlikely that increasing the duration of centrifugation would be sufficient to remove this interference.

In conclusion, despite likely variability in instantaneous rpm and corresponding RCF and a modestly increased lipemia index, we found that centrifugation using a manually operated consumer-grade salad spinner centrifugation is sufficient for the separation of diagnostic-quality serum from whole blood. For nearly all analytes, serum separated by salad spinner centrifugation yielded comparable results to those obtained by traditional centrifugation in both the domestic horse and southern white rhinoceros, eliminating one obstacle to obtaining biochemical data from wild animals in remote field settings. We recently showed that many serum analytes in elephants are stable for up to 10 days at sub-optimal temperature storage conditions.[45] Future studies that investigate the

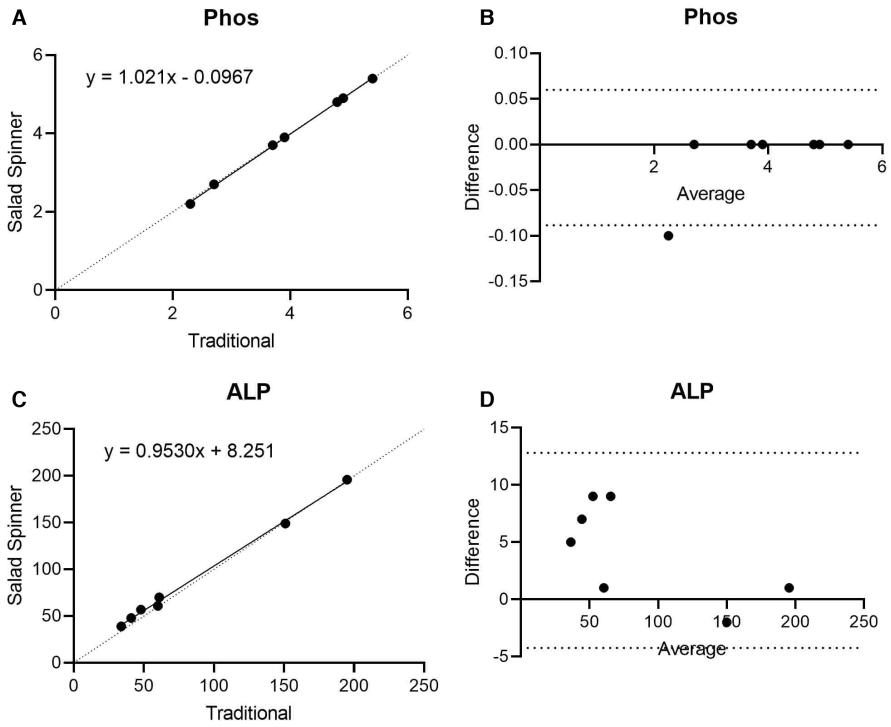


**FIGURE 3** Representative Deming and Bland–Altman plots comparing southern white rhinoceros (*Ceratotherium simum*) serum prepared by salad spinner and traditional laboratory centrifugation. A, Deming regression for phosphate (Phos). Each point represents one paired sample. The dotted line represents the line of unity ($y = x$). The solid line is the best-fit line. B, Bland–Altman plot for phosphate. The difference between the two methods is on the *y*-axis, and the average of the two measures is on the *x*-axis. Each point represents one paired sample. The dotted lines indicate a 95% confidence interval of agreement. C, As in (A) except for alkaline phosphatase (ALP). D, As in (B) except for ALP. $n = 7$.

combined effects of manual centrifugation and suboptimal storage conditions could have considerable practical value for veterinarians and researchers conducting field studies in remote locations. Additional research to determine if manual centrifugation is sufficient to separate serum for other diagnostic tests, including protein electrophoresis or measurement of hormones, acute proteins, or cytokine concentrations, would be warranted prior to using this method for these tests.

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## CONFLICT OF INTEREST STATEMENT

The author declares no conflicts of interest.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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