Clinical performance evaluation of total protein measurement by digital refractometry and characterization of non-protein solute interferences

Joshua J.H. Hunsaker, Sara P. Wyness, Taylor M. Snow, Jonathan R. Genzen

ARUP Institute for Clinical and Experimental Pathology, 500 Chipeta Way, Salt Lake City, UT 84108, United States
Department of Pathology, University of Utah, 15 North Medical Drive East, Suite #1100, Salt Lake City, UT 84112, United States

Abstract

Objectives: Refractometric methods to measure total protein (TP) in serum and plasma specimens have been replaced by automated biuret methods in virtually all routine clinical testing. A subset of laboratories, however, still report using refractometry to measure TP in conjunction with serum protein electrophoresis. The objective of this study was therefore to conduct a modern performance evaluation of a digital refractometer for TP measurement.

Design and methods: Performance evaluation of a MISCO Palm Abbe™ digital refractometer was conducted through device familiarization, carryover, precision, accuracy, linearity, analytical sensitivity, analytical specificity, and reference interval verification. Comparison assays included a manual refractometer and an automated biuret assay.

Results: Carryover risk was eliminated using a demineralized distilled water (ddH2O) wash step. Precision studies demonstrated overall imprecision of 2.2% CV (low TP pool) and 0.5% CV (high TP pool). Accuracy studies demonstrated correlation to both manual refractometry and the biuret method. An overall positive bias (+5.0%) was observed versus the biuret method. On average, outlier specimens had an increased triglyceride concentration. Linearity was verified using mixed dilutions of: a) low and high concentration patient pools, or b) albumin-spiked ddH2O and high concentration patient pool. Decreased recovery was observed using ddH2O dilutions at low TP concentrations. Significant interference was detected at high concentrations of glucose (≥4267 mg/dL) and triglycerides (≥580 mg/dL). Current laboratory reference intervals for TP were verified.

Conclusions: Performance characteristics of this digital refractometer were validated in a clinical laboratory setting. Biuret method remains the preferred assay for TP measurement in routine clinical analyses.

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
1. Introduction

Refractometry has been used for over 100 years to measure the density of biological solutions. Refraction is the change in direction of a light wave as it passes through the boundary of two mediums with different wave propagation speeds. The ratio of the wave speed in air versus its speed in another medium (e.g. water) is known as its refractive index. Refractometry has been particularly valuable in assessing urine concentrations through specific gravity (SG) measurements [1].

Refractometry has also been used in the assessment of serum and/or plasma total protein (TP) measurements [2–5]. In this capacity, refractometer scales are calibrated against normal serum, with the general assumption that many non-protein solutes (e.g. electrolytes) are at similar, relatively low concentrations across patient specimens [6]. Many substances do, however, vary significantly between individuals (e.g. lipids), and this may alter solution density such that refractometric assessment of TP may be inaccurate [7]. While handheld manual refractometers (developed in the 1960s) provided a rapid and accessible method for TP measurement, modern clinical chemistry analyzers now provide more sensitive, specific, and automated measurements of TP primarily through the biuret method [8]. Refractometry has therefore become regarded as a less acceptable technique for routine human serum or plasma TP measurement [9].

While “refractometer” remains an available TP method selection in the College of American Pathologists (CAP) General Chemistry and Therapeutic Drugs proficiency testing (PT) surveys [10], the overwhelming number of participant laboratories (99.6%) use automated biuret-based methods for TP measurement [11]. Refractometry-based TP measurement still maintains a presence, however, in select applications such as serum protein electrophoresis. For example, 2.4% (n = 20 of 842) participants in a recent CAP Electrophoresis PT survey used refractometry to determine TP concentration [12]. Presumably, these laboratories select refractometry for the convenience of rapid, inexpensive TP testing in close proximity to specialized electrophoresis instrumentation. While manual and digital refractometry is also used extensively in veterinary medicine for assessing TP, immunoglobulin G, and dissolved solid concentrations in serum, plasma, and colostrum [13–21], few studies have focused on the potential application of digital refractometry in modern analytical settings [22].

In a companion study to the present report [23] we conducted a clinical validation of a handheld digital refractometer to serve as a replacement for manual urine SG measurements in our laboratory. As a human blood (serum/plasma) TP scale was also available on this handheld device, we conducted the present experiments to: a) evaluate the performance of a digital refractometer versus a manual refractometer and a biuret method of TP measurement, and b) to investigate the potential for non-protein substances to interfere with TP measurement when using refractometry. By using a digital refractometer we excluded any contribution of subjective operator interpretation of a visual TP scale.

2. Materials and methods

A Palm Abbe™ model PA202X (MISCO; Solon, OH) was used as the digital refractometer for all experiments. The “Blood-Human-Total Protein By Refractometer (TPr)” scale (ID#108) was used and reports TP to 1 decimal place. While the instruments reportable range is stated to be 1.0–14.0 g/dL, instrument results down to 0.2 g/dL could be obtained and were recorded as the displayed numerical value. All studies were conducted with the instrument and materials at room temperature.

2.1. Specimens

Using an Institutional Review Board (IRB)-approved protocol (University of Utah IRB Protocol #0007275), previously collected clinical serum specimens at ARUP Laboratories (Salt Lake City, UT) were obtained from frozen storage (–20 °C) and de-identified for use in method validation experiments. Serum specimens obtained from healthy donors (collected using IRB Protocol #0007740) were also used for reference interval verification studies.

2.2. Device familiarization

Instrument familiarization studies consisted of running low (diluted Level 1) and high (Level 3) TP quality control (QC) materials (Multiqual® Liquid Unassayed; Bio-Rad Laboratories, Inc; Hercules, CA) in 10 replicates each.

2.3. Minimum volume

Minimum specimen volume parameters (60 μL) were adopted from concurrent studies validating the Palm Abbe for urine SG [23].

2.4. Carryover

Carryover was evaluated using: a) low (diluted Level 1) and high (Level 3) QC materials (Multiqual® Liquid Unassayed), and b) patient serum pools with low and high TP. Carryover studies were conducted using the following test order: low,
high, low (4 replicates of each set), both with and without in between ddH2O washes for QC studies, and with ddH2O washes for subsequent serum studies.

2.5. Precision

Precision experiments were conducted over 20 days, with 2 runs daily (run = 2 samples at each of 2 levels; each run separated by at least 2 h) using low (diluted Level 1) and high (Level 3) QC materials (Multiqual® Liquid Unassayed).

2.6. Accuracy

Accuracy experiments were conducted using residual patient serum specimens (n = 50) spanning the analytical measurement range (AMR) of TP, based on prior clinical TP testing on either cobas c502 (Roche Diagnostics; Indianapolis, IN) or Abbott ARCHICTECT ci8200 (Abbott Diagnostics; Abbott Park, IL) chemistry analyzers. Accuracy experiments were conducted over 5 days (n = 10 specimens per day) on the Palm Abbe, a manual refractometer (Cat. 10414; Reichert Scientific Instruments, Buffalo, NY), and an automated biuret chemistry assay (cobas c502; TP2, Total Protein Gen.2, Roche). Testing was performed in duplicate on all instruments, with reverse order between duplicate runs (i.e., first run: 1, 2, 3, 4; second run: 3, 2, 4, 1). Testing order on the c502, however, varied based on random access instrument operation. Data are plotted as the mean result of duplicate values. Between-method outliers (Palm Abbe versus c502, n = 1) and within-method outliers (Palm Abbe, n = 3; c502, n = 1; manual refractometer, n = 2) as identified by StatisPro™ 2.51 (a partnership of CLSI, Wayne, PA & Analyze-it® Software, Leeds, UK) were repeated on all platforms prior to analysis. Subsequent investigation of accuracy involved testing all 50 specimens [archived at –20 °C] by the Roche c502 glucose and triglyceride assays, as well as serum protein electrophoresis using the CAPILLARYS PROTEIN (E) 6 kit on a CAPILLARYS 2 instrument (Sebia; Norcross, GA).

2.7. Linearity

Linearity was assessed using mixtures of either: a) high patient serum pool diluted with a low patient serum pool (final measuring range, 4.4 g/dL–10.8 g/dL), or b) high patient serum pool spiked with bovine albumin (ALB) (AMRESCO; Dallas, TX) diluted with a low TP pool consisting of ddH2O spiked with ALB (final measuring range, 0.3–13.9 g/dL). Bovine source ALB was chosen due to decreased cost and similar molecular weight (~ 66.5 kDa) to human albumin. All dilutions were tested in triplicate. Assigned TP was based on percent-split dilution from original high and low TP pools. Percent recovery was calculated as:

\[
\text{Percent Recovery} = \left( \frac{\text{Observed Result}}{\text{Assigned Result}} \right) \times 100
\]

Additional serial dilution studies were then conducted. These were performed using human type AB serum (Corning, VWR; spiked with ALB to reach TP = 6.1 g/dL) diluted with either ddH2O or 0.9% sodium chloride (NaCl) (Baxter Healthcare; Deerfield, IL). This NaCl solution contains 154 mEq/L Na⁺ and 154 mEq/L Cl⁻. Pretesting of diluent solutions (ddH2O and 0.9% NaCl) on the Palm Abbe demonstrated that baseline “TP” measurements were below the instrument reportable range. Serial dilution results were fit using linear regression while percent recovery calculations were fit automatically using non-linear regression (Exponential Rise to Maximum, Double, 5 Parameter) with the Dynamic Curve Fit module in SigmaPlot 11 (Systat Software; San Jose, CA).

2.8. Analytical sensitivity

Analytical sensitivity was evaluated by comparing total error (TE) for seven de-identified patient serum specimens chosen with baseline TP measurements of approximately 4, 5, and 6 g/dL. De-identified clinical specimens were divided into two categories: a) “concordant”, Palm Abbe and c502 TP baseline results within ± 0.2 g/dL of each other; and b) “discordant”, Palm Abbe and c502 TP baseline results greater than ± 0.2 g/dL of each other. Bias for each specimen was set as the difference between baseline Palm Abbe and Roche TP results; imprecision was determined by testing each specimen in triplicate for three consecutive days on Palm Abbe. TE was then calculated for each specimen using the Westgard model: TE = |Bias| ± 2 SD. The TE(%) = (TE – biuret mean) × 100. Since a published percent total allowable error TAE(%) acceptability threshold for TP of 3.63% [24] is somewhat ambitious at low TP concentrations [the absolute TAE would approach instrument resolution of 0.1 g/dL] a TAE(%) acceptability threshold of 10% was also considered.

2.9. Analytical specificity

An interference screen was conducted using low TP and high TP pools created with human AB serum (Corning, VWR) spiked with ALB to reach TP = 6 g/dL and TP = 8 g/dL targets [25]. These pools were then spiked with conjugated bilirubin (BILI; Scripps Laboratories), anhydrous d-glucose (GLU; VWR, Radnor, PA), lyophilized human hemoglobin (Hb; Sigma Aldrich, St. Louis, MO), sodium chloride (NaCl; BDH Chemicals, VWR), or 20% Intralipid (Sigma Aldrich), with spiked-
interferent concentrations targeted to CLSI-specifications [25]. Reconstituted human Hb was found to be in methemoglobin form [23]. 5 aliquots were tested per interferent in alternating order of control and test specimens. Interference was suggested when results exceeded a TAE of 3.63% [24]. Characterization of identified interferences were then evaluated through dilution mixtures of low and high interferent concentrations dissolved in a 6 g/dL TP pool (tested with 5 replicates in alternating ascending and descending order). The level of significant interference was defined as the concentration (based on linear fit) where the upper 95% confidence limit for bias exceeded TAE of 3.63% [24].

2.10. Reference intervals (RIs)

The laboratory’s previously established RIs for TP (female and male: 6.3–8.2 g/dL) were evaluated using 20 female and 20 male serum samples obtained from healthy donors.

2.11. Data analysis

Data was analyzed using EP Evaluator® 10 (Data Innovations; South Burlington, VT), StatisPro™ 2.51, Microsoft Excel 2010 (Redmond, WA), and SigmaPlot 11 (Systat). Graphs were prepared using SigmaPlot. Data is presented as mean ± standard deviation (SD) unless otherwise indicated.

Table 1

| Material | Mean TP material (g/dL) | Total imprecision (SD) | Total imprecision (%CV) | Within run (SD) | Between run (SD) | Between day (SD) |
|----------|-------------------------|------------------------|-------------------------|-----------------|-----------------|-----------------|
| Low QC   | 2.35                    | 0.05                   | 2.2                     | 0.02            | 0.00            | 0.05            |
| High QC  | 10.64                   | 0.05                   | 0.5                     | 0.04            | 0.02            | 0.02            |

3. Results

Familiarization studies demonstrated that the Palm Abbe digital refractometer was easy to operate, a finding that was consistent with our companion study evaluating the device for urine SG measurement [23]. Carryover studies using low TP (2.4 g/dL) and high TP (10.7 g/dL) QC material – without ddH₂O washes between measurements - demonstrated an unacceptable specimen carryover of 8.5%. Carryover using QC material was eliminated with the introduction of single in between ddH₂O wash steps (carryover 0.0%). Using low TP (4.4 g/dL) and high TP (10.6 g/dL) patient pools with in between ddH₂O wash steps, minimal carryover ( < 2%) was observed.

Results from precision experiments over 20 days are presented in Table 1. Low total imprecision was demonstrated using both low QC (%CV, 2.2; mean TP 2.35 g/dL) and high QC (%CV, 0.5; mean TP 10.64 g/dL) material. Overall precision estimates (SD) met the manufacturer claims ( ± 0.1 g/dL) [26].

Accuracy was assessed by comparing Palm Abbe results to a manual refractometer (Fig. 1A–C) and a biuret method on a Roche cobas c502 chemistry analyzer (Fig. 1D–F). Open (white) symbols indicate specimens in which a monoclonal protein was identified by serum protein electrophoresis. A correlation to both manual refractometry (Deming regression, y = 1.092x – 0.907, r = 0.992) and the Roche TP chemistry assay (Deming regression, y = 0.989x + 0.431, r = 0.976) was observed. A negative bias was noted at lower concentration of TP versus manual refractometry (absolute bias, – 0.22 g/dL, Fig. 1B; percent bias, − 3.03%, Fig. 1C). An overall positive bias (absolute bias, + 0.35 g/dL, Fig. 1E; percent bias, + 5.01%, Fig. 1F) was observed versus the Roche chemistry assay.

The gray symbols shown in Fig. 1D–F indicate “outlier” specimens that had both a > 0.9 g/dL absolute bias (Fig. 1D) and > 12% percent bias (Fig. 1E) in Palm Abbe versus Roche biuret TP results. To determine whether non-protein solutes may contribute to this bias, all 50 specimens used in the accuracy studies (7 outliers, 43 non-outliers) were tested for triglyceride and glucose concentration. There was a clear increase in triglycerides in outliers (513.1 ± 577.5 mg/dL, range 142–1803 mg/dL) versus non-outliers (123.4 ± 65.2 mg/dL, range 49–311 mg/dL, p < 0.01). Furthermore 86% of outliers (6 of 7), but only 11.6% of non-outliers (5 of 43) had triglyceride concentrations > 200 mg/dL. There was a potential (but non-significant) trend toward increased glucose in outliers (152.6 ± 57.8 mg/dL, range 97–250 mg/dL) versus non-outliers (116.2 ± 49.9 mg/dL, range 67–298 mg/dL, p = 0.09). No outliers had a monoclonal protein.

Linearity was evaluated using mixtures of: a) low and high concentration patient pools (Fig. 2A,B; TP range tested, 4.4–10.8 g/dL; slope 1.009), and b) low TP pool (ddH₂O spiked with ALB) and high concentration patient pool spiked with ALB (TP range tested, 1.7–13.9 g/dL; slope 0.950; Fig. 2C,D). Linear regressions are shown in Fig. 2 A,C, while percent recovery for corresponding data is shown in Fig. 2 B,D. While instrument linearity was verified using both strategies, a trend toward decreased recovery was observed when using ALB-spiked ddH₂O matrix as a diluent at lower TP concentrations (Fig. 2D).

Serial dilution studies were then conducted using a 6.1 g/dL baseline pool (human AB serum matrix adjusted with bovine ALB) with either ddH₂O or 0.9% NaCl diluents (Fig. 3). Decreased recovery was observed using ddH₂O diluent (Fig. 3A, black circles; linear regression, y = 1.223x – 1.357, r = 0.999; Fig. 3A). Markedly decreased percent recovery was observed with
Fig. 1. Accuracy. Comparison of Palm Abbe versus manual refractometry (A-C) or Roche c502 (D-F) TP results. Solid line is unity; dotted line is Deming regression (A, D). B. Absolute bias (in g/dL) of Palm Abbe versus manual refractometry. E. Absolute bias (in g/dL) of Palm Abbe versus Roche c502. Solid line is zero bias reference; dotted line is absolute bias (B, E). C. Percent bias of Palm Abbe versus manual refractometry. F. Percent bias of Palm Abbe versus Roche c502. Solid line is zero bias reference; dotted line is percent bias (C, F). Open white symbols (all panels) indicate serum specimens which contain a monoclonal protein. Gray symbols (D-F only) indicate “outlier” serum specimens (described in Section 3. Results) where absolute bias (E) is > 0.9 g/dL and percent bias (F) is > 12%, when comparing Palm Abbe to the Roche biuret method.
additional dilutions (Fig. 3B; nonlinear regression, r = 0.999). To confirm that this decreased recovery was due to concurrent dilution of non-protein solutes, partially solute replete diluent (0.9% NaCl) was then used (Fig. 3A, B; open circles). As predicted, this improved the apparent overall recovery of TP as shown in Fig. 3A (open circles; linear regression, y = 1.095x – 0.583, r = 0.999) and Fig. 3B (open circles, nonlinear regression, r = 0.998). Palm Abbe TP measurements were significantly different between corresponding ddH2O and NaCl dilutions (p < 0.001; paired t-test of average results across each dilution).

Analytical sensitivity was evaluated by calculating TE for seven de-identified patient specimens chosen for baseline TP measurements of approximately 4, 5, and 6 g/dL (Table 2). As shown in Table 2, bias was a major contributor to TE. For example, in the three “discordant” specimens (based on c502 to Palm Abbe baseline TP differences greater than ±0.2 g/dL of each other), none of the specimens fell within a TAE(%) of 3.63%. At a TAE(%) of 10%, TE(%) for “discordant” specimens was either just above (Specimens E and F) or below (Specimen G) the acceptable threshold. Two of the four “concordant”
specimens (based on c502 to Palm Abbe baseline TP differences less than ± 0.2 g/dL of each other) met the TAE(%) threshold of 3.63%, while all met the threshold of 10%.

Analytical specificity was then evaluated by conducting an interference screen to look for potential endogenous substances (total dissolved solutes) that may affect TP measurement. GLU and Intralipid demonstrated the potential for TP interference at the screen concentrations tested (Table 3). Hb, BILI, and NaCl did not show significant interference (TE < 3.63%) at described concentrations.

Further characterization of interference for GLU and Intralipid was conducted using the dose-response series of interferent mixtures dissolved in a 6 g/dL serum TP matrix (Fig. 4). Significant interference was observed at high concentrations of GLU (> 267 mg/dL; Fig. 4A) and triglycerides (TRIG, > 580 mg/dL, Fig. 4B; corresponding Roche lipemia “L” index of > 257, Fig. 4C).

Our current laboratory adult reference intervals for TP (6.3–8.2 g/dL) were verified on the Palm Abbe refractometer using male (n=20) and female (n=20) specimens according to a CLSI EP28-A3c protocol for validation of transference (data not shown) [27].
Table 3
Interference screen.

| Interferent | Interferent Concentration | TP (g/dL) | Bias (g/dL) | % Difference |
|-------------|---------------------------|-----------|-------------|--------------|
| Hb          | 0.2 g/dL                  | 6         | +0.14       | 2.3%         |
|             | 0.2 g/dL                  |           | +0.16       | 2.0%         |
| BILI        | 15.9 mg/dL                | 6         | +0.06       | 1.0%         |
|             | 18.7 mg/dL                | 8         | +0.02       | 0.2%         |
| Lipemia (Intralipid) | Trig. 3829 mg/dL (L index 1647) | 6 | +1.06 | 17.7% |
|             | Trig. 3810 mg/dL (L index 1622) | 8 | +0.84 | 10.4% |
| NaCl        | Na 177 mmol/L; Cl 137 mmol/L | 6 | +0.20 | 3.3% |
|             | Na 184 mmol/L; Cl 143 mmol/L | 8 | +0.20 | 2.5% |
| GLU         | 1024 mg/dL                | 6         | +0.70       | 11.7%        |
|             | 1015 mg/dL                | 8         | +0.68       | 8.4%         |

4. Discussion

The present experiments demonstrate the analytical performance of a handheld digital refractometer for TP measurement. Overall precision was near the literature-described precision claims for TP by refractometry (± 1% [6] and < 2% [8]). Palm Abbe precision was similar to the performance data described for biuret methods. For example, intermediate (between-day) precision for TP on Roche c501/502 analyzers has been described as 1.7–2.5%CV [28]. An analytical performance characterization noted inter-assay precision on the Roche c701 TP assay to be 1.7–2.1%CV (for QC) and 1.5–2.4%CV (for serum) [29].

While correlation was observed between Palm Abbe, manual refractometry, and biuret results (Fig. 1), a fair amount of scatter was present and may reflect between-patient variability in non-protein solutes. The slight positive bias (Palm Abbe vs. biuret) may reflect different standardization/calibration of platforms. Given the observed bias, it is likely that published accuracy claims for TP by refractometry (± 0.1 g/dL) [6] could only be met using relatively purified solutions of TP (i.e. non-clinical materials). Additionally, the observed bias (Palm Abbe vs. biuret) does exceed published desirable accuracy specifications (1.36%) [24]. It should be noted, however, that the clinical significance of TP differences at these inaccuracy specifications is negligible. Trueness of a Roche biuret TP assay (c701; within desirable bias limits of 1.2%) has previously been described [29].

While assay linearity was verified (Fig. 2A,C), a decreased percent recovery was observed using ddH2O-matrix diluent at low concentrations (Fig. 2D). This finding was confirmed in separate serial-dilution studies (Fig. 3). Decreased percent recovery with ddH2O dilution is expected, however, as it is concurrent with dilution of non-protein solutes that also contribute to solution density. As confirmation of this, improved recovery was observed when using a partially solute-replete diluent (0.9% NaCl; Fig. 3A, B). Linearity on our comparative Roche biuret method (c502) was verified during our initial instrument validations (slope 1.001; data not shown). Linearity on the c502 TP assay is consistent with that observed in a separate, comprehensive analytical performance evaluation of the cobas 8000 system [29].

Individual specimen TP differences observed between refractometry and the biuret method (Fig. 1) may have several causes. Specimens were mixed thoroughly prior to testing, thus we do not believe that sample heterogeneity due to inadequate mixing contributed to scatter. Furthermore, the relatively tight correlation between Palm Abbe and manual refractometry results (Fig. 1A) argues against inadequate mixing and suggests that the presence of additional, non-protein solutes may contribute to differences versus biuret measurements. Indeed, the 7 discordant “outlier” specimens (Fig. 1D–F) had higher average triglyceride concentrations than the remaining 43 non-outlier specimens used in the accuracy study. Interference studies (Fig. 4) reinforce the fact that high concentrations of non-protein solutes (e.g. GLU and/or lipids) can contribute to elevation in “TP” measurements beyond the established TAE. Concentrations of these solutes may fluctuate over time within individual patients. This concern argues against the use of refractometry for TP measurement when a biuret alternative is available. Accuracy of TP measurements (Palm Abbe versus biuret) were similar in specimens with and without monoclonal proteins.

It has been stated that the use of refractometry for measurement of TP in low concentrations (e.g. < 3.5 g/dL) is inadvisable [8]. At these lower TP concentrations non-protein solutes have a greater relative contribution to solution density than in normal serum used to calibrate the refractometer scale. Such low TP concentrations in serum or plasma are exceedingly rare, making up less than 0.15% of clinical specimens at our facility (data not shown). It is also likely that some of these specimens represent IV-line contamination and inadvertent dilution. Using the overall approximate bias shown in accuracy studies (0.35 g/dL; Fig. 1D) and an imprecision of 0.05 g/dL (based on Tables 1,2), a TE of 0.45 g/dL can be calculated. Since this TE would be > 10% of any measured serum TP concentrations < 4.5 g/dL, we have estimated a lower limit of the measuring interval of 4.5 g/dL. Analytical sensitivity studies, however, show that variation in TE exists across specimens and TP concentrations (Table 2). Ultimately, bias represented a greater contribution to TE than did imprecision.

While it has been argued in the veterinary literature that refractometry can be used for low TP body fluid testing (at or
Fig. 4. Interference Studies. Effect of increasing concentration of (A) GLU, (B) TRIG, or (C) Roche lipemic index (“L”) on Palm Abbe TP results. Solid black line, linear regression; solid gray line, upper 95% confidence interval of linear fit; dotted black line, limit of allowable bias (3.63%) from baseline TP concentration; dashed gray line, intercept where upper 95% confidence exceeds allowable bias.
below 2–2.5 g/dL) [30,31], studies in human pleural fluid demonstrated a risk for erroneous readings [32]. Given that body fluids may contain dramatic differences in both protein and non-protein solutes (e.g. electrolytes, GLU, Hb, hyaluronic acid, lipids, etc), we concur that refractometry is not appropriate for routine clinical body fluid TP measurement.

While TP specimen results (between biuret and refractometry) are similar on CAP Electrophoresis PT surveys in terms of mean, SD, and %CV [12], the matrix of PT material may not completely reflect the complexity of fresh serum and plasma specimens. The nature of PT testing also does not embody between-patient biological variability that is expected in other non-protein analytes.

In conclusion, while the digital refractometer used in the present report demonstrated excellent precision, it did not introduce benefits in accuracy over manual refractometry in regards to potential interference due to non-protein solutes. Additionally, refractometry is vulnerable to bias at low TP concentrations. While some clinical laboratory settings may benefit from TP measurement by refractometry (particularly low-resource settings as there is negligible cost of operation), its use should be discouraged where alternate biuret methods are readily available.

Conflicts of interest

None.

Acknowledgments

This work was supported by internal funding from the ARUP Institute for Clinical and Experimental Pathology.

References

[1] N.A. Brunzel, Fundamentals of Urine & Body Fluid Analysis, 3rd ed. Elsevier Saunders, St. Louis, Mo, 2013.
[2] E. Reiss, Eine neue methode der quantitativen eiweissbestimmung, Arch. Exp. Pathol. Pharmacol. 51 (1903) 18–29.
[3] B. Neuhausen, D. Riech, The refractometric determination of serum proteins, J. Biol. Chem. 55 (1923) 353–356.
[4] M. Rubini, A.V. Wolf, Refractometric determination of total solids and water of serum and urine, J. Biol. Chem. 225 (1957) 869–876.
[5] C. Tranter, A. Rowe, The refractometric determination of albumin, globulin, and nonprotein, JAMA LXV (1915) 1433–1434.
[6] L. Kaplan, A. Pesce, S. Kazmierczak, Clinical Chemistry: Theory, Analysis, Correlation, 4th ed. Mosby, St. Louis, MO, 2003.
[7] N. Moore, D. Van Slyke, The relationships between plasma specific gravity, plasma protein content, and edema in nephritis, J. Clin. Invest. 8 (1930) 337–355.
[8] Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed., in: C. Burtis, E. Ashwood, D. Bruns, (Eds.), Elsevier Saunders, St. Louis, MO, 2006.
[9] Discussion Session III, Clin. Chem. 38, 1992, pp. 1268–1272.
[10] C-C 2015 Kit Instructions. General Chemistry and Therapeutic Drugs Survey. College of American Pathologists. Northfield, IL, 2015.
[11] H.W. Vveden, A. Noren, Comparison of a Schmidt and Haensch refractometer and an Atago PAE-USG Cat refractometer for determination of urine specific gravity in dogs and cats, Vet. Clin. Pathol. 43 (2014) 63–66.
[12] J.K. Paris, et al., Comparison of a digital and an optical analogue hand-held refractometer for the measurement of canine urine specific gravity, Vet. Rec. 170 (2012) 463.
[13] I. Elsaohaby, J.T. McClure, G.P. Keefe, Evaluation of digital and optical refractometers for assessing failure of transfer of passive immunity in dairy calves, J. Vet. Intern. Med. 29 (2015) 721–726.
[14] J.A. MacFarlane, et al., Identification and quantification of factors affecting neonatal immunological transfer in dairy calves in the UK, Vet. Rec. 176 (2015) 625.
[15] G.M. Hayes, et al., Refractometric total plasma protein measurement as a cage-side indicator of hypoalbuminemia and hypoproteinemia in hospitalized dogs, J. Vet. Emerg. Crit. Care 21 (2011) 356–362.
[16] S.M. Ziska, et al., Effects of serial harvest of plasma on total plasma protein and total immunoglobulin G concentrations in donor horses involved in a plasmapheresis program, Am. J. Vet. Res. 73 (2012) 770–774.
[17] A. Tamborini, et al., Comparison of manual and laboratory PCV and total protein using EDTA and lithium heparin canine samples, J. Small Anim. Pract. 55 (2014) 258–264.
[18] M. Chigerwe, J.V. Hagey, Refractometer assessment of collostral and serum IgG and milk total solids concentrations in dairy cattle, BMC Vet. Res 10 (2014) 176.
[19] S.M. Deelen, et al., Evaluation of a Brix refractometer to estimate serum immunoglobulin G concentration in neonatal dairy calves, J. Dairy Sci. 97 (2014) 3838–3844.
[20] A. Gupta, S.L. Stockham, Refractometric total protein concentrations in icteric serum from dogs, J. Am. Vet. Med. Assoc. 244 (2014) 63–67.
[21] C. Gray, M. Rodriguez, K.L. Arheart, Use of refractometry for determination of psittacine plasma protein concentration, Vet. Clin. Pathol. 37 (2008) 438–442.
[22] H. Anderle, A. Weber, Rediscovery and revival of analytical refractometry for protein determination: recombining simplicity with accuracy in the digital era, J. Pharm. Sci. 105 (2016) 1097–1103.
[23] S. Wynn, et al., Evaluation and clinical validation of a handheld digital refractometer for urine specific gravity measurement, Pract. Lab. Med. 5 (2016) 65–74.
[24] Desirable specifications for total error, imprecision, and bias, derived from intra- and inter-individual biologic variation. Westgard QC website. (http://www.westgard.com/biodatabase1.htm) (accessed 2.27.16).
[25] Interference Testing in Clinical Chemistry, Approved Guideline – Second Edition, CLSI Document EP07-A2. Vol 25, No 27, Clinical and Laboratory Standards Institute, Wayne, PA, 2005.
[26] Palm Abbe Digital Refractometer, Blood – Human – Total Protein By Refractometer (TPr) Scale #108, MISCO: Solon, OH. (https://www.misco.com/build-your-own-handheld) (accessed 2.27.16).
[27] Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory. Approved Guideline – Third Edition, CLSI Document EP28-A3c. Vol 28, No 30, Clinical and Laboratory Standards Institute, Wayne, PA, 2010.
[28] Total Protein Gen.2 (TP2) Package Insert, 2012-03 V6. Roche Diagnostics; Indianapolis, IN, 2012.

[29] D.A. Dalenberg, P.G. Schryver, C.G. Klee, Analytical performance specifications: relating laboratory performance to quality required for intended clinical use, Clin. Lab. Med. 33 (2013) 55–73.

[30] J.W. George, S.L. O’Neill, Comparison of refractometer and biuret methods for total protein measurement in body cavity fluids, Vet. Clin. Pathol. 30 (2001) 16–18.

[31] J.P. Braun, J.F. Guelfi, J.P. Pages, Comparison of four methods for determination of total protein concentrations in pleural and peritoneal fluid from dogs, Am. J. Vet. Res. 62 (2001) 294–296.

[32] R.W. Light, Falsely high refractometric readings for the specific gravity of pleural fluid, Chest 76 (1979) 300–301.