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

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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Objectives: Refractometric methods to measure total protein (TP) in serum and plasma specimens have been replaced by automated biuret methods in virtually all routine clinical testing.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 (ddH_2O) 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 ddH_2O and high concentration patient pool. Decreased recovery was observed using ddH_2O dilutions at low TP concentrations. Significant interference was detected at high concentrations of glucose (> 267 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.

Abbreviations: ; ***ALB***, albumin; ***AMR***, analytical measurement range; ***ARUP***, Associated Regional & University Pathologists; ***BILI***, bilirubin; ***CAP***, College of American Pathologists; ***CLSI***, Clinical & Laboratory Standards Institute; ***CSF***, cerebrospinal fluid; ***CV***, coefficient of variation; ***ddH_2O***, demineralized distilled water; ***GLU***, glucose; ***Hb***, hemoglobin; ***IRB***, Institutional Review Board; ***LOQ***, limit of quantitation; ***NaCl***, sodium chloride; ***PT***, proficiency testing; ***QC***, quality control; ***RI***, reference interval; ***SD***, standard deviation; ***SG***, specific gravity; ***TAE***, total allowable error; ***TE***, total error; ***TP***, total protein; ***TRIG***, triglycerides

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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 innaccurate [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,