Clinical Biochemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Fecal electrolyte testing for evaluation of unexplained diarrhea: Validation of body fluid test accuracy in the absence of a reference method

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ARTICLE INFO

Article history:
Received 4 January 2015
Received in revised form 22 May 2015
Accepted 10 June 2015
Available online xxxx

Keywords:
Chronic diarrhea
Body fluid
Validation
Electrolytes
Cobas
ICP-OES

ABSTRACT

Background: Validation of tests performed on body fluids other than blood or urine can be challenging due to the lack of a reference method to confirm accuracy. The aim of this study was to evaluate alternate assessments of accuracy that laboratories can rely on to validate body fluid tests in the absence of a reference method using the example of sodium (Na^+), potassium (K^+), and magnesium (Mg^{2+}) testing in stool fluid.

Methods: Validations of fecal Na^+, K^+, and Mg^{2+} were performed on the Roche cobas 6000 c501 (Roche Diagnostics) using residual stool specimens submitted for clinical testing. Spiked recovery, mixing studies, and serial dilutions were performed and % recovery of each analyte was calculated to assess accuracy. Results were confirmed by comparison to a reference method (ICP-OES, PerkinElmer).

Results: Mean recoveries for fecal electrolytes were Na^+ upon spiking = 92%, mixing = 104%, and dilution = 105%; K^+ upon spiking = 94%, mixing = 96%, and dilution = 100%; and Mg^{2+} upon spiking = 93%, mixing = 98%, and dilution = 100%. When autoanalyzer results were compared to reference ICP-OES results, Na^+ had a slope = 0.94, intercept = 4.1, and R^2 = 0.99; K^+ had a slope = 0.99, intercept = 0.7, and R^2 = 0.99; and Mg^{2+} had a slope = 0.91, intercept = −4.6, and R^2 = 0.91. Calculated osmotic gap using both methods were highly correlated with slope = 0.95, intercept = 4.5, and R^2 = 0.97. Acid pretreatment increased magnesium recovery from a subset of clinical specimens.

Conclusions: A combination of mixing, spiking, and dilution recovery experiments are an acceptable surrogate for assessing accuracy in body fluid validations in the absence of a reference method.
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Introduction

Laboratories are often asked to analyze body fluid types that are not listed in the intended use claims per an assay manufacturer's packaging. Validation of such testing is of vital importance to ensure accurate results are produced, not to mention maintaining regulatory compliance. Laboratories may struggle in determining which experiments are necessary in order to meet these requirements. Sparse availability of certain body fluid types for validation studies compounds the challenge. Many laboratory accrediting agencies require validation that includes assessment of precision, accuracy, reportable range, reference interval, analytical sensitivity, and analytical specificity/interferences [1–3]. Most laboratories can adequately address validation for precision, reportable range, analytical sensitivity, and interferences in alternate specimen types. However, assessing accuracy and providing reference intervals can pose more of a challenge.

Quantifying electrolytes and osmolality in liquid stool specimens can be used to differentiate osmotic vs. secretory causes of chronic diarrhea [4,5]. Sodium and potassium concentrations are used to calculate an osmotic gap where a gap >50 mOsm/kg is suggestive of an osmotic etiology while <50 mOsm/kg suggests secretory [6]. Our laboratory recently validated a fecal electrolyte panel which includes measurements of sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), chloride (Cl^-), and phosphorus (PO_4^{3-}) performed on an automated chemistry analyzer. Together with calculated osmotic gap and osmolality determined using an osmometer, these form a comprehensive panel that can be used to differentiate among several causes of idiopathic chronic

Abbreviations: Na^+, Sodium; K^+, Potassium; Mg^{2+}, Magnesium; HCl, Hydrochloric acid; ICP-OES, Inductively coupled plasma-optical emission spectrometry; ISE, Ion-specific electrodes.

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http://dx.doi.org/10.1016/j.clinbiochem.2015.06.011
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Please cite this article as: Voskoboev NV, et al, Fecal electrolyte testing for evaluation of unexplained diarrhea: Validation of body fluid test accuracy in the absence of a reference method, Clin Biochem (2015), http://dx.doi.org/10.1016/j.clinbiochem.2015.06.011

diarrhea [7]. The previous method for Na^+, K^+, and Mg^{2+} quantitation in our laboratory employed inductively coupled plasma-optical emission spectrometry (ICP-OES) which is considered a reference method for measurement of metals in biological specimens [8]. Although accurate and precise, issues with instrument availability and cost led us to validate alternative testing on a chemistry autoanalyzer, adapting methods already used for urine testing.

Validating the accuracy of body fluid tests, especially when a standard reference method is not available, is potentially problematic. Thus, many laboratories have resorted to method comparison studies with laboratories that use similar platforms, or comparison to peer groups on proficiency testing surveys [9]. This approach could fail to detect issues with accuracy or identify interfering substances. In the current study, we describe an alternative approach to assess accuracy in a fecal fluid matrix using spike recovery, mixing recovery, and dilution recovery. The acceptability of this approach was assessed by comparison to a reference method (ICP-OES).

Materials and methods

Specimen processing and analytical methods

Validation was performed using residual liquid stool specimens submitted for clinical testing. The study was reviewed by the Mayo Clinic Institutional Review Board and deemed exempt. Specimens were received frozen, thawed at ambient temperature, and then blended (Waring Commercial 7011S, New Hartford, CT) for 5 minutes until homogenous. Specimens were then divided into two tubes. One aliquot was incubated 30 minutes with 6 N HCl, centrifuged at 3,000 rpm for 10 minutes, and the supernatant analyzed for Na^+, K^+, and Mg^{2+} using ICP-OES (PerkinElmer, Shelton, CT). The second aliquot was centrifuged at 14,000 rpm for 60 minutes, and the supernatant analyzed on a Roche cobas 6000 c501 (Roche Diagnostics, Indianapolis, IN) analyzer using MG2 reagent (Mg^{2+}) and indirect ISE (Na^+, K^+). Performance characteristics of the Na^+, K^+, and Mg^{2+} assays on the cobas c501 were verified and met the manufacturer's specifications for use with urine.

Experiments

Spiked recovery was performed by adding standard solutions of NaCl, KH_2PO_4, or $MgSO_4$ (<10% by volume) into fecal specimens post-centrifugation. Standard solutions were prepared by dissolving solid salts (NaCl (Sigma S7653-1 kg or Fisher S271-500), KH_2PO_4 (Fisher L-D539), $MgSO_4$ (Sigma–Aldrich M1880-1 kg)) in sterile water. Spike recovery was evaluated in three specimens for Na^+, six specimens for K^+, and eleven specimens for Mg^{2+}, using at least 3 different concentrations each. Serial dilution was performed using water (purified using Thermo Barnstead NANOpure Diamond system, Dubuque, IA to 18 mOhm) in five specimens for Na^+, eleven specimens for K^+, and six specimens for Mg^{2+}. Mixed recovery was performed by mixing fecal specimens with high and low analyte concentrations in equal volume ratios to create a series of five samples [10]. Five series were prepared for Na^+, eight for K^+, and four for Mg^{2+}. The concentrations of Na^+, K^+, and Mg^{2+} were measured in all samples using the cobas c501. A minimum of 50 specimens spanning the measuring ranges were analyzed for method comparison studies.

Data analysis

Mean (range) % recovery was calculated as (measured/expected × 100%) for mixing and dilution studies [11] where:

measured = analyte concentration measured on the cobas c501 in each diluted/mixed sample
expected (dilution) = undiluted analyte concentration/dilution factor

For mixed recovery, $[\text{analyte}]_{mid}$ is the concentration of the analyte in the 50:50 mixture of the high and low sample and (Δave) is the average difference in analyte concentration between consecutive samples in the series.

1. expected (mixed) = $[\text{analyte}]_{mid} - 2(\Delta\text{ave})$
2. expected (mixed) = $[\text{analyte}]_{mid} - (\Delta\text{ave})$
3. 50:50 mixed high/low = $[\text{analyte}]_{mid}$
4. expected (mixed) = $[\text{analyte}]_{mid} + (\Delta\text{ave})$
5. expected (mixed) = $[\text{analyte}]_{mid} + 2(\Delta\text{ave})$

Mean (range) % recovery was calculated as ((measured − initial)/added × 100%) for spiking studies taking into account the initial analyte concentration and the concentration and volume of spiking solution added which accounts for dilution. Clinically derived criteria used to assess acceptable assay performance were +/−5% for Na^+ and K^+ and +/−20% with analytical concordance >90% using 110 mg/dL as the clinical decision limit for Mg^{2+}. Linear regression analysis was performed on method comparison data to obtain a slope, intercept, and correlation coefficient (R^2). The osmotic gap was calculated as:

$$290\ \text{mOsm/kg} - 2 * ([Na^+] + [K^+])$$

[7,12]

Microsoft Excel (version 14.0, Microsoft, Redmond, WA, USA) with Analyse-It plug-in (version 3.53, Analyse-it Software, Ltd, Leeds, LS3 1HS, United Kingdom), OriginPro (version 9.0, OriginLab Corporation, Northampton, MA, USA), or JMP statistical software (version 9.0, SAS Institute, Cary, NC, USA) were used for all data analyses.

Results

The range of concentrations measured in fecal specimens over a 1-month period (October 2012) by the current reference method (ICP-OES) was used to assess the most appropriate application settings on the Roche cobas c501 for fecal electrolyte testing. Since the Na^+ concentrations ranged from 5 to 200 mmol/L (n = 121), K^+ from 5 to 160 mmol/L (n = 118), and Mg^{2+} from 5 to 120 mg/dL (2–49 mmol/L) (n = 82), the urine applications were more appropriate than the serum applications for this testing.

Comparison studies: Na^+ and K^+, and osmotic gap

The Roche urine Na^+ ISE method was compared to the ICP-OES method across the measuring range of 11–150 mmol/L. Linear regression analysis from this method comparison (n = 51) revealed a slope of 0.94, intercept of 4.1, and R^2 of 0.99 (Supplemental Fig. 1A). A negative proportional bias was offset by a positive constant bias. Similarly, a Bland–Altman plot revealed an overall mean bias of 3.4% (Fig. 1A). The percent differences ranged between −16% and 29% within the analytical measuring range. Marked positive percent differences were observed for the c501 at a low Na^+ concentration (below the limit of quantitation, 11 mmol/L). The urine K^+ ISE method was compared to the ICP-OES method across the measuring range of 2–165 mmol/L. Linear regression analysis from this method comparison (n = 50) demonstrated a slope of 0.99, intercept of 0.7, and R^2 of 0.99 (Supplemental Fig. 1B). Similarly, a Bland–Altman plot revealed a mean bias of 0.3% (Fig. 1B). The percent differences ranged from −16% to 38%.

The stool osmotic gap is a calculation that uses the Na^+ and K^+ concentrations in liquid stool to estimate osmolality. The Na^+ and K^+ concentration is doubled to account for unmeasured anions and subtracted from 290 mOsm/kg, the assumed normal stool osmolality [7]. Notably, measured osmolality is not reliable for calculating the gap since it is affected by fermentation by stool microbes during storage and transport, especially at ambient temperature, and for this reason, normal stool osmolality is assumed to be close to serum. However, the

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