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An approach to analytical validation and testing of body fluid assays for the automated clinical laboratory

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ABSTRACT

Background: Biochemical analysis of body fluids may lend insight into the pathogenesis of disease. Most commercially-available clinical laboratory methods are intended for blood-derived specimens and/or urine and laboratories must characterize the analytical performance of these methods for other specimen types such as body fluids. The aim of this work is to demonstrate one approach for characterizing analytical performance of assays for clinical analysis of body fluids.

Methods: Residual waste samples were obtained from clinically ordered testing. Validation studies were performed for 8 chemistry analytes on Roche Cobas 6000 c501 (Roche Diagnostics, Inc.) analyzers. Accuracy, precision, analytical measurable range, reportable range, analytic sensitivity, interferences, analyte stability were assessed. Laboratory workflow for body fluid handling was designed based on results obtained.

Results: Sample matrix interferences were not observed for the body fluids tested and assay reportable ranges used for serum were validated for body fluids. Dilution of body fluid specimens with saline demonstrated non-linear recovery of some enzyme activities. Assay imprecision was comparable to the manufacturer's claims for serum. The serum index thresholds for interference from hemoglobin and lipemia were lower for body fluids compared to manufacturer's stated limits for serum. Pretreatment of body fluid samples with hyaluronidase caused a 28% false increase in lipase activity, and 13% increase in total protein concentration. Ambient temperature analyte stability in body fluids was ≤ 24 h for most analytes compared to manufacturer's stated stability of 7 days for serum. In contrast to serum, lactate dehydrogenase (LDH) was labile in frozen body fluid specimens.

Conclusions: Validation of analytical methods for body fluid testing is a necessary exercise to exclude potential matrix effects and set pre-analytic specimen quality criteria.

1. Introduction

Non-standard body fluids often referred to simply as body fluids, are specimen types received and analyzed in clinical laboratories using assays in which the manufacturer has not listed that fluid type as acceptable in the “Intended Use” portion of their product insert. This typically includes those fluids not derived from blood or urine, which may include cerebrospinal fluid (CSF), drain fluids, wound fluids, and other fluids usually obtained by ultrasound guided aspiration (synovial, amniotic, pleural, peritoneal, pericardial, etc). The pathologic build-up of fluids in the body may occur as a consequence of a multitude of clinical abnormalities. Extravascular fluid volume increases due to an increase in fluid production or a decrease in fluid absorption. Increased fluid production may be caused by increases in intravascular hydrostatic pressure (i.e. congestive heart failure, kidney disease), decreased oncotic pressure (i.e. malnutrition, severe burns, nephrotic syndrome, liver cirrhosis), or increased capillary permeability (i.e. inflammation, infection, burns, nephritis). Decreased fluid absorption may occur due to lymphatic obstruction, often secondary to malignancy or impaired drainage due to elevations in systemic venous pressures (i.e. congestive heart failure) [1].

Body fluids have routinely been tested for a variety of analytes by the chemistry laboratory over a period of decades [2–8]. However, in recent years, laboratory accreditation agencies such as the College of

Abbreviations: LDH, lactate dehydrogenase; CSF, cerebrospinal fluid; LIS, laboratory information system; AMR, analytical measuring range; QC, quality control; CAP, College of American Pathologists; BSA, bovine serum albumin; SD, standard deviation; SAAG, serum ascites albumin gradient; BCG, bromcresol green; BF, body fluid; PT, proficiency testing; CV, coefficient of variation; PI, package insert

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American Pathologists (CAP) have increased their oversight of body fluid testing by incorporating a checklist specific to the assessment of body fluid analytical validation parameters including accuracy, precision, reportable range, analytic sensitivity and analytic interferences [9]. Although this checklist is helpful, there continues to be some trepidation and uncertainty within clinical laboratories due to the extra work required and the unknown complexity of such a validation. Despite previous work to analytically validate these assays [10–13], the scope has been fairly narrow within the individual reports. The purpose of this report highlights one approach to the analytical validation of multiple body fluid tests and specimen types which might be conducted when a laboratory initially validates several analyte and body fluid type combinations. Analytical validation includes studies which address accuracy (trueness), precision (repeatability), analytical measuring range (measuring interval), reportable range, analytic sensitivity, analytical specificity (interferences), and analyte stability. While reference intervals or decision limits are required to complete a validation, they are outside the scope of this publication. We also present the first report of a workflow for handling body fluid testing in an automated laboratory.

2. Materials and methods

2.1. Specimens and analyzers used for validation

Residual waste samples for validation were obtained from clinically ordered body fluid specimens which were collected in plain polypropylene or non-additive, non-gel blood collection tubes and were then analyzed and reported as part of routine clinical protocols at the Mayo Clinic (Rochester, MN). The studies decribed occurred from March 2010 until June 2011. All body fluids were centrifuged 10 min at 3200 rpm prior to analysis and studies were conducted within 7 days of receipt while in refrigerated storage. The most prevalent fluid types were identified via laboratory information system (LIS) queries over a six-month period between January–June 2009 and the more prevalent fluid types were prioritized over less prevalent fluid types. Studies were conducted for many analytes and body fluid types at the same time, therefore the physician order patterns and residual volume influenced the ability to conduct the studies. Consequently, the specimen types varied between the studies and the range of concentrations were limited in some cases but attempts were made to span the measuring range or assess presumed clinically relevant concentration ranges when available (Tables 1 and 2). All attempts were made to use individual specimens, however specimens of like type were pooled when increased volume was needed.

Validation was performed on Roche Cobas c501 (Roche Diagnostics, Indianapolis, IN) chemistry analyzers. Roche reagents were used for albumin (bromcresol green), amylase, creatinine (enzymatic), glucose, lactate dehydrogenase, total protein, urea nitrogen. For lipase, reagent was obtained from Genzyme -Sekisui Diagnostics (Framingham, MA). The assays reported herein have been validated to have acceptable performance when testing the approved matrix (ie. serum).

2.2. Accuracy (trueness)

To evaluate for the presence of non-serum matrix interference, accuracy (trueness) was indirectly assessed by calculating the percent analyte recovery after spiking (< 10% by volume) of three samples of each body fluid specimen type with four increasing concentrations of either a standard solution, calibration verification material, or serum with known concentration of analyte. Commercial linearity material was used to spike albumin, creatinine, glucose, total protein, and urea nitrogen (PreciLin, Roche Diagnostics, Inc.). Residual serum samples were used to spike lipase and LDH. A standard solution of amylase (1400 U/L) was prepared using lyophilized α-amylase (*Aspergillus oryzae*, Sigma-Aldrich). Concurrently, dilution control samples were prepared by adding an equal amount of deionized water to a similar volume of body fluid sample for comparative analysis. The mean ± SD % recovery was calculated for the 4 concentrations of spiked standard where %recovery = [spike conc − initial conc]/[theoretical spike conc] ∗ 100. Recoveries of 100 ± 10% were considered acceptable.

A second indirect method to assess accuracy was conducted by calculating analyte recovery upon serial dilution (experiments described in Section 2.4).

A method comparison for albumin was conducted in peritoneal and pleural fluids using the bromcresol green automated assay (Roche Diagnostics c501), nephelometry (BN™II, Siemens), and protein electrophoresis (SPIFE 3000, Helena Laboratories) with nephelometry considered the gold standard reference method (x-axis). These comparisons were conducted with body fluids over a period of 2 days (n = 20) and serum specimens monthly for 12 months. Mean CAP proficiency testing results (three surveys) for nephelometry (n = 10 to 12 laboratories) and Roche Cobas bromcresol green (n = 556 to 562 labs) albumin peer groups were plotted for comparison (surveys C 2012, A&B 2013). Results of the method comparison were analyzed with Deming regression and Bland-Altman bias plots. Albumin bias ≤0.1 g/dL was considered acceptable.

2.3. Precision and analytical sensitivity

Intra-assay precision was determined in a single run (n = 20) and inter-assay precision was assessed in a single run each day over a minimum of 20 days (n = 20) with one or more body fluid types and quality control (QC) material (Bio-Rad Laboratories, Unassayed Chemistry controls) at two concentrations. The manufacturer's specifications for precision in sera were used for comparison and CV's < 10% were considered acceptable. Analytical sensitivity was verified by assessing intra-assay precision at concentrations within 60% of the lowest reportable concentration stated in the manufacturer's package insert for each assay with CV's < 20% considered acceptable.

2.4. Analytical measurable range (AMR) and reportable range

The AMR for body fluid assays were adopted from the respective serum assays which had previously been verified using commercial linearity materials (PreciLin, Roche Diagnostics, Inc.). Conversely, linearity of body fluids were assessed using linear regression analysis calculating slope, y-intercept, and R^2 by plotting the measured (x-axis) vs expected (y-axis) of serially diluted body fluids (a minimum of 3 different types) with saline (2-fold, 4-fold, 8-fold, and 16-fold), which was the assay manufacturer's recommended diluent for all analytes. Slope 0.95–1.05, y-intercept < 50% of lower limit of AMR, and R^2 > 0.95 were considered acceptable. Pancreatic cyst fluid specimens were diluted in saline using an alternate scheme of (20-fold, 40-fold, 80-fold, and 160-fold) due to the increased pancreatic enzyme activity in this fluid type. In addition to saline, 7% bovine serum albumin (BSA) in saline (Ortho Clinical Diagnostics, Inc.; Cat no. 8262487), was used as a diluent for enzyme assays as it more closely represents a serum or plasma matrix. Pancreatic cyst fluid specimens were diluted (100-fold, 200-fold, 400-fold, and 800-fold) with 7% BSA solution. Samples with concentrations near the upper limit of the measuring range were selected to attempt to establish a maximum dilution, however samples with mid-measuring range concentrations were also included to represent instances when samples might be diluted for other reasons such as due to presence of interference or for troubleshooting purposes.

2.5. Analytical specificity (interferences)

The effect of hemolysis (measured as H-Index on Roche Cobas 6000 c501) was assessed by adding known concentrations of hemoglobin to three body fluid samples, as follows: albumin in pericardial, drain and pleural fluids; amylase and total protein in drain, peritoneal and pleural