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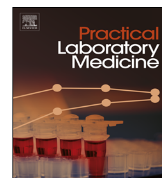

Filling in the gaps with non-standard body fluids ☆

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ABSTRACT

Objectives: Body fluid specimens other than serum, plasma or urine are generally not validated by manufacturers, but analysis of these non-standard fluids can be important for clinical diagnosis and management. Laboratories, therefore, rely on the published literature to better understand the validation and implementation of such tests. This study utilized a data-driven approach to determine the clinical reportable range for 11 analytes, evaluated a total bilirubin assay, and assessed interferences from hemolysis, icterus, and lipemia in non-standard fluids.
Design and methods: Historical measurements in non-standard body fluids run on a Beckman Coulter DxC800 were used to optimize population-specific clinical reportable ranges for albumin, amylase, creatinine, glucose, lactate dehydrogenase, lipase, total bilirubin, total cholesterol, total protein, triglyceride and urea nitrogen run on the Beckman Coulter AU680. For these 11 analytes, interference studies were performed by spiking hemolysate, bilirubin, or Intralipid® into abnormal serous fluids. Precision, accuracy, linearity, and stability of total bilirubin in non-standard fluids was evaluated on the Beckman Coulter AU680 analyzer.
Results: The historical non-standard fluid results indicated that in order to report a numeric result, 4 assays required no dilution, 5 assays required onboard dilutions and 2 assays required both onboard and manual dilutions. The AU680 total bilirubin assay is suitable for clinical testing of non-standard fluids. Interference studies revealed that of the 11 total AU680 analyte measurements on non-standard fluids, lipemia affected 1, icterus affected 3, and hemolysis affected 5.
Conclusions: Chemistry analytes measured on the AU680 demonstrate acceptable analytical performance for non-standard fluids. Common endogenous interference from lipemia, icterus, and hemolysis (LIH) are observed and flagging rules based on LIH indices were developed to help improve the clinical interpretation of results.
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1. Introduction

Analysis of abnormally accumulated body fluids is used to diagnose and manage the pathological conditions underlying their formation. To date, most commercial chemistry assays are only FDA-approved for serum, plasma, and urine, with a few exceptions for cerebrospinal and pleural fluids such as glucose, lactate and pH. Individual clinical laboratories interested in

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offering non-standard fluids testing must, therefore, complete extensive studies to evaluate assay performance characteristics with alternative fluid types. For some laboratories, attaining regulatory compliance has impeded or ceased the implementation of this important clinical testing service. In particular, laboratory accreditation agencies, such as the College of American Pathologists, regard analysis of these abnormal body fluids as a laboratory-developed test and require validation of accuracy, precision, analytical sensitivity, analytical specificity, interferences, and reportable ranges [1]. Furthermore, demonstration of clinical claims and commutability between non-standard fluids and FDA-cleared specimen types is needed.

To improve resource utilization in meeting these complex regulatory requirements, laboratories can limit their testing menu of non-standard fluid analytes to those with clinical significance [2,3]. For example, Light's criteria includes measurement of total protein and lactate dehydrogenase in pleural effusions to differentiate transudate and exudate. These two types of effusions have distinct analyte profiles: transudates form as an ultrafiltrate caused by imbalance of hydrostatic or oncotic pressure and exudates form due to local inflammation leading to increased capillary permeability or impaired lymphatic fluid reabsorption. Clinical management of these pathological fluid accumulation depends on correct classification. Similarly, albumin concentrations in serum are compared to albumin concentrations in peritoneal effusions, known as serum ascites albumin gradient (commonly referred to as “SAAG”), to differentiate clinical conditions related to changes in portal hypertension. Many other analytes have also demonstrated clinical utility, albeit less general, such as cholesterol and triglyceride for chylothorax and pseudochylothorax, amylase and lipase for pancreatitis, creatinine and urea nitrogen for urinary leakage, glucose for parapneumonic or malignant effusions, and total bilirubin for detection of biliary leaks.

Validating non-standard fluids for chemistry testing is challenging and requires strategic planning. The limited published studies on non-standard fluid validation have provided useful, but incomplete, practical guidelines for clinical laboratories to design and complete their own validations [4–6]. In the present study, our objective was to address some of these literature gaps. Specifically, we established a data-driven approach to determine the clinical reportable range for non-standard fluid analytes, evaluated the total bilirubin assay for non-standard fluids, and assessed interferences from hemolysis, icterus, and lipemia on the performance of 11 non-standard fluid analytes measured on the Beckman Coulter AU680 chemistry analyzer.

2. Materials and methods

2.1. Specimens, instrument and chemistry assays

This quality improvement project, conducted at the University of Washington Medical Center (UWMC, Seattle, WA), was granted an exemption by the UW Medicine institutional review board. Residual ascites, pelvic, pericardial, and pleural fluids submitted to UWMC for testing were used to evaluate the performance characteristics of 11 Beckman Coulter AU680 serum chemistry assays for non-standard fluids. The 11 assays were: albumin, amylase, bilirubin (total), cholesterol (total), creatinine, glucose, lactate dehydrogenase, lipase, total protein, triglyceride, and urea nitrogen. Specific test parameters were programed for each assay in order to define different onboard dilution factors for non-standard fluids relative to serum. The remaining test parameters (ie. – sample volume, reaction monitoring, associated calibration curve or enzyme blank, etc.) programmed for non-standard fluids were configured identically to the specific test parameters recommended by the manufacturer for serum except that “other-1″ was designated for specimen type. All non-standard fluid specimens were stored at 4 °C and utilized within 2 months of collection. Prior to analysis on the Beckman Coulter AU680 chemistry analyzer, all specimens were visually inspected and filtered through a 200–300 µmol filter (Fisher Scientific, No. 1,138,750) or centrifuged at 4500*g* for 10 min to remove large debris.

2.2. Laboratory data and clinical reportable range

Historical results for the 11 non-standard fluid analytes generated between January 1st, 2013 and December 31st, 2014 using the Beckman Coulter DxC800 were retrieved from the laboratory information system (Sunquest, Version 7.1). Prior to analysis, these results were de-identified after excluding specimens submitted without a description or for proficiency testing. The AU680 onboard dilution factor (0, 2, 3, 5, or 10; manufacturer recommends less than 5-fold) and pre-defined manual dilution factor (0, 2, 3, 11, or 51) for each analyte were selected to include the majority of historical results.

2.3. Total bilirubin

The performance characteristics for all 11 AU680 assays for non-standard fluids were evaluated, but only total bilirubin is described here (information on remaining AU680 assays can be found in Lin et al. [5]). Intraday and interday precision studies were performed using 2 ascites and 2 pleural fluids, with low and high bilirubin concentrations collected from 4 different individuals. Each non-standard fluid specimen was analyzed 20 consecutive times to obtain the intraday precision and 4 consecutive times per day for 5 consecutive days to obtain the interday precision. To define the lower limit of blank, the mean plus 3 SD was calculated from 20 consecutive measurements of a 0.9% saline solution. For the analytical measurement range, Verichem Bilirubin Standards A (0.565 mg/dL) and F (28.4 mg/dL) were diluted into an ascites fluid

Table 1
Analysis of historical results for 11 analytes measured in non-standard fluids over a 2-year period. With the exception of lipase, all results above the upper limit of onboard measurement range were manually diluted until a numerical number within the analytical measurement range was obtained. OB range: onboard measurement range; and CRR: clinical reportable range.

		Beckman Coulter DxC800			Results					Distribution of numerical results		
Non-standard fluid analyte	Unit	Lower CRR	Upper OB range	Upper CRR	N	< Lower CRR (%)	Numerical (%)	> Upper CRR (%)	Manual dilution (%)	Minimum	Median	Maximum
Albumin	g/dL	1.0	6.0	N/A	998	54.0	46.0	0.0	0.0	1	1.7	3.4
Amylase	U/L	5	2400	N/A	1009	5.1	94.9	0.0	18.7	5	48.5	804,465
Bilirubin, total	mg/dL	0.1	30	N/A	223	7.2	92.8	0.0	5.4	0.1	2.5	123.5
Cholesterol, total	mg/dL	5	972	N/A	99	1.0	99.0	0.0	0.0	6	52.5	145
Creatinine	mg/dL	0.1	25	N/A	1012	0.1	99.9	0.0	2.6	0.1	1.1	145
Glucose	mg/dL	10	1200	N/A	1908	3.4	96.6	0.0	0.0	10	101	4560
Lactate Dehydrogenase	U/L	5	2700	N/A	1054	5.1	94.9	0.0	2.9	6	160.5	47583
Lipase	U/L	10	396	> 396	87	4.6	67.8	27.6	0.0	11	24	396
Total protein	g/dL	1.0	12	N/A	1659	17.4	82.6	0.0	0.0	1	2.9	8.8
Triglyceride	mg/dL	10	1000	N/A	348	15.5	84.5	0.0	5.8	10	55	11640
Urea nitrogen	mg/dL	1	300	N/A	75	26.7	73.3	0.0	0.0	1	10	150

Table 2
Optimized Beckman Coulter AU680 AMR, onboard dilution, manual dilution and clinical reportable range for 11 analytes measured in non-standard fluids. Simulated results were computed by applying these optimized AU680 parameters to the 2-year historical results. *Due to the lower DxC clinical reportable range (CRR), the number of numerical results and results greater than the upper limit of CRR for lipase could not be calculated. AMR: analytical measurement range; OB dilution: onboard dilution; and CRR: clinical reportable range.

		Beckman Coulter AU680					Results (Simulated)				
Non-standard fluid analyte	Unit	Lower AMR/CRR	Upper AMR	OB dilution	Manual dilution	Upper CRR	*N*	< Lower CRR (%)	Numerical (%)	> Upper CRR (%)	Manual dilutions (%)
Albumin	g/dL	1.0	6	N/A	N/A	6	998	54.0	46.0	0.0	0.0
Amylase	U/L	10	2000	× 5	× 51	102,000	1009	12.9	85.0	2.1	12.3
Bilirubin, total	mg/dL	0	30	× 3	N/A	90	223	0.0	99.1	0.9	0.0
Cholesterol, total	mg/dL	25	700	N/A	N/A	700	99	16.2	83.8	0.0	0.0
Creatinine	mg/dL	0.2	25	× 5	N/A	125	1012	0.2	99.6	0.2	0.0
Glucose	mg/dL	10	800	× 3	N/A	2400	1908	3.4	96.5	0.1	0.0
Lactate Dehydrogenase	U/L	25	1200	× 5	× 51	61,200	1054	6.2	93.8	0.0	1.0
Lipase	U/L	10	600	N/A	N/A	600	87	4.6	N/A*	N/A*	0.0
Total protein	g/dL	1	12	N/A	N/A	12	1659	17.4	82.6	0.0	0.0
Triglyceride	mg/dL	10	1000	× 5	N/A	5000	348	15.5	83.3	1.2	0.0
Urea nitrogen	mg/dL	2	130	× 3	N/A	390	75	36.0	64.0	0.0	0.0

with low bilirubin concentration to produce a dilution curve that contained 9 data points (concentration 0.53–28.4 mg/dL), with water as the zero blank. Each linearity specimen was measured in triplicate. The accuracy of total bilirubin measured in non-standard fluids was determined using a recovery study whereby a high bilirubin serum specimen was spiked into a low bilirubin ascites fluid at a volume ratio of 1 to 9. Percent recovery was calculated by dividing the measured total bilirubin by the expected total bilirubin concentration and then multiplying by 100. The stability of bilirubin in non-standard fluids at 4 °C was determined using 3 residual ascites fluids received on the day of collection. Total bilirubin was quantified for 5 consecutive days following collection, starting with day 0. Since our laboratory policy does not include precautionary protocols to shield non-standard fluid specimens from light, specimens for the bilirubin stability study were not protected from light exposure. Percent recovery, calculated by dividing the average measured total bilirubin at a given time point by the average measured total bilirubin on the initial day of collection and multiplying by 100, was used to assess analyte stability.

2.4. Interference studies

Interference studies for the 11 analytes were performed on 2 non-standard fluids – pericardial fluid from 1 individual and pooled ascites fluids from 2 individuals – to obtain a high and a low concentration for each analyte. To mimic interference from endogenous hemoglobin, hemolysates were prepared. Red blood cells (RBCs) were separated from plasma by centrifugation at 4500*g* for 10 min then washed 10 times with 0.9% saline. The washed RBCs were lysed by 3 freeze-thaw cycles and the supernatant was collected after pelleting cellular debris by centrifugation after each cycle. The hemoglobin concentration in the supernatant (22 g/dL) was quantified using the Radiometer ABL90 blood gas analyzer (Radiometer) after a 20-fold dilution with water. To replicate icterus, a bilirubin solution was prepared by dissolving bilirubin powder (Sigma-Aldrich, No. B4126) in 0.1 M NaOH. After 1-h incubation at 4 °C with periodic mixing, bilirubin concentration (2.4 g/mL) of the stock solution was measured using the total bilirubin assay on the AU680 after a 100-fold dilution with water. To simulate lipemia, 20% Intralipid® was used (Kabivitrum Inc.) [7]. The Intralipid® concentration (47,500 mg/dL) was established using the triglyceride assay on the AU680 after a 100-fold dilution with water.

The degree of analyte interference in non-standard fluids due to hemolysis was determined by spiking water or hemolysate into 1 mL of non-standard fluid at varying volumes, without exceeding 5% of total volume. A series of 6–7 paired specimens were produced, where one set of specimens contained a range of hemoglobin concentrations that spanned the hemolysis index of the AU680, while the other set compensated for any dilutional effects. Each specimen was measured one time. The degree of hemolysis interference was analyzed by calculating the absolute and percent difference between the hemolyzed and non-hemolyzed paired specimens for the 11 analytes. A similar approach was applied to examine icterus and lipemia analytical interference, except that 0.1 N NaOH was used to correct for any dilutional effects in the icterus study and 2 mL of non-standard fluid was used in the lipemia study. The concentrations of hemoglobin, total bilirubin, and triglyceride evaluated ranged from 33–641 mg/dL, 0.3–39.5 mg/dL, and 55–1576 mg/dL, respectively.

Lipemia, icterus, and hemolysis (LIH) indices were also measured on all specimens to develop LIH-based flagging rules. These rules were used to determine test cancellation, specimens requiring ultracentrifugation prior to analysis, and results needing an interpretative comment prior to release into the electronic medical record. The AU680 LIH indices are semi-quantitative estimates of lipid turbidity, bilirubin, and hemoglobin concentrations in a specimen to replace visual grading system. To obtain the LIH indices, the Beckman Coulter AU680 analyzer measures the absorbance of a saline diluted specimen at 6 unique wavelengths. These spectrophotometric results approximate the levels of lipid turbidity, bilirubin, and hemoglobin, which have distinct absorption spectra (Supplemental Table I).

2.5. Statistics

Data analysis and calculations were performed using Microsoft Excel (Version 2010, Microsoft, Seattle, WA), GraphPad Prism (Version 6.0, *GraphPad* Prism Software for Science, San Diego, CA, USA), and EP Evaluator (Build 11.1.0.26, Data Innovations, LLC, South Burlington, VT).

3. Results

3.1. Optimization of Beckman Coulter AU680 measurement ranges for non-standard fluid analytes

Our previously published data-driven approach to optimize dilutions parameters of chemistry analytes in serum/plasma specimens was applied to non-standard fluids [8]. Specifically, historical results for the 11 non-standard fluid analytes measured on the Beckman Coulter DxC analyzers validated for non-standard fluids (Table 1) were used to develop optimized population-specific analytical measurement range (AMR), onboard (OB) and manual dilution factors, and clinical reportable range (CRR) for the Beckman Coulter AU680 analyzer (Table 2). For example, the reported concentration of fluid amylase measured using the DxC ($N=1009$) ranged from 5 to 804,465 U/L over a 2-year period. A fraction of these results (5.1%) was below the lower limit of the CRR and reported as "< 5″. Another fraction (18.7%) was above 2400 U/L, the upper limit of the OB measurement range, and required manual dilution to obtain a numerical value. Using this information, we aimed to maintain a similar reporting percentage of exact numerical results for the AU680 amylase assay while minimizing manual dilutions. For a validated AMR of 10–2000 U/L on the AU680, 5-fold OB and 51-fold manual dilution factors were selected to maximize the number of DxC results within the upper limits of OB measurement range and CRR. The optimized OB and manual dilutions were then applied to the historical data set to evaluate the impact on the AU680 workflow and reporting system. Our analysis showed that extending the amylase OB measurement range to 10,000 U/L eliminated the need for 39% of manual dilutions ($N=74$). Furthermore, the CRR of 10–102,000 U/L for amylase would minimally impact reporting of results with 12.9% reported as below the CRR and 2.1% reported as above the CRR. Similar analysis was applied to the remaining 10 analytes to determine their optimized OB and manual dilution factors. Overall, of the 11 analytes, 7 require OB dilution, 2 require manual dilution, and 4 require no dilution protocol. Once we determined the optimized OB and manual dilution factors, we validated these dilution factors by confirming that the corrected dilution results were within 10% and 15%, respectively, of the neat results. Taking all 11 analytes together, implementing the optimized AU680 AMR and dilution factors reduced the number of manual dilutions by 50% while minimally increasing the percentage of

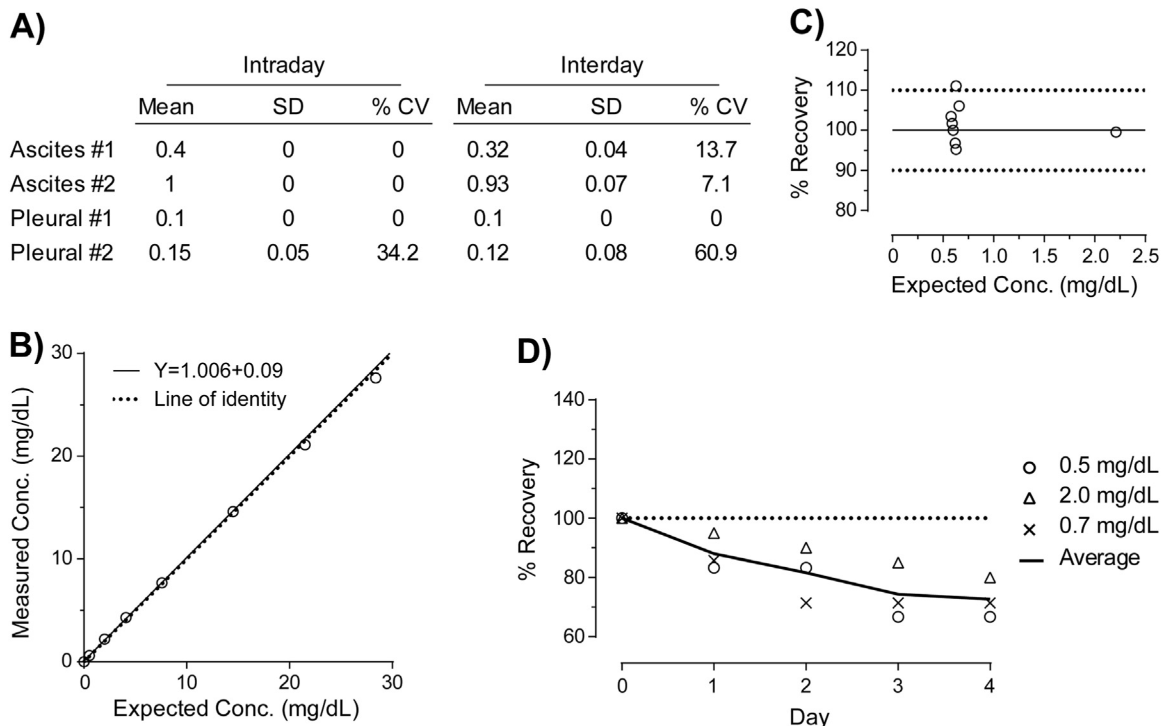

Fig. 1. Validation of total bilirubin assay for non-standard fluids on Beckman Coulter AU680. A) Intraday and interday precision of the total bilirubin assay using 2 ascites and 2 pleural fluids, with low and high bilirubin concentrations(N=20). B) Verichem Bilirubin Standards A (0.565 mg/dL) and F (28.4 mg/dL) diluted into an ascites fluid with low bilirubin concentration to produce a dilution curve with water as zero blank. C) Recovery study from spiking 9 high bilirubin serum specimens into a low bilirubin ascites fluid at 1:9 vol ratio (N=9). The average bilirubin concentration and recovery were 0.59 mg/dL and 101.7%, respectively. D) Stability of bilirubin in ascites fluids was examined for 5 consecutive days (N=3). Recovery was calculated by comparing bilirubin concentration measured at each time point to the initial bilirubin concentration measured on day 0.

results below and above the CRR.

3.2. Validation of Beckman Coulter AU680 total bilirubin assay for non-standard fluids

Analytical validation of the total bilirubin assay for non-standard fluids included precision, accuracy, linearity, and analyte stability. The intraday and interday precision coefficients of variation (CVs) for total bilirubin are listed in Fig. 1A. With the exception of pleural fluid #2, which had large apparent CVs due to a combination of low endogenous bilirubin concentration and results being reported only to 1 decimal point, the observed CVs were all within the precision criteria. The analytical measurement range (AMR) for total bilirubin was validated from 0.0 to 30.0 mg/dL with lower limit of the blank at 0.0 mg/dL (Fig. 1B). Once we established assay precision and linearity, accuracy was evaluated with a spike-and-recovery experiment. The average total bilirubin percent recovery of 9 different serum specimens mixed with a single ascites fluid at a 1 to 9 ratio (v/v) was calculated to be 101.7% (Fig. 1C). Based on 3 different ascites fluids stored at 4 °C, total bilirubin in non-standard fluids showed average percent recovery of 74.3% at 3 days (Fig. 1D).

3.3. Development of LIH flagging rules for non-standard fluid analytes

The bias observed from lipid, bilirubin, and hemoglobin interferences on 11 analytes in two different non-standard fluids are summarized in Table 3. For amylase, total cholesterol, and lipase, one of the paired specimens was excluded from the bias analysis because the low endogenous analyte concentration (below the AMR) caused a large apparent percent difference (Supplemental Fig. 1). Despite the significant bias observed for total cholesterol in the presence of hemoglobin, we still excluded the low endogenous analyte specimen from the analysis because the parallel bias was observed for the high analyte specimen.

3.3.1. Lipemia

Albumin was the only analyte affected by the addition of Intralipid® (Table 3). The interference was determined to be proportional to the concentration of Intralipid® (Supplemental Fig. 1) and independent of endogenous albumin concentration (Supplemental Fig. 2). A ≥ 2 L index-flagging rule was implemented to ultracentrifuge the specimen prior to measurement (Table 4).

Table 3
Biases observed with lipemia, icterus, and hemolysis interferences on 11 Beckman Coulter AU680 chemistry assays in non-standard fluids. Endogenous starting analyte concentrations of the pooled ascites (top) and pericardial fluids (bottom) with no detectable lipemia, icterus, or hemolysis were used in this experiment. *Analyte excluded from analysis due to low endogenous concentration. α: proportional; ↑: positive bias observed; ↓: negative bias observed; ALB: albumin; AMY: amylase; TBIL: bilirubin, total; CHOL: cholesterol, total; CRE: creatinine; GLU: glucose; LDH: lactate dehydrogenase; LP: lipase; TP: total protein; TRIG: triglyceride; and UN: urea nitrogen.

		ALB	AMY	TBIL	CHOL	CRE	GLU	LDH	LP	TP	TRIG	UN
	Ascites	0.8	453	4.2	9*	1.72	123	577	1961	1.3	36	50
	Pericardial	2.5	9*	0.4	86	1.61	137	157	3*	3.6	38	23
		(g/dL)	(U/L)	(mg/dL)	(mg/dL)	(mg/dL)	(mg/dL)	(U/L)	(U/L)	(g/dL)	(mg/dL)	(mg/dL)
Lipemia	[Lipid]	α	No bias*	No bias	No bias*	No bias	No bias	No bias	No bias*	No bias	N/A	No bias
	Direction	↑										
Icterus	[Bilirubin]	No bias	No bias*	N/A	α	α	No bias	No bias	No bias*	α	No bias	No bias
	Direction				↓	↑				↓		
Hemolysis	[Hemoglobin]	α	No bias*	Variable	α	No bias	No bias	α	No bias*	α	No bias	No bias
	Direction	↑		Variable	↑			↑		↑		

Table 4
Lipemia, icterus, and hemolysis flagging rules for the 11 Beckman Coulter AU680 chemistry assays for non-standard fluids.

Analyte	**Hemolysis**	**Icterus**	**Lipemia**
Albumin	4 to 5-comment bias direction; 6-cancel	N/A	≥ 2-airfuge prior to measurement
Amylase	N/A	N/A	N/A
Bilirubin, total	≥ 3-comment interference	N/A	N/A
Cholesterol, total	1–3-comment bias direction; ≥ 4-cancel	≥ 2-cancel	N/A
Creatinine	N/A	≥ 3-comment bias direction	N/A
Glucose	N/A	N/A	N/A
Lipase	N/A	N/A	N/A
Lactate Dehydrogenase	2-comment bias direction; ≥ 3-cancel	N/A	N/A
Total protein	≥ 3-comment bias direction	≥ 3-cancel	N/A
Triglyceride	N/A	N/A	N/A
Urea nitrogen	N/A	N/A	N/A

3.3.2. Icterus

The following assays displayed interference proportional to bilirubin concentration: total cholesterol, creatinine and total protein (Table 3 and Supplemental Fig. 1). Total cholesterol and creatinine also exhibited bias that depended on the endogenous analyte concentrations, with cholesterol showing a negative and creatinine showing a positive bias as the analyte concentration increased (Supplemental Fig. 2). Total protein, in contrast, showed a proportional negative bias such that the magnitude was independent of the endogenous total protein concentration. A middleware rule was implemented to cancel total cholesterol and total protein when I index is ≥ 2 and 3, respectively, and to append a comment about the bias direction for creatinine when I index is ≥ 3 (Table 4).

3.3.3. Hemolysis

Hemolysis affected 5 of the 11 assays evaluated in non-standard fluids: albumin, total bilirubin, total cholesterol, lactate dehydrogenase and total protein (Table 3). For albumin, total cholesterol, lactate dehydrogenase, and total protein, interference was proportional, with the bias increasing with increased concentration of hemoglobin (Supplemental Figs. 1 and 2). Total bilirubin also exhibited a proportional interference from hemoglobin, but the direction of the bias was variable at the two different concentrations evaluated. Since bilirubin exists in complex forms, the variability in hemolysis interference likely reflects biological differences in the concentration of conjugated and unconjugated bilirubin forms in the two individual specimens [9]. H index-flagging rules included comments for bias direction for all 5 analytes and canceling tests for albumin, total cholesterol, and total protein (Table 4).

4. Discussion

With increasing regulatory oversight of laboratory-developed tests, validating commercial chemistry assays for non-standard fluids has evolved into a complex operation. In this study, we focused on expanding the validation outlined by Lin et al. for the Beckman Coulter AU680 analyzers to improve testing services for non-standard fluids. First, we used a data-driven approach in concert with analytical constraints to establish our population-specific clinical reportable ranges for analytes in non-standard fluids. Second, we assessed the performance specifications of total bilirubin in non-standard fluids. Third, we present the first published interference data in non-standard fluids examining the most common pre-analytical interferents (lipemia, icterus, and hemolysis).

A conundrum for laboratories validating non-standard fluid testing is the establishment of a clinical reportable range. There are published resources addressing the utility of non-standard fluid testing, but the precise context that quantitative results generated from non-standard fluids are used to guide clinical decisions are not generally specified. To overcome this limitation, one objective of this validation was to maximize reporting of exact numerical results for non-standard fluid analytes in order to accommodate the needs of the ordering providers. We approached this by optimizing the clinical reportable ranges for the AU680 using historical data to simulate the percentage of numerical and non-numerical results for the 11 non-standard fluid analytes. In addition, we monitored the changes in simulated manual dilutions to improve workflow efficiency.

Another focus of this validation was to establish if lipemia, icterus, and/or hemolysis interfered with the analytes measured in non-standard fluids and to use these data to develop analyte-specific LIH flagging rules. An analyte was considered unaffected by these substances when the percent differences between the paired specimens with and without an interferent was less than 15% (Supplemental Fig. 1). Due to the absence of industry standards or publications defining total allowable error for analytes in non-standard fluids, a 15% difference between paired specimens with and without the potential interfering substrate was selected as reasonable threshold. These data were then integrated with the published clinical utility to designate analyte specific LIH indices corresponding to the need to append an interpretive comment or when to cancel testing (Table 4). For example, a total cholesterol of 45 mg/dL in pleural fluid has been defined as a diagnostic cutoff to differentiate transudate and exudate [2]. Our data revealed that in the presence of bilirubin at 27.5 mg/dL (I index of 4), measured total cholesterol decreased from 86 to 43 mg/dL (Supplemental Fig. 2). As a precautionary measure, we cancel total cholesterol testing on all non-standard fluids with I index ≥ 2 to prevent misinterpretation of the laboratory result. Overall, several Beckman Coulter AU680 serum chemistry assays for non-standard fluids showed interference patterns with addition of exogenous bilirubin or hemoglobin, but minimal effect with addition of Intralipid®. It should be noted that our interference study used Intralipid® to simulate lipemia/turbidity since there are no available standard lipoprotein preparations that mimic the composition of physiologic lipids [7]. Therefore, we cannot exclude the possibility that clinical lipemia interferes with these assays in ways not predicted by our data.

Our validation data and interference studies with lipids, bilirubin, and hemoglobin for all 11 analytes revealed parallel assay performance for non-standard fluids and serum/plasma. Thus far, published studies using varying methodologies and platforms have not observed matrix effect from commonly received non-standard fluids for frequently requested chemistry tests [5,6]. Consequently, assumptions were generally made that the common pre-analytical interferents perturb testing of analytes in non-standard fluids in a similar manner to that of FDA-approved specimen types. Our data support this hypothesis and the emerging picture is that systematic matrix effects are minimal in non-standard fluid testing. Comparison of hemolysis index flagging and cancellation rules between serum/plasma and non-standard fluids showed good agreement with identical bias directions despite slight variation in the specific hemolysis index cutoffs. For example, serum total cholesterol is canceled when H index is ≥ 2 but non-standard fluid total cholesterol is only canceled when H index is ≥ 4, with positive interference bias comment appended to the laboratory results when H index is 1–3. These differences are a function of clinical indication rather than analytical robustness.

This study, along with the previous study by Lin et al., provides a blueprint for analytical validation of non-standard fluid analytes for the Beckman Coulter AU680 chemistry analyzer. More broadly, the validation concepts presented here can aid the implementation of this clinically important laboratory-developed testing for other chemistry analyzers.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.plabm.2016.03.003.

References

[1] CAP. COM.40620 Body Fluid Validation. October 2015.
[2] CLSI. Analysis of Body Fluids in Clinical Chemistry. Approved Guideline. C49-A. Vol. 27, No. 14. (March 2009).
[3] D.R. Block, A. Algeciras-Schimnich, Body fluid analysis: clinical utility and applicability of published studies to guide interpretation of today's laboratory testing in serous fluids, Crit. Rev. Clin. Lab. Sci. 50 (2013) 107–124, http://dx.doi.org/10.3109/10408363.2013.844679.
[4] D. Block, Practical guide to the analytical validation of body fluid chemistry testing, Mayo Med. Lab. Commun. (2013).
[5] M.J. Lin, C. Hoke, R. Dlott, T.S. Lorey, D.N. Greene, Performance specifications of common chemistry analytes on the AU series of chemistry analyzers for miscellaneous body fluids, Clin. Chim. Acta Int. J. Clin. Chem. 426 (2013) 121–126, http://dx.doi.org/10.1016/j.cca.2013.08.011.
[6] W.E. Owen, et al., Body fluid matrix evaluation on a Roche cobas 8000 system, Clin. Biochem. 48 (2015) 911–914, http://dx.doi.org/10.1016/j.clinbiochem.2015.05.012.
[7] CLSI. Hemolysis, Icterus, and Lipemia/Turbidity. Indices as Indicators of Interference in Clinical. Laboratory Analysis; Approved Guideline. C56-A. Vol. 32, No. 10. (July 2012).
[8] S.Y. Lo, G.S. Baird, D.N. Greene, A roadmap to defining the clinical reportable ranges of chemistry analytes: increasing automation efficiency and decreasing manual dilutions, Clin. Chim. Acta Int. J. Clin. Chem. http://dx.doi.org/10.1016/j.cca.2015.10.006.
[9] D.N. Greene, et al., Development of an equation to correct for hemolysis in direct bilirubin measurements, Clin. Chim. Acta Int. J. Clin. Chem. 429 (2014) 194–197, http://dx.doi.org/10.1016/j.cca.2013.12.023.