REVIEW

A Review of Current Practices and Future Trends in Body Fluid Testing

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Background: Body fluid testing in the clinical chemistry laboratory is a cornerstone in the diagnostic workup of pathological effusions. Laboratorians may not be aware of the preanalytical workflows used in the collection of body fluids though the value is evident whenever processes change or issues arise. The analytical validation requirements can vary depending on the regulations dictated by the laboratories' jurisdiction and accreditor requirements. Much of analytical validation hinges on how useful testing is to clinical care. Usefulness of testing varies with how well established and incorporated the tests and interpretation are in practice guidelines.
Content: Body fluid collections are depicted and described so clinical laboratorians have a basic appreciation of what specimens are submitted to the laboratory for testing. A review of validation requirements by major laboratory accreditation entities is presented. A review of the usefulness and proposed decision limits for common body fluid chemistry analytes is presented. Body fluid tests that show promise and those that are losing (or lost long ago) value are also reviewed.
Summary: The total testing process from collection to result interpretation can be complicated and easily overlooked by the clinical laboratory. This review aims to improve the understanding and awareness of collections, validation, result interpretation, and provide an update on recent trends.

IMPACT STATEMENT

The total testing process from collection to result interpretation can be complicated and easily overlooked by the clinical laboratory. This review provides an overview of common body fluid collection procedures to improve the understanding and awareness of collections, analytical validation requirements by major accreditation agencies to ensure regulatory compliance, fluid-specific interpretive information to guide utilization, and reviews current literature and provides an update on recent trends in body fluid testing.

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INTRODUCTION

Analytical validation and clinical utility are 2 key pillars that ensure quality in the field of laboratory medicine. Laboratory professionals are tasked with balancing regulatory requirements with clinical needs when it comes to accepting alternative specimen types for testing. Body fluid testing presents unique challenges in almost every phase of the total testing process, from ordering, collection, analysis, and interpretation. This is compounded by constraints in analytical verification studies for method performance due to specimen availability, lack of reference methods, and matrix variability. These challenges require evaluation of the complete picture as it relates to regulatory, analytical, and clinical needs to support diagnostic testing for these specimens.

Where Do Body Fluids Come From?

The preanalytical phase of body fluid testing can be somewhat of a mystery to the clinical laboratory, yet it is of utmost concern as it influences the quality of specimens received for testing. For example, much effort is spent educating phlebotomists and nurses on the best techniques for avoiding hemolysis and intravenous fluid contamination when collecting blood. Our concern for the collection of body fluids should be no different, however, the challenges are often unique compared to blood specimens. To this end, laboratorians should be aware of the processes and procedures used to collect body fluid specimens sent for testing. Additionally, laboratorians can be an asset in designing workflows to help communicate the appropriate collection-specific information including specimen containers, fluid volumes, labeling, etc. Figure 1 presents a flowchart of steps taken in the evaluation of a patient presenting with a pathologic effusion who undergoes a specimen collection procedure for diagnostic purposes. These procedures may be performed urgently in locations such as the emergency department or at the bedside for patients experiencing life-threatening symptoms. Ambulatory patients are typically sent to procedure areas where a variety of visualization modalities may be available and procedural specialists perform the collection.

The body fluid compartments that contain serous fluid are located within the space created between two mesothelial membranes (1). These membranes include the visceral membrane that surrounds the organ and the parietal membrane that surrounds the body wall. Each membrane is comprised of a secretory epithelial layer and a layer of connective tissue underneath. The visceral pleura, pericardium, and peritoneum surround each lung, the heart, and abdominal organs, respectively. The parietal pleura, pericardium, and peritoneum surround the chest wall, the mediastinum where the heart lies, and the abdominal wall, respectively. Between these visceral and parietal membranes, the pleural, pericardial, and peritoneal cavities form and normally hold a small volume of serous fluid. The fluid in this space serves to decrease surface tension and facilitate movement such as breathing. Under normal circumstances, the balance between hydrostatic (e.g., blood pressure) and oncotic (e.g., protein concentration) pressures contribute to a small volume of interstitial fluid production which is returned to circulation (e.g., vascular system) through the lymphatic system.

Pathologic Effusions

Pathological processes can lead to an imbalance in normal movement of fluid in and out of the interstitial space which forms a pathologic effusion. They result from an imbalance between hydrostatic and oncotic pressures, inflammation or infection increasing capillary permeability, and/or lymphatic obstruction by tumors impeding normal drainage of these compartments. The volume of fluid depends on the magnitude of imbalance and its duration. Excess volume of fluid

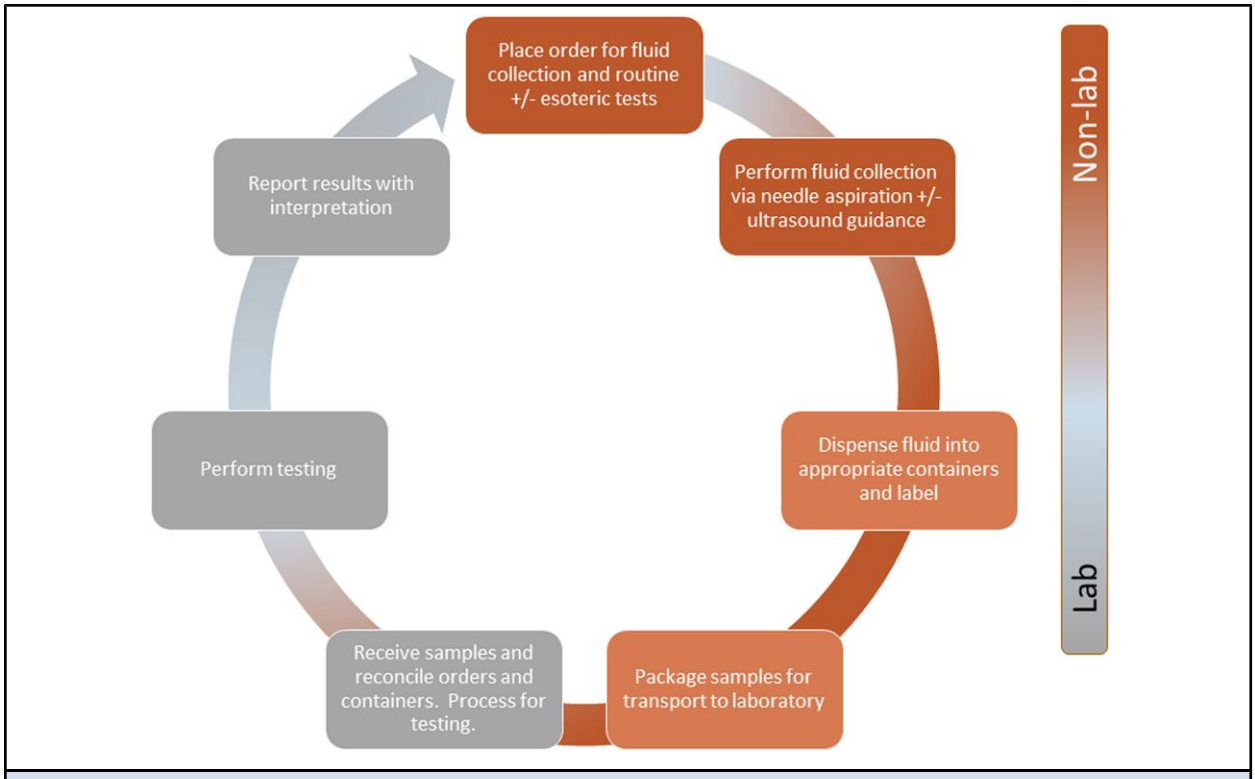

Fig. 1. Preanalytical steps taken by non-laboratory (orange) and laboratory staff (gray) in the collection of a body fluid specimen for the diagnostic workup of a pathologic effusion.

indicates a problem and can manifest with various symptoms. Removal of this excess volume of fluid can serve 2 purposes. First, to relieve symptoms such as shortness of breath (pleural effusion), early satiety (ascites), painful joint (synovial effusion), or cardiac tamponade (pericardial effusion). Second, the fluid can be tested for diagnostic purposes to help identify the underlying condition that may be causing the effusion to guide treatment.

In general, the fluid is collected through a catheter which enters the space containing the fluid percutaneously (through the skin) using ultrasound guidance. The path that the needle will follow is anesthetized using 1% lidocaine. The length of the catheter is procedure dependent, and the gauge of the catheter depends on the volume of fluid to remove. Most fluids collected for diagnostic purposes can be collected in a syringe. Once collected, it is best practice to dispense the fluid as soon as possible into containers that are appropriate for the desired tests to be transported to the laboratory. Chemistry analytes are usually stable in plain, nonadditive containers. For microbial culture of fluid specimens, blood culture bottles have been shown to significantly increase detection rates relative to conventional agar plate media or broth solutions (2–7). The use of fluid instead of blood in culture bottles may be outside of the bottle's approved indication so a documented comparison of culture bottles with conventional culture methods may be warranted. Culture bottles are ideally inoculated at bedside using sterile technique or sent in sterile containers for inoculation by the microbiology laboratory. Specimens sent for cell count and

Fig. 2. Percutaneous procedures use to collect: (A), pleural fluid via thoracentesis; (B), pericardial fluid via pericardiocentesis; (C), peritoneal fluid via paracentesis; (D), synovial fluid via arthrocentesis; (E), cerebrospinal fluid via lumbar puncture; (F), cyst fluids originating from pancreas; and (G), liver.
Panel A from Mayo Clinic Patient Education. Pleural Effusion (MC6380-01). Rochester, MN: Mayo Clinic, 2012; p. 5 used with permission of Mayo Foundation for Medical Education and Research, all rights reserved (9). Panel B from Mayo Clinic Patient Education. Pericarditis and Pericardial Effusion (MC5394). Rochester, MN: Mayo Clinic, 2004, p. 12; used with permission of Mayo Foundation for Medical Education and Research, all rights reserved (10). Panels C–G used with permission of Mayo Foundation for Medical Education and Research, all rights reserved.

differential should be dispensed into anticoagulant-containing tubes (e.g., liquid EDTA) to prevent clotting at the point of collection (8). Alternately, single containers of fluid may be sent, which the laboratory will accession, and the proper number of aliquots made using the appropriate container types.

Common percutaneous procedures used to collect body fluids include thoracentesis, paracentesis, pericardiocentesis, arthrocentesis, and lumbar puncture. Thoracentesis is the procedure to collect pleural fluid through a needle inserted into the posterior intercostal space typically just above the 7th to 9th rib from a patient that is leaning forward in a seated position. A typical volume of 50 to 100 mL of pleural fluid is removed with a syringe. Thoracentesis is performed for diagnostic purposes to identify the nature and potential causes of new-onset pleural effusion or for therapeutic purposes to alleviate dyspnea and other symptoms (Fig. 2, A) (9). Pericardiocentesis is the procedure to collect pericardial fluid under fluoroscopic, ultrasound, or echocardiographic guidance by inserting an 18-gauge needle or sheathed catheter into the pericardial space. Therapeutic pericardiocentesis is utilized to relieve symptoms of cardiac tamponade while diagnostic pericardiocentesis can help differentiate the cause of an effusion (Fig. 2, B) (10). Paracentesis is the procedure to collect peritoneal fluid using a 1.5 to 3.5 inch, 15- to 22-gauge needle advanced from below the umbilicus into the lower right or left quadrant. Diagnostic paracentesis is performed on patients presenting with new-onset ascites or to evaluate new symptoms concerning infection. Therapeutic paracentesis is performed to relieve symptoms related to increased intraabdominal pressure such as dyspnea and early satiety (Fig. 2, C). Arthrocentesis is the procedure to collect synovial fluid by aspirating a joint for diagnostic or therapeutic purposes. The procedure is common in emergency rooms to assess acutely painful, swollen hot joints for the diagnosis of septic and crystal-induced arthropathies (Fig. 2, D). Lumbar puncture (LP) is the procedure to collect cerebrospinal fluid (CSF) from the subarachnoid space of the spinal cord by inserting a needle between the 3rd and 4th or 4th and 5th lumbar vertebrae. A manometer is often placed on the spinal needle first to measure the opening pressure. The fluid is allowed to drain from the manometer before being removed and an additional 4 mL of CSF is collected for routine testing. Upwards of 40 mL is collected if cytology or cultures are desired. The CSF is allowed to drip from the spinal needle into sequentially numbered tubes. Urgent LPs are performed often in emergency departments when infection or hemorrhage are on the differential while nonurgent LP can be done in an ambulatory setting to investigate neurological symptoms of possible demyelinating disease or malignancy (11) (Fig. 2, E). Uniquely located fluid collections can be aspirated from almost anywhere in the body, often in conjunction with surgical procedures. These include abscess fluids which would be sent for culture and susceptibility testing, or cyst fluids to distinguish origin and potential for malignancy, for example, in the pancreas (Fig. 2, F) or liver (Fig. 2, G).

Managing Body Fluid Testing in the Clinical Laboratory

Radiologists performing imaging studies to localize disease within an organ before the whole body is impacted is akin to testing a sample of fluid from a localized compartment of the body that assists in the identification and treatment of the underlying pathologic process. Body fluid testing has been supported by laboratories for many decades. Many seminal studies were published as early as the 1970s which are part of current clinical guidelines and used by practitioners today as a standard part of care (6, 7, 12–14).

The conundrum for laboratories supporting these well-established practices is that many of the analytes measured are not included in the

intended use section of the assays by the manufacturers, which presents risk in offering the tests and requires validating them in compliance with accreditation standards as an alternate specimen type. There are a small number of analytes which are included in the intended use section of assays. They include some point-of-care tests, CSF protein, glucose, and lactate, body fluid cell count on automated hematology analyzers, and pleural fluid pH on blood gas analyzers. Conducting validation studies when few samples, often of low volume, are typically sent to the laboratory can make even straightforward work more challenging.

Some laboratories with more limited test menus may consider outsourcing these tests to larger laboratories that may service larger hospitals with critically ill patient populations and perform outreach testing to bolster volumes that justify the added work. There are a couple of caveats that are worth consideration. First, some analytes and sample types may not be stable enough to be transported, such as pleural fluid pH or body fluid cell counts. Second, in some patient populations and situations, a shorter turnaround time for results may be expected to make treatment decisions such as lactate dehydrogenase LDH) and total protein in pleural fluid to differentiate transudative from exudative effusions (15, 16). These decisions are not easy, nor will they be overwhelmingly popular with all stakeholders.

Analytical Validation

Once the laboratory decides to offer a test, it must be validated. Clinical laboratories typically seek accreditation to align with local and federal regulations. The Clinical Laboratory Improvement Act of 1988 493.1253(b)(2) (CLIA) provides the regulatory framework in the United States and Conformité Européenne (CE) marking is in use in the European Union, as examples. Accreditation demonstrates that the clinical laboratory meets these regulations from entities such as the College of American Pathologists (CAP), the Joint Commission (JC), the Commission on Laboratory Accreditation (COLA), or the International Organization for Standardization (ISO), as examples (17–20). A summary of validation expectations is provided in Table 1 which demonstrates they are rather similar overall, with the most notable differences arising in the nomenclature utilized by ISO compared to the other accreditors. One such example is accuracy, where ISO also includes trueness and uncertainty, whereas accuracy alone is referenced by COLA, CAP, and JC. Also, ISO does not mention reference interval, but instead suggests diagnostic sensitivity and specificity be evaluated or documented for a modified test. Interestingly all mention analytical sensitivity, which is eloquently defined as the slope of the calibration curve of a measuring system and clearly differentiated from detection limit or quantitation limit by the Clinical and Laboratory Standards Institute (CLSI) (21). Analytical sensitivity is not addressed by the CLSI documents referenced, perhaps because it is a term that is no longer promoted and functionally addressed with measurement range and other precision studies. Resources, such as previously published analytical validations and other guidelines, are available to assist clinical laboratories to meet these expectations (22, 31). Ultimately, these resources are intended to help clinical laboratories ensure they maintain quality work and report results that are accurate and actionable for clinical diagnostic purposes.

The CAP checklist contrasts the testing for routine tests and fluid types with unique sources. The CAP considers fluid analytes on the laboratory's test menu and orderable in the electronic health record as routine tests needing analytical validation in contrast to clinically unique specimens encountered infrequently where performing robust method performance studies would be challenging. It is not uncommon for laboratories to encounter situations when test requests are placed that involve a sample type that is not

Table 1. Summary of analytical validation requirements from major laboratory accreditation bodies.

	COLA	CAP	ISO	JC	CLSI reference to study design
Preamble	Prior to patient testing, the performance specifications for each the US Food and Drug Administration (FDA)-approved but modified, non-FDA approved, or in-house developed test system, must be established and documented for:	Methods for body fluid analysis have been validated or verified and metrics for interpretation have been established. This requirement applies directly to body fluid testing that the laboratory offers as a routine, orderable test.	The laboratory shall validate examination procedures derived from the following sources: (*a*) non-standard methods; (*b*) laboratory designed or developed methods; (*c*) standard methods used outside their intended scope; (*d*) validated methods subsequently modified.	When adding or replacing a modified test, method, or instrument, the laboratory establishes written performance specifications that include the following:	
Trueness			X		CLSI (21, 22)
Accuracy	X	X	X	X	
Precision	X	X	X (repeatability)	X	CLSI (23–25)
Uncertainty			X		
Reportable range	X	X	X (measuring interval)	X	CLSI (26)
Detection limit			X		CLSI (27)
Quantitation limit			X		CLSI (27)
Reference range	X	X			CLSI (28)
Analytical sensitivity	X	X	X	X	Unclear
Analytical specificity, including interfering substances	X	X	X	X	CLSI (29)
Diagnostic sensitivity			X		CLSI (30)
Diagnostic specificity			X		CLSI (30)
Notes	Any other performance characteristics required for accurate test performance	Any other performance characteristic required to ensure analytical test performance	The validation shall be as extensive as is necessary and confirm, through the provision of objective evidence	Modified tests, methods, or instruments include the following: (*a*) Test procedures with modifications to FDA–approved use for	

(continued)

Table 1. Continued

	COLA	CAP	ISO	JC	CLSI reference to study design
			(in the form of performance characteristics), that the specific requirements for the intended use of the examination have been fulfilled.	specimen type, reagents, instrument, procedural steps, or other components; (*b*) Tests or methods developed in the laboratory with no FDA evaluation; (*c*) Tests, methods, or instruments not subject to FDA clearance	
Reference	COLA (18)	CAP (17)	ISO (20)	JC (19)	

Abbreviations: ACOLA, Commission on Laboratory Accreditation; CAP, College of American Pathologists; ISO, International Organization for Standardization; JC, Joint Commission.

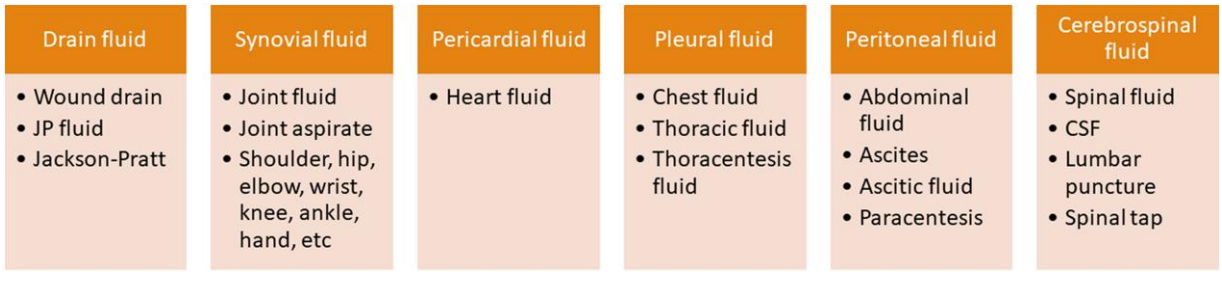

Fig. 3. Common body fluid aliases and synonyms encountered when accessioning body fluid orders.

routinely received in the laboratory. Sometimes it is a fluid type that comes with a specific name such as abdominal drain 1, perihepatic, pelvic, peripancreatic head/tail, etc. It is reasonable to suggest that if drain fluid or abdominal fluid is a validated source, then specifying drain 1, or some other detail, does not make the fluid unique and it can be tested under the laboratory's routine protocols. However, fluids such as perihepatic, peripancreatic head, or other very specifically named fluid, even though it may derive from an anatomical location that is validated (abdomen), the fluid could have unique properties and possibly unique reasons for measuring analytes that should be tested under the laboratory's unique specimen protocol. At minimum, this protocol should account for both the accuracy of the measurement and alternative interpretation of results. A serial dilution or spiking recovery study should be conducted to exclude matrix interference thus ensuring the analyte concentration is measured accurately. Several approaches to addressing accuracy and trueness for body fluids are published that outline spiking studies, mixing studies, interference studies, and stability assessment in more detail, as well as comparison of analyte concentrations within body fluids measured on 5 major chemistry platforms (32–35). Laboratories can choose to reference published literature where applicable and verify through studies those

aspects that may be patient, sample, or method specific. Emergent requests to test an unvalidated sample type and test combination are tricky to handle. Most requests, if clinically valid, can be accommodated by a reference laboratory or can be declined as investigational and lacking clinical validity. Most requests are declined once the requester understands the test is unvalidated and considered research use only whereby the results should not be used for medical decision-making. In those ultra-rare scenarios when testing is performed, these disclaimers should accompany the results.

The interpretation of results may not be well established so a disclaimer that states effectively "The reference range has not been established for this test in this fluid. Serial dilution/spiking recovery of the sample confirmed that [measurand] recovery was acceptable. The concentration of [measurand] in body fluids may be compared to serum and should be integrated into the clinical context for interpretation." Each laboratory should devise a list of fluids considered validated for routine testing along with the synonyms that staff can reference when deciding which fluids are indeed unique vs routine. Figure 3 summarizes commonly encountered body fluid source aliases for reference.

Body Fluid Test Utility and the Laboratory's Role in Guiding Interpretation

The clinical laboratory is responsible for providing a means to interpret results for clinical tests, and body fluid tests are not an exception. There are many challenges to consider and overcome to be successful. First, body fluids are rarely if not impossible to collect from healthy, normal individuals so the concept of establishing a reference interval is not applicable. The volume of fluid present in the spaces where it normally exists is simply too small to adequately visualize and collect safely. There are also ethics to consider, such as whether an institutional review board would consider the risk to the patient greater than the potential gains in knowledge. Healthy subjects consenting to venipuncture to donate blood to establish a reference interval is quite different than lumbar puncture to donate CSF for example. The next best alternative is an interpretation supplied by derivation of a decision limit. A decision limit is a concentration above or below which the sensitivity for detecting a condition or specificity for ruling out a condition can be ascertained. There are a few approaches to deriving decision limits once a definition of presence and absence of disease/condition is settled. The concentration of one or more analytes in the body fluid is compared to one or more of the following: an arbitrary concentration, the concentration considered "normal" in blood, or the concentration of a contemporaneously collected blood sample. When compared to a blood measurement, a ratio or gradient may be calculated. The utility of chemistry testing in body fluid samples has been covered extensively in several excellent resources and is beyond the scope of this review [see (36–39)]. Table 2 summarizes the interpretive information currently accepted for body fluids derived from widely recognized practice guidelines, consensus derived from meta-analysis of multiple studies, or single reports derived from single or multiple studies conducted independently.

Emerging Body Fluid Analysis

Laboratory medicine has seen testing trends change over time as new tests become available and evidence-based practices provide insight into their clinical utility. This has resulted in notable trends in body fluid testing practices which rise and fall as new research is published. Understanding this ebb and flow allows the field of laboratory medicine to critically evaluate new testing modalities for body fluids as they emerge.

Body Fluid Tests with Declining Utility

Fetal lung maturity testing in amniotic fluid. One prominent fluid analysis that has declined to the

Table 2. Summary of chemistry analytes measured in the diagnostic evaluation of pathologic effusions.

Analyte	Body fluid	Use	Decision limit	Reference type
Adenosine deaminase	Pleural	Surrogate marker to rule out tuberculous pleural effusion in low-incidence populations.	<40 U/L	Practice guideline Hooper et al. (6)
	Peritoneal	Indirect test for tuberculosis amongst patients with suspected tuberculosis and nonportal hypertension causes of ascites.	<35 U/L	Consensus and practice guideline Biggins et al., Tao et al. (7, 40)
	Pericardial	Indirect test for tuberculosis amongst patients with suspected tuberculous pericarditis and pericardial effusion.	<40 U/L	Consensus and practice guideline Adler et al., Xie et al. (14, 41)
Albumin	Peritoneal	Used in combination with serum albumin to calculate serum-ascites albumin gradient (SAAG) in patients presenting with their first episode of ascites.	SAAG ≥1.1 g/dL indicates portal hypertension.	Practice guideline Biggins et al. (7)
	Pleural	Used in combination with serum albumin to calculate serum-effusion albumin gradient in patients presenting with pleural effusion.	>1.2 g/dL is consistent with a transudative effusion in patients receiving diuretics.	Consensus Romero-Candeira et al. (42)
Amylase	Peritoneal including drain fluids	Evaluate pancreatic injury or disease contributing to ascites.	Approximately 5-fold greater than serum.	Single report Runyon et al. (43)
	Pleural	Evaluate uncommon causes of pleural effusion including esophageal rupture and pancreatitis, pancreatic pseudocyst, or pleural malignancy.	Concentrations greater than serum.	Practice guideline Hooper et al. (6)
Bilirubin	Peritoneal including drain fluids	Evaluate biliary injury or disease contributing to ascites including choleperitoneum or following liver transplant or other abdominal injury or surgery.	Fluid to serum ratio >1 is abnormal. >5 is highly sensitive and specific for biliary leak.	Single reports Darwin et al.; Runyon et al.; DeBenedet et al. (44–46)
Creatinine and/or urea nitrogen	All	Evaluate kidney, urinary tract, or bladder injury or disease following surgery whereby urinary leakage may be contributing to formation of effusion.	Fluid concentration > serum or ratio ≥1.2.	Consensus Manahan et al.; Toubes et al.; Karcher et al.; Wang et al.; Williams et al. (47–51)

(continued)

Table 2. Continued

Analyte	Body fluid	Use	Decision limit	Reference type
Cholesterol and triglyceride	Pleural	Differentiate chylothorax from pseudochylothorax in patients with pleural effusions that appear milky after centrifugation.	Chylothorax Trigyceride > 110 mg/dL, cholesterol usually low, crystals absent, chylomicrons usually present. Pseudochylothorax cholesterol >200 mg/dL (5.2 mmol/L), crystals often present, chylomicrons absent.	Practice guideline Hooper et al. (6)
	Peritoneal	Differentiate chylous from pseudochylous ascites in patients with peritoneal effusions that appear milky. Chylous ascites is caused when an intestinal lymphatic system is obstructed or damaged causing chylomicron-rich fluid to leak associated with malignant, inflammatory, or infectious processes, and trauma. Pseudochylous ascites is caused when an effusion remains in situ for a long duration often associated with malignant ascites.	Chylous ascites triglyceride > serum. malignant ascites triglyceride low; cholesterol > 46 to 93 mg/dL (1.2 to 2.4 mmol/L).	Consensus Burgess et al. (52)
Glucose	Pleural	In the absence of disease, pleural glucose is equivalent to blood concentrations. Low glucose is associated with complicated parapneumonic effusions, empyema, rheumatic pleurisy, tuberculosis infection, malignancy, and esophageal rupture. Very low concentrations are often associated with rheumatoid arthritis and empyema.	Low glucose <61 mg/dL (3.4 mmol/L). Very low glucose <30 mg/dL (1.6 mmol/L).	Practice guideline Hooper et al. (6)
	Pericardial	Differentiate purulent (very low glucose) from tuberculous or malignant pericarditis.	Fluid to serum ratio <0.5.	Practice guideline Adler et al.; Meyers et al. (14, 53)
	Synovial	Differentiate septic arthritis (low glucose) from other causes.	Normal synovial fluid glucose is within 10 mg/dL of fasting serum concentration.	Consensus Margaretten et al. (54)
	Amniotic	Low glucose increases risk of intrauterine infection and inflammation in patients with premature rupture of membranes.	<10 to 16 mg/dL (0.6 to 0.9 mmol/L).	Single reports Kacerovsky et al.; Gonzalez-Bosquet et al. (55, 56)

(continued)

Table 2. Continued

Analyte	Body fluid	Use	Decision limit	Reference type
	CSF	Differentiate causes of meningitis. Decreased ratio indicates bacterial or fungal infections.	Normal CSF/serum ratio: >0.4 to 0.5 Pyogenic: low *L. monocytogenes*: normal Aseptic (viral): normal Tubercular: low Fungal: low	Practice guideline Deisenhammer et al.; Bamberger et al. (57, 58)
Lactate dehydrogenase (LDH)	Pleural	Differentiate transudative from exudative pleural effusions.	Exudate Fluid to serum ratio > 0.6 or > 0.67 the upper limit of normal serum LDH.[a]	Clinical guideline Hooper et al. (6)
	Peritoneal	Differentiate spontaneous from secondary bacterial peritonitis.	Not recommended.	Clinical guideline EASL (59)
	Synovial	Differentiate septic arthritis from other causes.	>1900 U/L (AUC: 0.82)	Single report Lenski et al. (60)
Lipase	Peritoneal including drain fluids	Evaluate pancreatic injury or disease contributing to ascites.	Hypothetically similar to amylase.	No reports
pH	Pleural	Parapneumonic effusion requiring tube drainage.	< 7.2	Clinical guideline Hooper et al. (6)
Total protein	Pleural	Differentiate transudative from exudative pleural effusions.	Exudate fluid to serum ratio > 0.5.[a]	Clinical guideline Hooper et al. (6)
	Peritoneal	Measured in patients presenting with their first episode of ascites. Low protein predisposes cirrhotic patients to develop spontaneous bacterial peritonitis.	>2.5 g/dL (25 g/L) suggests a cardiac source of ascites <1.5 g/dL (11 g/L).	Practice guideline Biggins et al. (7)
	CSF	Blood–CSF barrier integrity assessment. High protein is associated with bacterial meningitis.	Normal: <45 g/dL, varies with age and method Pyogenic: >100 mg/dL *L. monocytogenes*: >50 mg/dL Aseptic (viral): <200 mg/dL Tubercular: >100 mg/dL Fungal: varies	Practice guideline Deisenhammer et al.; Bamberger et al. (57, 58)

[a]In combination with total protein and LDH.

point of near extinction in recent years is amniotic fluid assessment of fetal lung maturity which has been elegantly reviewed elsewhere (61). Early studies linked inadequate production of surfactant by immature lung cells to increased risk for respiratory distress syndrome (RDS) in premature newborns. The conventional approach to assess lung maturity was measurement of surfactant molecules in amniotic fluid (AF) including lecithin to sphingomyelin (L/S) ratio, phosphatidyl glycerol (PG), or surfactant to albumin (S/A) ratio (61, 62). However, manufacturer support for these assays,

including reagents and instrumentation, waned with time, and many laboratories responded by replacing them with the lamellar body (LB) count test. LBs are a packaged form of surfactant produced by the type II pneumocytes (63). They are approximately the same size as platelets, therefore assays employing automated cell counters can be used to measure LBs in amniotic fluid in place of the now obsolete methods (63, 64). Meanwhile, 2 improvements for mitigating risk of poor neonatal respiratory outcomes became standard practice. First is antenatal treatment of mothers with corticosteroids, which accelerates the production of lung surfactants (65). The second is surfactant replacement therapy to treat both neonatal RDS and severe meconium aspiration (66). These medical advancements along with recognition that the best prognostic indicator of fetal outcome is gestational age, leads to the conclusion that fetal lung maturity testing lost its clinical utility. Notably, CLSI C58-A:2011 "Assessment of Fetal Lung Maturity by the Lamellar Body Count" was officially archived in July 2020.

Brain natriuretic peptide in pleural fluid. Another body fluid test that is either declining or has been discouraged from ever gaining favor is measurement of brain natriuretic peptide (BNP) or N-terminal propeptide of BNP (NT-proBNP) in pleural fluid. Pulmonologists rely on the Light criteria to distinguish transudates from exudates (67). However, Light criteria in the setting of heart failure misclassifies pleural effusions as exudates up to 28% of the time, especially after diuretic administration (68). To address this diagnostic dilemma, several studies reported measurement of NT-proBNP or BNP to discriminate pleural effusions of cardiac origin (69, 70). Despite reported high diagnostic sensitivity and specificity of pleural fluid NT-proBNP measurement, an often-overlooked feature is the near perfect correlation of paired pleural fluid and serum NT-proBNP values (71). This strong correlation confirms there is little additional value measuring NT-proBNP in pleural fluid.

Uric acid in synovial fluid. The differentiation of septic arthritis from other causes of joint effusion is a popular endeavor with many studies published given the criticality of making this important determination to avoid morbidity and mortality. The standard workup for crystal arthropathies is to perform cell count, Gram stain, and culture to rule out infection and perform microscopy to look for monosodium urate (consistent with gout) or calcium pyrophosphate dihydrate (consistent with pseudogout) crystals in the synovial fluid by polarizing microscopy which is well documented in clinical guidelines (12). Microscopy is the preferred test, however, when it is not available or crystals are not observed in the setting of high clinical suspicion, uric acid measurement in synovial fluid has been proposed. An aged and underpowered study (n = 27, 8 with gout) demonstrated potential which further studies have attempted to replicate with mixed success (60, 72). Ultimately, serum and synovial fluid uric acid concentrations in each study appear to be very similar between patients that were ultimately diagnosed with gouty arthritis or some other cause. The reported increases in synovial fluid uric acid compared to matched serum are exceedingly small and arguably within the imprecision of the test (e.g., 0.1 mg/dL). The mean ± 1 standard deviation of the ratio of uric acid concentrations in synovial fluid to serum overlap extensively (1.1 ± 0.1 for gout compared to 1.0 ± 0.1 for osteoarthritis and pseudogout and 0.9 ± 0.1 for rheumatoid arthritis and ankylosing spondylitis) despite being statistically significant (72). There is minimal evidence to support uric acid testing beyond serum.

Most chemistry analytes in synovial fluid. Notably, the efficacy of measuring many chemistry analytes in synovial fluid is rather marginal, particularly total

protein and LDH (73). Many studies have investigated biomarkers to replace or enhance sensitivity and specificity of leukocyte count and % polymorphonuclear (PMN) cells for septic joint diagnosis of both native and prosthetic joints. Such biomarkers include lactate, glucose, a host of cytokines, leukocyte esterase measured by dipstick methodology, procalcitonin, total protein, uric acid, C-reactive protein, and alpha defensin with most performing equally to slightly worse than leukocytes and % PMNs (74–76). Since the diagnosis is not always straightforward, having some additional tests to rule in or rule out the joint infection could be considered helpful, however, they should not replace cell count and crystal evaluation (74).

Most chemistry analytes in pericardial fluid. The 2 most common causes of pericardial effusion in developed areas of the world are infection and malignancy (14). The laboratory diagnosis relies firmly on cell count, culture, and PCR to identify infectious etiologies and cytology for malignancy. More than 60% of pericardial effusions in developing areas of the globe are related to tuberculosis (TB) infection where measurement of adenosine deaminase has proven value for screening (41). Interestingly, the task force for the diagnosis and management of pericardial diseases for the European Society of Cardiology (ESC) has a long history of suggesting measurement of pericardial fluid chemistry analytes such as protein, LDH, specific gravity, and glucose, as well as a long list of tumor markers in suspected cases of malignancy be considered, though the evidence to support their use was not well established (77). In the most recent update, tumor markers are acknowledged to have controversial utility and high concentrations of protein and LDH are cited as commonly interpreted as exudates like in pleural fluid, though their use in pericardial fluid has not been validated; see Table 15 of reference (41). Therefore, there are minimal tests that add diagnostic value beyond cell count and cytologic examination for infection (including TB) or malignancy.

Occult blood in discharge fluid. Measurement of occult blood in nipple discharge is yet another fluid analysis that should be discouraged by the clinical lab. Originally proposed in 1982 for detection of cancer, the analysis of discharge using point-of-care hemoccult cards became routine and persists today (78). Clinical practice varies widely, sometimes without following evidence-based guidelines. The accessibility of hemoccult point-of-care cards and "waived" status makes testing an easy target for misuse and noncompliance in the outpatient setting. The intended use is limited to detection of blood in stool for colorectal cancer screening and is recommended every year for individuals over 50 (79, 80). The test's application for occult blood screening in nipple discharge is fraught with both regulatory and analytical issues. The diagnostic accuracy of hemoccult testing in nipple discharge for ductal carcinoma is reported as 50% sensitivity and 0% specificity (81). The recommended workup for pathologic nipple discharge includes only ultrasound or mammography depending upon age for both males and females (82).

Body Fluid Tests with Promising Utility

Adenosine deaminase measurement in pleural fluid for tuberculosis. Adenosine deaminase (ADA) measurement in pleural effusions has been shown to be a useful predictor of TB (83). In geographic locations where TB is endemic, ADA is a cost-effective screening tool for TB effusions. The diagnostic accuracy in low-, intermediate-, and high-prevalence areas has been evaluated. It demonstrated similar performance using a cutoff of ≥40 U/L when coupled with lymphocyte measurement >50% (84). Together these markers have sensitivity of 86.3% and specificity of 98.3%. Very recently, the performance of ADA was evaluated in low-prevalence regions using a machine learning approach to classify 3 types of effusions as either

TB, malignant, or other using ADA coupled with other clinical markers (85). The algorithm including ADA with other biochemical markers performed in a similar manner as ADA ≥40 U/L and lymphocytes >50% for diagnosis of TB effusion with the added benefit of detecting malignant pleural effusion. Similar value for measuring ADA in pericardial fluid and ascites to detect tuberculous effusions has been reported (40, 41).

Homocysteine in pleural fluid for evaluation of malignant effusions. Several independent retrospective studies have reported diagnostic performance of homocysteine (HCY) measurement and traditional tumor markers in pleural fluid for discrimination between malignant and benign effusions (86, 87). The first study reported precision performance of HCY in pleural fluid on the BNII analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY), area under the curve (AUC), optimal cutoff, sensitivity, and specificity of HCY, cancer antigen (CA) 125, CA19.9, CA15.3, and carcinoembryonic antigen (CEA) or each marker in conjunction with HCY. HCY at a cutoff of 13.6 mcmol/L performed better than CA15.3 in their cohort to differentiate benign and malignant effusions (n = 133) with AUC = 0.846 and AUC = 0.778, respectively. Using a combination of HCY and CEA provided significant improvement in performance with sensitivity of 86.5% and specificity of 97.5%. It is thought that accumulation of HCY in pleural fluid occurs due to consumption of B vitamins and degradation of methionine by rapidly dividing tumor cells which interrupts normal metabolic conversion of HCY to methionine or breakdown to cysteine. A second study with 194 specimens found diminished performance of HCY alone with an AUC of 60% compared to approximately 85% in the previous study (88). The discrepancy remains unknown, but it was noted that the prevalence of TB was much higher in the cohort with an AUC of 60% (14.4% vs 4%).

Duodenal fluid bicarbonate for assessment of pancreatic insufficiency. Laboratories may encounter duodenal fluid collected via endoscopic ultrasound (EUS) for the evaluation of exocrine pancreatic insufficiency in the setting of chronic pancreatitis. The diagnosis of chronic pancreatitis recently shifted from pathological evidence of chronic inflammation and irreversible fibrosis to a mechanistic definition to identify early manifestations of pancreatic disease. This offers the advantage of earlier detection and management (89). The primary evaluation for chronic pancreatitis includes cross-sectional imaging via computed tomography (CT) or magnetic resonance imaging (MRI) followed by analysis of duodenal fluid if the diagnosis is unclear on imaging. The updated American College of Gastroenterology (ACG) guidelines recommend stimulated endoscopic exocrine pancreatic function testing through measurement of bicarbonate in timed duodenal fluid samples following secretin stimulation or volume and enzyme quantitation following cholecystokinin stimulation (89). A cutoff of >80 mmol/L bicarbonate at 60 min post-secretin stimulation has been proposed to indicate normal pancreatic function (90). As the diagnostic criteria evolve for chronic pancreatitis, the inclusion of bicarbonate analysis may shift over time due to changing sensitivity and specificity (91). A limited number of studies have evaluated the performance of duodenal fluid bicarbonate measurement on modern chemistry analyzers (92, 93). Enzyme measurement is less established acknowledging the limited availability of testing. Little is documented regarding stability of enzymes post-collection. Gastric fluid contamination is known to decrease pH, thereby influencing enzyme activity (94).

Cyst fluid CEA and glucose for pancreatic neoplasms. Requests for measurement of pancreatic cyst fluid CEA and glucose are not uncommon for clinical laboratories. Pancreatic lesions identified via imaging need risk stratification to guide management. Surgery should only be considered in cases where lesions have a high risk for malignancy. Differentiation of mucinous from non-mucinous

lesions helps to identify premalignant neoplasms (95). CEA and more recently glucose have been evaluated as markers to identify malignant or premalignant cysts (96–99). Recent studies with CEA estimate sensitivity and specificity at 63% and 88% to differentiate mucinous and non-mucinous cysts with a cutoff of >192 ng/mL. Surprisingly, pancreatic cyst glucose measurement showed improved performance in 2 systemic reviews and meta-analysis compared to CEA (97, 98). Both meta-analyses calculated pooled sensitivities >90% for cyst fluid glucose alone using a cutoff of >50 mg/dL. The analysis of cyst fluid is challenging for clinical laboratories because the volume of sample is small and often shared with the cytology or molecular laboratory. Cyst fluids often have high viscosity, therefore enzymatic pretreatment with hyaluronidase can be attempted but may impact measurement of key analytes such as lipase (32, 100).

Electrolytes and beta-2-microglobulin in vesicocentesis fluid for evaluation of lower urinary tract obstruction. Another emerging, yet controversial, fluid request that may be encountered is analysis of electrolytes and beta-2-microglobulin in fetal urine acquired via vesicocentesis, or collection of urine from a catheter inserted into the fetal bladder. Lower urinary tract obstruction (LUTO) is a rare but serious condition that complicates approximately 2.2/10 000 pregnancies. Mortality ranges from 60% to 80% if oligohydramnios or anhydramnios is identified in the second trimester due to outflow obstruction of the fetal bladder (101). Measurement of sodium, chloride, osmolarity and beta-2-microglobulin (which does not cross the placenta) in fetal urine collected via vesicocentesis was proposed to predict postnatal renal function; however, a systemic review found poor predictive value (102, 103). Despite this finding, favorable fetal urine biochemistry can be used to select which fetuses would benefit from vescico-amniotic shunting (VAS) surgery (101). A staging classification system for LUTO defines favorable fetal urine as sodium <100 mmol/L, chloride <90 mmol/L, osmolarity <200 mmol/L, and beta-2-microglobulin <6 mg/dL (104). Due to the scarcity and minimal residual volume for these specimens, formal studies exploring variation in specimen matrix have not been published.

Cortisol and cortisone in saliva for assessment of adrenal insufficiency. The clinical utility of saliva as an alternative specimen type for several analytes has cycled in popularity over time. Measurement of cortisol and cortisone in saliva remains popular for both diagnostic purposes and as general markers of stress (105). Saliva resembles an ultrafiltrate of plasma which theoretically allows free cortisol concentrations to be evaluated in the absence of cortisol-binding globulin, albumin, and other proteins. Unbound cortisol is converted to cortisone by 11β-hydroxysteroid dehydrogenase (11βHSD) type 2 expressed in saliva glands. Therefore, salivary cortisone correlates most with free serum cortisol (106). Preanalytical issues complicate steroid analysis in saliva. Smoking, caffeine, diet, and the collection device may influence physiologic concentrations, recovery, and analysis. Oral fluid collection devices designed for drugs of abuse testing, metabolomics, and metagenomics all have different preservatives which are optimized for downstream analysis. The use of salivary cortisol and cortisone has been investigated for diagnosis of adrenal insufficiency using stimulated and unstimulated protocols with varying success (107–110). Laboratories that offer testing in this matrix should optimize and then standardize the preanalytical variables to the extent possible, and include the collection device in the validation for their specific assay. Applications of saliva analysis beyond adrenal insufficiency using HLA genetics, microbiome, and nutrient markers have shown surprising correlations with suicidal ideation in a university-age population (111).

Amniotic fluid glucose measurement as a marker of chorioamnionitis. The measurement of amniotic

glucose has been proposed as a marker of intraamniotic infection that is associated with preterm premature rupture of membranes which influences preterm delivery decisions (112, 113). Awaiting culture results can delay the identification of microbial invasion of the amniotic sac (e.g., chorioamnionitis). Glucose measurement <15 ng/mL has been shown to provide moderate sensitivity (73%) and specificity (88%) in detection of chorioamnionitis compared to Gram stain or bacterial culture (112). When combined, amniotic glucose measurement and Gram stain have sensitivity of 66% and specificity of 100% (114). A recent study of 142 singleton pregnancies calculated the performance of cutoffs ranging from 5 to 20 mg/dL for amniotic glucose and at a cutoff of 15 mg/dL had sensitivity and specificity of 39% and 70%, respectively, for intrauterine microbial invasion in women with preterm labor (55). Amniotic fluid is not included in manufacturer package inserts for glucose assays, therefore, as with many other body fluid tests, validation of the method performance specifications should be investigated in support of testing.

Additional Body Fluid Testing Matters

Measurement range and method selection. Selecting an appropriate measurement range for a body fluid assay is a critical step. It is common to perform body fluid testing using the serum version of an assay. It is important to recognize the approximate concentrations expected in body fluids for each analyte and select the instrument application that aligns most closely. For example, total protein measures in the g/dL range for serum-based applications and in mg/dL for CSF- and urine-based applications. It is also important to recognize the differences between assay manufacturers for similar applications and how frequently they may elicit greater than or less than results when testing body fluids. As a case in point, the total protein assay lower limit of quantitation varies in the package insert between 0.1 g/dL (Roche Diagnostics) and 3.0 g/dL (Beckman Coulter) (35). Body fluid total protein was found in a cohort of samples to be significantly lower with a median (range) concentration of 2.6 g/dL (<0.2 to 3.6 g/dL, n = 10 366) compared to serum with median concentration of 6.6 g/dL (2.7 to 16.2 g/dL, n = 100 232, $P < 0.0001$) (115), suggesting the Beckman Coulter lower limit of quantitation is most appropriate for serum testing but may produce a significant number of less than results if the measurement range is not extended lower for testing body fluids, which has ramifications for not only the analytical validation but also ongoing calibration verification activities.

Quality control practices. When the laboratory shares instruments and methods between serum/plasma testing and body fluid testing many aspects of daily operations and maintenance can be shared. This includes quality control testing. It is advisable to use matrix-specific materials where they are available, such as chemistry controls for spinal fluid and urine. If they do not exist, it is reasonable to apply the serum/plasma quality controls to both serum and body fluid testing. The laboratory should consider the relevant decision limits in body fluids (see Table 2) and how well the serum controls assess variation within a "normal" and "abnormal" concentration range. Alternate practices such as diluting an existing control or testing body fluid-specific controls may be an option.

Nonstandard Abbreviations: CSF, cerebrospinal fluid; LDH, lactate dehydrogenase; CAP, College of American Pathologists; ISO, International Organization for Standardization; NT-proBNP, N-terminal propeptide of brain natriuretic peptide; TB, tuberculosis; ADA, adenosine deaminase; HCY, homocysteine: AUC, area under the curve; CEA, carcinoembryonic antigen.

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