EDUCATIONAL COMMENTARY – BODY FLUID ANALYSIS

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Learning Outcomes

On completion of this exercise, the participant should be able to

- describe some of the more common nonstandard body fluids and associated testing requested in the clinical chemistry laboratory;
- define requirements for body fluid analysis for laboratories accredited by the College of American Pathologists;
- identify some of the challenges associated with nonstandard body fluid analysis in the clinical chemistry laboratory;
- discuss available resources and ways to maintain regulatory compliance for the validation and implementation of nonstandard body fluid testing;
- discuss new advances in testing of body fluids.

Introduction

The pathologic accumulation of fluids in the body often warrants laboratory testing to aid in the diagnosis and management of certain disease states. Although most chemistry tests are approved by the U.S. Food and Drug Administration (FDA) for serum, plasma, or urine specimens, testing on various other body fluids has been performed in the clinical chemistry laboratory for years. Before 2009, the accuracy of test results in relation to differences in matrices, stability of the specimen and analyte, and interfering substances were not considered when reporting patient results from specimens that were not intended to be used for chemistry assays. Only recently have the implications and risks of performing testing on fluids other than serum, plasma, or urine been recognized and acknowledged. In 2009, the College of American Pathologists (CAP) stated that for an FDA-cleared/approved test, the use of body fluids other than the fluids listed as suitable for analysis in the manufacturer’s package insert under Intended Use must be treated as a modification, with subsequent validation and metrics for interpretation established. As a result, despite the clinical utility of many of these tests, laboratories must decide whether implementation of nonstandard body fluid testing is worth the time and trouble.

Body Fluids

Most commercial chemistry assays are developed for use with serum, plasma, or urine. Fluids other than those listed by the manufacturer as intended for use are considered nonstandard or off label. Although
cerebrospinal, peritoneal, pericardial, pleural, synovial, and amniotic fluids are nonstandard fluids frequently submitted to the laboratory for testing, a number of other fluids may be submitted as well. Laboratories may see testing requests for a multitude of fluid types, including fine-needle aspirate washout, drain, wound, or cyst fluids; semen; and saliva. Many of these nonstandard fluids are usually considered irreplaceable owing to the invasive nature of their collection. They each have a unique composition of proteins, electrolytes, and lipids that can affect assay performance and prevent laboratories from being able to assume that the assay will perform the same on the nonstandard body fluids as on the standard body fluids.

Cerebrospinal fluid (CSF) is produced by the choroid plexus and ependymal lining cells of the ventricles and found between the arachnoid mater and the pia mater. It functions to lubricate and protect the brain and spinal column and works as a transport medium to maintain a stable chemical environment as well as to aid in the removal of metabolic waste products. In healthy adults, the total volume ranges from 85 to 150 mL; in healthy neonates, volume ranges from 10 to 60 mL. Examination and testing of CSF aids in the diagnosis of diseases such as meningitis, demyelinating diseases like multiple sclerosis and Guillain-Barré syndrome, central nervous system cancers, and subarachnoid hemorrhage. Lumbar puncture is performed for the removal of up to 20 mL (or approximately 15% of the total volume) of CSF in adults with normal CSF pressure. In addition to physical, immunologic, microbiologic, and microscopic examination, chemical examination routinely includes quantitation of CSF glucose, total protein, and lactate.

Serous fluid is a collective term for those plasma ultrafiltrates formed in the serous/body cavities with a composition similar to serum. Serous fluids are found in small volumes in healthy individuals and act as lubricants in the pleural, peritoneal, and pericardial spaces. Fluid volumes are maintained in healthy individuals by several factors: parietal membrane capillary permeability, hydrostatic pressure, oncotic pressure, and absorption by the lymphatic system. Disease may lead to an accumulation, known as an effusion, in any of these spaces. Laboratory testing is used to classify the type of effusion by differentiating between an exudate and a transudate to identify its cause. Exudates are effusions resulting from inflammatory processes that cause increased vascular permeability and decreased lymphatic absorption. Exudates are typically caused by infections, malignant neoplasms, and trauma. Transudates, on the other hand, are the result of a noninflammatory process that cause an increased hydrostatic pressure or decreased plasma oncotic pressure. Transudates can be seen in individuals with congestive heart failure, kidney disease, and hepatic cirrhosis. Light criteria are traditionally used to differentiate transudates and exudates in pleural fluid by calculating the pleural fluid protein to serum protein ratio, and the pleural fluid lactate dehydrogenase to serum lactate dehydrogenase ratio.
analytes routinely tested on serous fluids include but are not limited to glucose, albumin, cholesterol, triglycerides, amylase, creatinine, electrolytes, pH, and bilirubin.

Synovial or joint fluid is a viscous ultrafiltrate of plasma across the synovial membrane. It functions to not only lubricate joints, but to nourish and remove debris from the joints. In healthy individuals, synovial fluid volumes depend on the size of the joint but are generally quite small, between 0.1 and 3.5 mL. With disease, synovial fluid accumulation can occur and arthrocentesis may be performed to differentiate among noninflammatory, inflammatory, septic, and hemorrhagic disease. Physical, microscopic, and chemical examination all play a role in the diagnosis of diseases such as osteoarthritis, gout, pseudogout, rheumatoid arthritis, and infection. Routine chemical testing usually includes glucose, total protein, uric acid, cholesterol, triglycerides, and lactate.

Amniotic fluid enables a fetus to move freely throughout gestation, provides a cushion against injury to the fetus, and helps to maintain a constant temperature for the fetus. Amniocentesis may be performed to detect congenital and genetic disorders, assess fetal lung maturity, and identify Rh isoimmunization or infection. During the course of gestation, the composition of the amniotic fluid changes considerably, allowing for a variety of testing at different stages of gestation. Common laboratory testing includes tests for fetal lung maturity, bilirubin, and α-fetoprotein.

Regulatory Requirements

According to the CAP All Common Checklist, any test that the laboratory alters, including changes in specimen type, is considered modified, no longer FDA cleared/approved, and requires validation by the laboratory. Section COM.40620 states that laboratories must provide records of validation or verification studies for all methods of body fluid testing that are offered as routine, orderable tests to ensure that reliable results are obtained. Validation includes analytic accuracy, precision, interferences, analytic sensitivity, analytic specificity, sample stability, and reportable range. The standards also state that performance specifications for blood specimens may be used for the body fluid if matrix effects can be excluded using a dilution study with mixtures of samples and spiking. In addition, labs must perform alternative performance assessments semiannually for any tests not required by CAP for proficiency testing. These alternative assessments can be accomplished through external proficiency testing programs, split sample analysis, chart review of clinical validation, or other appropriate and documented means. All laboratory results must be reported with defined reference intervals unless the analyte concentration is reported with a corresponding blood sample concentration. While requiring that all body fluid results not used in comparison with blood specimen results be reported with reference intervals or interpretations, CAP recognizes that many body fluid analytes have no published data for reference
intervals and it is difficult to obtain normal fluids for establishing a lab’s own reference intervals. Therefore, CAP allows for interpretations, as appropriate, if the laboratory cannot provide a record of a reference interval study, verification of the manufacturer’s stated interval, verification of published reference intervals, or a method approved by the laboratory or section director.\(^1\) Results reported in comparison with serum, plasma, or blood samples must be accompanied by a comment stating that there are no established reference intervals for the analyte in the specific body fluid, and that comparison of the result with the concentration in the blood, serum, or plasma is recommended.

**Challenges**

As one would imagine, there are many challenges associated with nonstandard body fluid testing and the regulatory requirements associated with the reporting of results. Although myriad studies have been done over the years on analyte testing using serum and plasma, data are limited with regard to many aspects of nonstandard body fluid testing. For example, questions arise as to the appropriate collection container and sample handling conditions for different body fluids. Once collected, both analyte and sample stability and the lack of information available must be considered as well. Validation and verification are costly and cumbersome, requiring additional laboratory resources that may not be available. Until recently, materials for body fluid linearity were largely unavailable, and obtaining enough in-house samples to perform validation studies was difficult. One of the most troubling concerns is the lack of reference intervals, clinical utility, and interpretive data for many of the analytes in nonstandard body fluids. Because accumulation occurs with disease states and not in healthy populations, it seems almost impossible that reference intervals will be associated with many of the body fluid analytes, making it necessary to interpret test results based on the patient’s clinical history.\(^6\)

**Resources for Regulatory Compliance**

Navigating the regulatory requirements regarding nonstandard body fluid analysis and its associated challenges may be difficult, but there are several resources available that may be helpful to those laboratories implementing testing. The CAP All Common Checklist is a necessary tool for CAP-accredited laboratories looking for guidance on the regulatory requirements for implementing procedures, validating methods, reporting results, and documenting compliance. The Clinical and Laboratory Standards Institute Guidelines for Body Fluids (document C49-A) offers direction for nonstandard body fluid testing implementation, recognition of analytic variability, reporting, and interpretation.\(^7\) There are many studies available in which the authors outline their procedures for validating nonstandard body fluid testing. A recent article by Block et al. outlines their approach to validating performance characteristics of numerous assays in several nonstandard fluids.\(^8\) Although the article does not address reference intervals or decision limits, it does give the reader insight into their laboratory’s validation of accuracy,
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precision, analytic measuring range, analytic specificity, and analyte stability. Lo et al. also documented their laboratory's experience with the evaluation of performance characteristics and interferences in bilirubin testing using serous fluid. Accuracy was assessed in both studies by calculating the percent analyte recovery in spiked fluid specimens. Body fluid specimens were spiked with known varying concentrations of the analyte from either a standard solution, calibration verification material, or serum with a known concentration. Comparative analysis was performed using controls having an equal volume of deionized water and body fluid specimen. Method comparison was performed using the gold standard reference method, when appropriate and available. Linearity was assessed by serially diluting spiked specimens. Intra-assay precision was determined in both studies by running a single specimen 20 consecutive times in a single run; interassay precision was run over 20 consecutive days in Block’s study and 4 consecutive times per day over 5 days in Lo’s study. The studies assessed the effects of hemolysis, icterus, and lipemia by spiking fluid samples with known concentrations of hemoglobin, conjugated bilirubin, and 20% lipid emulsion, respectively. Stability was assessed by calculating percent recovery of the analyte under varying conditions with consecutive analysis over the course of 5 to 7 days after collection.

New Advances in Testing of Body Fluids

Although implementing nonstandard body fluid testing in the clinical chemistry lab may seem like a daunting task, research continues to show astounding advancements in the use of nonstandard fluids for diagnosis and management of disease. Some of the most intriguing studies have looked at the use of nonstandard body fluids and the assessment of cancer biomarkers. Carcinoembryonic antigen (CEA), associated with adenocarcinomas, has been found useful in the differentiation of malignant from nonmalignant causes of serous effusions. More specifically, CEA has been evaluated in conjunction with cytologic examination of pleural fluid in the diagnosis of lung, breast, ovarian, and colorectal cancers; and of peritoneal fluid from patients with metastatic colorectal cancer. Studies have found that in patients with pleural effusions, elevations in CYFRA 21-1 (a fragment of cytokeratin 19) are a sensitive tumor marker for non–small-cell lung cancer. Both cancer antigen 19-9 (CA19-9) and cancer antigen 15-3 (CA15-3) have also been studied in serous effusions and found useful for differentiating malignant from nonmalignant causes, when used in conjunction with cytology. In addition, fine-needle aspirate washouts can be used to assess metastases or recurrences in patients with thyroid cancer using thyroglobulin measurements, or to detect medullary thyroid cancer with procalcitonin measurements.

Summary

Recent changes in regulatory requirements have caused many laboratories to question whether offering nonstandard body fluid testing is worth the effort. The task may seem daunting and the challenges many,
but nonstandard body fluid testing plays an important role in the diagnosis and management of disease states. When faced with the decision to validate specific assays on nonstandard body fluids, one should evaluate the laboratory’s test-ordering volumes and the clinical utility of the test being considered. Laboratories struggling with the time and resources to implement nonstandard body fluid testing should consider only those tests with known clinical utility that are ordered most commonly. Changes in the regulations may have added a burden on laboratories, but laboratories should take advantage of the resources available to them to maintain compliance and reduce risk to their patients.

References


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