The role of laboratory investigations in identifying body fluids

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In diverse clinical situations it may be important to establish the nature and origin of fluid samples. This article sets out to provide some practical guidance on the role of laboratory investigations in this process.

The nature and origin of fluid samples collected from patients can be of critical importance in determining clinical management. Clinicians usually seek to establish what a particular fluid sample is and where it has come from, and rely on laboratory analysis and diagnostic imaging to provide answers to these questions. While it may not always be practical or feasible to do so, laboratory investigations undoubtedly have a role to play in helping to elucidate the nature of unidentified fluid samples.

The purpose of this article is to clarify this role by examining what is known about the biochemical composition of various body fluids, with particular emphasis on those unique characteristics of specific fluids that may help to identify them. It is intended to be a practical guide rather than a comprehensive survey of body fluids and their biochemical composition.

AMNIOTIC FLUID

Amniotic fluid fills the amniotic cavity surrounding the fetus throughout intrauterine life, and is released following rupture of the fetal membranes. Premature rupture is associated with both maternal and fetal/infant morbidity and mortality, so its timely recognition is important. Clinical diagnosis is usually easy, but occasionally it may be necessary to distinguish amniotic fluid from maternal urine, vaginal fluid, cervical mucus or blood.

Sideroom tests may be used — these include dipstick testing for protein and glucose (amniotic fluid is positive for both, urine normally negative), or pH (vaginal fluid and urine are acidic, amniotic fluid neutral), or testing for 'ferning' (when amniotic fluid is dried on a glass slide, crystals appear because of its salt content, and these can be viewed under a low power microscope where they resemble the branches of a fern) (*Table 1*). However, these tests are not specific and not always reliable.

Fetal fibronectin is relatively specific to amniotic fluid. The fibronectin family of proteins is found in the plasma and extracellular matrix, and a unique moiety has been identified in amniotic fluid, extracts of placental tissue and some malignant cell lines. However, fetal fibronectin may be found in the cervix and vagina even when the membranes appear to be intact.

The basis for this apparent paradox is unclear and possible mechanisms are discussed elsewhere (Lockwood et al, 1991). The consequence is that the presence of fetal fibronectin cannot be used to diagnose rupture of the membranes. Its detection in the cervix or vagina is

TABLE 1.
Methods of identifying body fluids

Fluid	Property or marker
Amniotic fluid	Protein
	Glucose
	pH
	'Ferning'
	Fetal fibronectin
CSF	β_2-transferrin (tau band)
Chyle	Chylomicrons
	Triglycerides
Synovial fluid	Mucin clot test
	Metachromatic staining
Urine	Urea
	Creatinine

Only fetal fibronectin and β_2-transferrin are specific markers, for amniotic fluid and CSF respectively

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nevertheless useful, since it appears to act as a marker for both term and preterm labour.

ASCITES

Ascites refers to the accumulation of fluid in the peritoneal space. Its pathogenesis is complex and contributory factors include increased portal venous pressure, decreased plasma oncotic pressure, increased hepatic lymph formation and secondary hyperaldosteronism. The composition of ascites varies depending on the underlying cause (Runyon et al, 1992), but peritoneal fluid arises in most cases by plasma ultrafiltration.

Identification of ascites is rarely required. Clinical examination is usually sufficient to establish the presence of fluid in the peritoneal space, but in doubtful cases diagnostic imaging may be helpful. It may be necessary to distinguish peritoneal fluid and urine. Measurement of fluid urea and creatinine is usually sufficient, although it is important to compare the results with simultaneous serum and urine measurements.

The role of laboratory investigations in the differential diagnosis of ascites is a separate issue from the unique identification of ascites and will be covered in a future article.

BILE

The presence in bile of pigments (bilirubin, indocyanin green, sulphobromophthalein) ensures that in normal circumstances it is readily recognizable by visual inspection, obviating the need for laboratory investigations. There is a significant literature on the composition of bile (Paumgartner and Sauerbruch, 1983), because abnormal bile composition may lead to the formation of gallstones. (It is necessary to distinguish between hepatic bile and gallbladder bile; the latter is much more concentrated.) Crucially, the concentrations in hepatic bile of readily measurable components (bilirubin, lipids and electrolytes) do not differ greatly from those observed in plasma (Thureborn, 1962).

CEREBROSPINAL FLUID

Unique identification of cerebrospinal fluid (CSF) is sometimes required in cases of otorrhoea or rhinorrhoea, usually after trauma or surgery to the head and neck. Oberascher (1988) described a method of identifying CSF that involves electrophoresis, immunofixation and silver nitrate staining of the protein β-transferrin. One isoform of this protein, β_2-transferrin, is specific to CSF, giving rise to a second distinctive band (the so-called tau band) in addition to the β_1-transferrin band, when CSF is analysed using this method. Bands that migrate to the same position as the tau band have been identified in the serum of several patients with cirrhosis, and also in one family. For this reason, a sample of the patient's serum is run, along with a positive standard (5 μl pure CSF), at the same time as the putative CSF sample. Using this method, Oberascher (1988) claims that CSF can be identified using as little as 1 μl pure CSF, or 100 μl contaminated CSF, per 1 ml of secretion (nasal, wound, etc).

More sensitive methods exist based on fluorescein detection, and these may be used to identify as little as 2 μl contaminated CSF per 1 ml of secretion. These combine intrathecal injection of fluorescein with placement of absorptive sponges in the nasal cavity or pharynx. For obvious reasons, they are less widely used.

CHYLE

Chyle is the fluid found in the intestinal lymphatics during absorption of food postprandially; it is therefore a particular kind of lymph (see below). Whereas lymph is usually colourless, chyle appears milky because of the presence of a fine emulsion of fats. It drains from the intestinal lymphatic system into the thoracic duct, and from there into the venous system.

There are two main clinical scenarios where identification of chyle is potentially useful — chylothorax and chylous ascites. Chylothorax may be defined as the presence of lymphatic fluid (chyle or lymph) in the pleural space, and results from a leak of the thoracic duct or one of its major divisions. Chylous ascites refers to the accumulation of lymphatic fluid in the peritoneal space, and it is sometimes (but not always) associated with lymphatic obstruction.

Unfortunately there is no unique marker for chyle. However, chylomicrons are usually present in thoracic duct lymph and are present in blood only postprandially, except in pathological states, so the presence of chylomicrons in pleural or ascitic fluid is usually indicative of a chylous origin. For practical reasons, lipoprotein electrophoresis is not widely available, and triglyceride determination may be used as a screening test.

Based on their series of 141 patients, Staats et al (1980) estimated that pleural fluid with a triglyceride concentration of greater than 110 mg/dl (1.24 mmol/litre) has a less than 1% chance of not being chylous, whereas fluid with a triglyceride concentration less than 50 mg/dl

(0.56 mmol/litre) has no more than a 5% chance of being chylous. Although there are no similar data for chylous ascites, it seems reasonable to conclude that where the triglyceride concentration in the ascitic fluid is significantly greater than in the serum, the ascites is indeed chylous; where required, lipoprotein electrophoresis may be carried out to confirm the diagnosis.

ENDOLYMPH

Of specialist interest to otologists, the cochlear endolymph is unique among extracellular fluids in having a high potassium and low sodium content, resembling intracellular fluid. Glycerol and other dehydrating agents are used in the investigation of Menière's disease — the changes in endolymph induced by these agents are followed by transtympanic electrocochleography. The use of this technique reflects the fact that measurement of endolymph composition is technically difficult and is not routinely available.

LYMPH

Lymph is formed as a result of opposing hydrostatic and osmotic forces at capillary level, and drains into lymphatic vessels that permeate the extracellular spaces of the body. Lymphatics thus effectively drain the extracellular fluid as it forms, eventually returning it to the blood via ever-larger lymph vessels, culminating in the thoracic duct, which empties into the venous system at the junction of the jugular and subclavian veins.

Several observations may be made about the composition of lymph. First, lymph from any tissue (except the kidney medulla and choroid plexus, both of which lack lymph vessels) contains all of the proteins that can be detected in plasma, albeit at different (usually lower) concentrations (Lindena and Trautschold, 1983). Second, the concentration of lymph increases as it ascends the lymphatic tree because of ultrafiltration (Hargens and Zweifach, 1976). Third, pressure variation within the central lymphatic system affects the composition of lymph (Elk and Laine, 1990). These factors collectively make it very difficult to unequivocally identify lymph.

A fourth fundamental difficulty arises precisely because there is a continual cycle of fluid from plasma to tissue fluid to lymph and back to plasma. Because the lymphatic system is constantly delivering lymph into the bloodstream, there is no marker that is unique to lymph.

PLEURAL AND PERICARDIAL FLUID

Pleural and pericardial fluid is formed by the filtration of plasma through the capillary endothelium, and is reabsorbed by the lymphatic system. Normally there is just enough to facilitate movement of the parietal and visceral pleura against each other (or, in the case of the pericardial cavity, the serous membranes that line the heart and pericardial sac), but greater amounts (effusions) may be seen in various pathological conditions.

Many of the comments made about ascites may also be applied here. There is no unique marker for either fluid, although laboratory investigations have a role to play in differential diagnosis (Sahn, 1987).

SALIVA

Saliva is readily accessible and unique identification is rarely required. A monoclonal antibody has been produced that specifically inhibits salivary α-amylase without any detectable cross-reactivity against pancreatic α-amylase. An assay of its analytical performance has been described that, in principle, could be used to identify saliva (Gerber et al, 1987).

SYNOVIAL FLUID

Synovial fluid is an ultrafiltrate of plasma combined with a mucopolysaccharide — hyaluronate — secreted by cells of the synovial membranes that line joint spaces. Identification of synovial fluid is required only where there is doubt about the nature of fluid aspirated during arthrocentesis. When only a small amount of fluid is obtained it may be difficult to decide whether it represents synovial fluid, fluid from subcutaneous tissue or local anaesthetic.

Testing for mucin formation and staining for metachromasia are two simple methods for rapid detection of trace amounts of synovial fluid, both of which depend on the presence in synovial fluid of hyaluronate. In the mucin clot test, mucin (a complex of hyaluronate and protein) is precipitated with acetic acid, and the specimen examined for turbidity or a mucin clot. Metachromatic staining involves spotting filter paper with synovial fluid and staining with toluidine blue. These methods have been compared with each other (Goldenberg et al, 1973). Metachromatic staining is more sensitive, but is not suitable for analysing fluid exposed to heparin (a mucopolysaccharide like hyaluronate). However, both are capable of identifying as little as 0.5 μl undiluted synovial fluid.

TEARS

As with saliva, unique identification of tears is rarely required. Human tears are a mixture of secretions from the lacrimal gland and other smaller glands. Biochemically they contain electrolytes, glucose, urea and lipids, as well as a variety of proteins. At least one protein appears to be specific to tears (Bonavida et al, 1969), and could in principle be used for identification. Tear-specific prealbumin has been characterized by a variety of electrophoretic techniques (Janssen and Van Bijsterveld, 1981), and appears to be heterogeneous (Gachon et al, 1979).

URINE

Urine is often suspected as a contaminant of fluids sent for identification. The simplest way to establish the presence or absence of urine in a specimen is to compare measurements of urea and/or creatinine in the specimen with simultaneous measurements in serum and, ideally, urine. Because the urinary concentrations of urea and creatinine exceed the serum concentrations by several orders of magnitude, contamination is easily detected. Where there is severe renal failure, serum concentrations of creatinine in particular may approach those of normal urine; comparisons with simultaneous serum and urine samples are essential.

TABLE 2.
Fluids not normally identified by laboratory investigation

Fluid	Comment
Ascites	No unique marker. Laboratory investigations useful in differential diagnosis
Bile	Usually identifiable by visual inspection. Bile pigments could be used to identify
Endolymph	Inaccessible. Composition usually studied indirectly by transtympanic electrocochleography
Lymph	No unique marker
Pleural fluid	No unique marker. Laboratory investigations useful in differential diagnosis
Pericardial fluid	No unique marker. Laboratory investigations useful in differential diagnosis
Saliva	Readily accessible. Monoclonal antibody to salivary α-amylase exists that could be used to identify if required
Tears	Readily accessible. Tear-specific prealbumin assay available
Vitreous humour	Identification rarely required. Metachromatic staining could in theory be used

VITREOUS HUMOUR

Vitreous humour fills the posterior cavity of the eye. It is occasionally sampled for glucose post-mortem but in-vivo measurements are virtually never required. It is a hydrogel composed mainly of water; collagen provides a framework, with hyaluronate filling the interfibrillar space and providing the gel properties. Thus in theory metachromatic staining (see above) could be used if identification were required.

CONCLUSION

The laboratory has a role to play in clarifying the origin of unidentified fluid samples, but the importance of this role varies from one clinical situation to another. From a practical viewpoint, knowing which body fluids can be identified readily and how — and just as importantly, which ones cannot — may be very useful (*Table 2*). However, a comprehensive knowledge of the biochemical composition of body fluids is not required. **HM**

KEY POINTS

- Fetal fibronectin is relatively specific for amniotic fluid, but the assay is not routinely available.
- The tau band (β_2 transferrin) test for cerebrospinal fluid is now available as a supra-regional assay in the UK.
- Serum triglycerides are the most convenient test for chyle.
- When testing for synovial fluid, both the mucin clot test and metachromatic staining are readily adaptable to the clinical setting.
- When looking for contamination with urine, urea and creatinine must be compared in the sample, serum and if possible urine.

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