The biochemistry of body fluids

ACBI
Scientific
Committee
Guidelines

The Biochemistry of Body Fluids

Association of Clinical Biochemists in Ireland

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Preface

These Guidelines are the latest in a series commissioned and produced by the Scientific Committee of the Association of Clinical Biochemists in Ireland, to promote appropriate and effective use of the laboratory service. They are intended to be a concise reference document to assist practitioners in the Clinical Biochemistry field and those who order tests from the laboratory service, whether hospital- or community-based.

This volume deals with the analyses of body fluids other than blood and urine. It covers analytical issues, interpretation of results and limitations of testing. A section on sweat testing for cystic fibrosis is included in view of the high prevalence of this condition in Ireland, but it is emphasised that for best practice the performance of this test should be confined to specialised centres of excellence.

On behalf of ACBI Council, I thank the Scientific Committee and the individual authors for their work on this project, and also Dr. Yvonne O'Meara who kindly reviewed the penultimate draft, and made many helpful suggestions. Council is also grateful to Randox Laboratories Ltd. for their generous financial contribution to the printing costs.

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Introduction

We are all familiar with the use of blood and urine as interpretative tools in the diagnosis of disease. These fluids can tell us much about a patient from presence or absence of disease to its severity and the prognosis for the patient. However there are other bodily fluids with a story to tell.

Most fluids are ultra-filtrates of blood that have undergone processing by the relevant tissues while some are produced by active transport. These fluids may contain bio-markers that are not found in blood or are at different concentrations than in blood. Some fluids are present in the healthy population while some are only found in the disease state. Amniotic fluid is only found in pregnancy while pleural fluid is usually only seen in noticeable quantities in disease.

Analytical Issues

Although the presence or absence of a bio-marker in a fluid may be sufficient to diagnose disease, in many conditions the concentration of the analyte in disease relative to the concentration in health is itself diagnostic. This may be problematic in rarer fluids where reference ranges have not been established. Comparison of the fluid level of an analyte with the level of that analyte in the patient's serum has also proved of value.

Matrix (i.e. the components of a sample other than the analyte) is a very important factor in chemical analysis. Different matrices seen in the various body fluids affect biochemical assays in potentially two main ways – the assays themselves and the methods used for assay quality assurance.

Quality assurance may be an issue because internal quality control material and external quality assessment material are usually serum or urine based. Matrix effects may not be taken into consideration if assay performance is monitored using these controls.

In such circumstances it may be necessary to exchange samples with other sites performing similar assays if a diagnostic service is to be offered.

With the exception of blood, urine and CSF, method validation for other fluids has not been carried out and this could prohibit their use in routine diagnosis. However the valuable information that may be obtained through using less common body fluids helps the individual patient and our understanding of the disease processes.

New techniques may improve the usefulness of these fluids. Mass spectrometry and proteomics will soon become the most powerful diagnostic tools in medicine. Fluids containing biomarkers at low concentration or of unknown significance will benefit from more sensitive techniques.

Notwithstanding the difficulties potentially arising from the lack of manufacturers' validation of biochemical analyses in fluids, the considerable experience which has been built up for the most commonly used tests makes

them valuable clinical aids when used appropriately.

This booklet aims to explain the use of bichemical tests for fluids, other than blood and urine, and how they may help us in the diagnosis of patients and thus facilitate appropriate and effective treatment.

A table of factors affecting analysis is included for easy reference.

Fluid	Common Tests	Pre-analytical Factors	Analytical Factors	Post-analytical Factors	Interpretative Notes
Amniotic Fluid	Bilirubin	Protect from light	Centrifuge before analysis	Use Liley or Queenan chart to interpret absorbances	Gestation date required
CSF	Glucose	Preserve glucose			
	Protein			Note red cell count	
	Bilirubin	Protect bilirubin from light			
Pericardial Fluid	Protein LDH				Light's Criteria
Peritoneal / Ascitic Fluid	Protein Albumin LDH Amylase Triglyceride Haematology Microbiology	Microbiological tests should be performed first before distributing to other laboratories. Blood and fluid samples should be taken concurrently	Fluid and blood albumin measured to calculate SAAG		Light's Criteria Blood should also be analysed for liver function tests (including Albumin, total protein), renal function tests, and amylase
Pleural Fluid	Protein LDH				Light's Criteria Measure plasma / serum LDH and Protein simultaneously
	Glucose	Preserve glucose			
	pH	As soon as fluid is collected, take a sample into 'blood gas' tube and expel all air	Analyse anaerobically as blood gas		
Saliva	Free hormones Drugs	Use commercial collecting device	Freeze to remove viscosity	Dehydration or oral contamination may invalidate results	Cut offs for dynamic function tests need to be established
Seminal Fluid	Enzymes	Sample must be received within 2 hours of collection			
Sweat	Chloride	Infants should be more than 2 weeks and 3kg in weight Secretion rate of sweat not less than $1g/m^2/min$ required	Elute sweat from filter paper for at least 40 minutes		Sodium should not be interpreted without chloride

Cerebral Spinal Fluid

Physiology

The cerebral spinal fluid (CSF) is a clear bodily fluid that occupies the space between the arachnoid mater (meninges) and the pia mater. It constitutes the content of all intra-cerebral ventricles, cisterns and sulci, as well as the central canal of the spinal cord. It is formed in the choroid plexus by both filtration and active transport. In normal adults approximately 20mL is produced each hour, and the CSF volume is 125mL to 150mL. CSF has multiple functions - it protects the brain from sudden changes in pressure, it maintains a stable chemical environment and it removes waste products of cerebral metabolism.

Pathology

Afflictions of the CNS causing changes in the macroscopic, microscopic and chemical composition of CSF are diverse, each with its own pathogenetic mechanism. Some categories of primary CNS pathology reflected in CNS analysis include, haemorrhage, infections, malignancy and demyelinating disease[1].

Haemorrhage, with red blood cells in the CSF, may be secondary to:

- Hypertensive intracerebral haemorrhage into ventricles,
- rupture of a berry aneurysm with bleeding into the subarachnoid space,
- extension of a traumatic haematoma,
- bleeding from vascular malformations.

Meninigitis is an inflammation of the lepto meninges, usually caused by infection. Infecting organisms include bacteria, viruses, fungi and parasites. Microorganisms may reach the brain by haematogenous spread or by direct extension from sinuses, accessory structures (teeth) and via peripheral nerves.

Malignant tumours may shed cells into the CSF. Primary tumours e.g. gliomas may spread along the subarachnoid space. They are more common in ventricular than lumbar fluid [2]. Metastatic (secondary) tumours reach the brain by haematogenous spread and may involve the parenchyma or meninges.

Demyelinating diseases may produce CSF abnormalities by several mechanisms. Products of demyelination may be present in the fluid (eg myelin basic protein); leucocytes from lesional tissue may shed into the fluid; and increased oligoclonal immunoglobulins produced by local synthesis at the site of lesional tissue may be washed into the fluid.

Diagnostic Use

Cerebrospinal fluid can be tested for the diagnosis of a variety of neurological diseases. Cell count, levels of protein and glucose, and cell culture are the most frequently requested tests on CSF, which is obtained by lumbar puncture (LP). These parameters are helpful in the diagnosis of subarachnoid hemorrhage and central nervous system infections. More sophisticated markers, such as oligoclonal bands, are characteristic of an ongoing inflammatory condition (e.g multiple sclerosis). A β2 transferrin assay is highly specific and sensitive for the detection for CSF leakage.

Typical laboratory findings in viral meningitis include:

- The CSF white blood count (WBC) is usually less than 250/μL, and almost always less than 2,000/μL [3]. The differential shows a predominance of lymphocytes [4].
- The CSF protein concentration is typically less than 1.5g/L. It has been estimated that a CSF protein concentration >2.2g/L reduces the probability of viral infection to 1% or less [3].
- The CSF glucose concentration is usually more than 50% of serum concentration, but moderately reduced values are occasionally seen with herpes simplex virus, mumps, some enteroviruses, and lymphocytic choriomeningitis virus.

Typical laboratory findings in bacterial meningitis include.

- A CSF WBC count above 1000/μL, usually with a neutrophilic predominance
- A CSF protein concentration above 2.5 g/L
- A CSF glucose concentration below 2.5 mmol/L

Note that the range of CSF values in bacterial meningitis is so wide that there is substantial overlap with the findings in viral meningitis.

Lumbar puncture can also be performed to measure the intracranial pressure, which may be increased in certain types of hydrocephalus.

Biochemical Tests Performed Routinely on CSF

Protein

Proteins are largely excluded from the CSF by the blood-CSF barrier. Proteins gaining access to the CSF primarily reach the CSF by transport within pinocytotic vesicles traversing capillary endothelial cells. The normal CSF protein concentration in adults ranges from 0.15 to 0.45 g/L [5]. CSF protein concentrations in premature and term neonates normally range between 0.2 and 1.7g/L [6]. CSF protein can be falsely elevated in the presence of RBCs from subarachnoid haemorrhage or traumatic LP. The presence of CSF bleeding results in approximately 0.01g of protein/L per 1000 RBCs/μL. When assessing the potential effect of CSF bleeding on an elevated CSF protein concentration, the CSF protein concentration and RBC count should be performed on the same sample of CSF.

Elevations in the CSF protein concentration can occur in both infectious and non-infectious conditions, including conditions associated with obstruction to CSF flow. CSF protein elevations may persist for weeks or months following recovery from meningitis and have little utility in assessing cure or the response to therapy [3]. Elevated protein levels may aid in diagnosis of inflammatory conditions such as Guillain Barré Syndrome, where levels of over 1g/L are often seen.

Glucose

The glucose concentration in CSF is maintained by both facilitated transport and simple diffusion. Glucose is removed from the CSF by transport across capillaries and arachnoid villi but also is utilized by cells lining the ventricular cavities and subarachnoid spaces. As a result, it normally takes several hours for the serum glucose to equilibrate with the CSF glucose. The CSF-to-serum glucose ratio is approximately 0.6 in normal individuals; ventricular CSF has a higher glucose concentration than CSF in the lumbar space by 0.33 to 1.0 mmol/L [7].

The CSF glucose concentration may be altered in a variety of pathologic conditions. Abnormally low CSF glucose concentrations can occur in bacterial meningitis and mycobacterial and fungal CNS infections. During recovery from meningitis, CSF glucose concentration tends to normalize more rapidly than do the CSF cell count and protein concentration. Low CSF glucose concentrations can also be observed in CNS infections due to *M. pneumoniae* and non-infectious processes, including malignant processes infiltrating the meninges, subarachnoid haemorrhage, and CNS sarcoidosis. However, CSF glucose concentrations less than 1.0 mmol/L are strongly predictive of bacterial meningitis. The CSF glucose concentration is typically normal during viral CNS infections, although exceptions have been reported in patients with meningoencephalitis due to mumps, enteroviruses, lymphocytic choriomeningitis (LCM), herpes simplex, and herpes zoster viruses. The CSF-to-serum glucose ratio has limited utility in neonates and in patients with severe hyperglycemia. CSF glucose concentrations rarely exceed 16 mmol/L, even in patients with severe hyperglycemia.

Biochemical Tests Performed in Specific Clinical Circumstances

Immunoglobulins

Immunoglobulins are almost totally excluded from the CSF in healthy individuals. The normal blood to CSF ratio of IgG is 500:1 or more. Elevations in immunoglobulin concentrations in CSF may occur in any disorder that disrupts the blood-brain barrier. Thus, an elevated CSF IgG concentration has limited diagnostic utility.

CSF IgG Index

Increased levels of CSF IgG can be due to excess production of IgG within the CNS (multiple sclerosis and several other diseases) or it can be due to leakage

of plasma proteins into the CSF (inflammation or trauma). To discriminate between these two possibilities, the IgG index is calculated from IgG and albumin measurements performed in CSF and serum [8] using the following equation:

IgG index = [IgG (CSF) / IgG (serum)] / [Albumin (CSF) /Albumin (serum)]

An elevated IgG index (>0.66), which indicates increased production of IgG within the central nervous system, is found in about 90% of cases of MS.

Lactate

The CSF lactate level normally parallels blood concentrations. With biochemical alterations in the CNS, CSF lactate values change independently of blood values. Increased CSF concentrations are noted in CVA, intracranial haemorrhage and epilepsy. CSF lactate concentration has been observed to rise in experimental and clinical cases of bacterial meningitis [9, 10]. In one study of infection following neurosurgical procedures, lactate levels had a higher sensitivity and specificity than determinations of the ratio of CSF-to-blood glucose [10]. Despite these data, testing for CSF lactate levels is not often performed in clinical practice because many physicians perceive that this test does not offer substantially more information than standard CSF analysis for the diagnosis of bacterial meningitis and there have been inconsistencies in the reported diagnostic power of the test [11].

Respiratory Chain Disease

For investigation of respiratory chain disorders quantitative amino acids in plasma and urinary organic acids are the investigations of choice. Where CSF is available an elevated CSF to blood lactate ratio is considered a useful test in the investigation of suspected mitochondrial disorders. In these conditions the blood lactate may be normal or only slightly raised with an inappropriately high CSF lactate. The finding of a normal concentration of lactate in blood and CSF does not always exclude a respiratory chain defect. Where there is a strong clinical suspicion of such a defect, pre- and post-prandial lactate may be measured (sometimes a glucose tolerance test is performed with simultaneous determination of blood lactate). Under these conditions, lactate concentrations in blood remain nearly constant or increase only slightly, but in a respiratory chain defect a pathological increase may occur [12].

Xanthochromia

Xanthochromia, a yellow or pink discoloration of the CSF, represents most often, the presence of haemoglobin degradation products and indicates that blood has been in the CSF for at least two hours (e.g subarachnoid haemorrhage). Following haemorrhage into CSF red blood cells rapidly undergo lysis and phagocytosis. The breakdown of haemoglobin first to oxyhaemoglobin (pink), and later to bilirubin (yellow), leads to a discoloration of the CSF known as xanthochromia. In most patients,

xanthochromia is first evident two to four hours after RBCs have entered the subarachnoid space, is visible within 12 hours in over 90 % of patients with a subarachnoid haemorrhage, and persists for two to four weeks. Other causes of xanthochromia include increased CSF concentrations of protein (≥1.5 g/L), systemic hyperbilirubinaemia (serum bilirubin >200 μmol/L approx), and traumatic lumbar puncture.

A recognised indication for LP is suspected subarachnoid haemorrhage in a patient with a negative CT scan. Since RBCs in the CSF can reflect a traumatic tap, an important finding in this setting is xanthochromia. Timing of the LP is critical, and should be performed >12hr after the onset of symptoms. Although xanthochromia may be confirmed visually, evidence indicates that this is not reliable, and laboratory confirmation of the presence of bilirubin using spectrophotometry is more sensitive and highly recommended [13,16]. Clearing of blood (a declining RBC count with successive collection tubes) is purported to be a useful way of distinguishing a traumatic LP from SAH. However, this is an unreliable sign of a traumatic tap, since a decrease in the number of RBCs in later specimens can occur in SAH. This method can reliably exclude SAH only if the last or final collection specimen is normal.

CSF Oligoclonal Bands

The immunoglobulins in CSF are compared with those in serum by isoelectric focussing and 'oligoclonal' bands identified (as opposed to 'monoclonal' bands such as are produced in serum by a myeloma or the 'diffuse polyclonal bands' of normal immunoglobulins). It is possible to identify whether the oligoclonal immunoglobulins originate from outside or from within the CNS. Intrathecal synthesis of oligoclonal immunoglobulins is associated with inflammation within the CNS. It is typically found in demyelinating diseases such as MS but may also be seen in infections and autoimmune diseases.

Electrophoresis and Isoelectric Focusing are two methods for separating the proteins in a biological fluid. A patient's CSF and serum are run side-by-side using either of these two techniques. Following the separation step, a protein stain is applied to both specimens, and the banding patterns of the proteins in CSF and serum are compared to one another. The presence of two or more IgG bands in CSF that are not present in serum is a positive test for oligoclonal banding. About 90% of MS patients show oligoclonal banding in their CSF.

Myelin Basic Protein

Myelin basic protein is a major component of myelin. Increased concentrations of myelin in CSF indicate that demyelination is taking place. This process is not specific for MS, as other inflammatory diseases of the CNS can also cause elevation of myelin basic protein. However, this test may be useful in assessing disease activity in cases of established Multiple

Sclerosis.

Amyloid Beta 42 peptide and Tau protein (Alzheimer biomarkers)

Amyloid Beta 42 peptide and Tau protein can be measured in the CSF of patients with dementia, to help discriminate between Alzheimer's disease and other forms of dementia. However, currently these tests are applicable to the research setting only and information on how to interpret the tests is limited.

Other Laboratory Tests

Macroscopic Examination

Colour: Normal CSF is clear and colourless. Both infectious and non-infectious processes can alter the appearance of the CSF. As few as 200 white blood cells (WBCs) or 400 red blood cells (RBCs)/μL will cause CSF to appear turbid. CSF will appear grossly bloody if ≥6000 RBCs/μL are present [14].

Microscopic Examination

The CSF is normally acellular, although up to 5 WBCs and 5 RBCs are considered normal in adults when the CSF is sampled by LP; newborns, in contrast, may have up to 20 WBCs/μL in the CSF. More than 3 polymorphonuclear (PMNs) leukocytes/μL are abnormal in adults and despite a higher total WBC in newborns, PMNs /μL remain low [15].

Cytology

Cytology is occasionally useful for the diagnosis of malignancy involving the CNS.

Gram Stain

Gram staining of the CSF is an integral part of the evaluation of patients with suspected meningitis or encephalitis.

Analytical Factors

Traumatic Tap

Accidental trauma to a capillary or venule may occur during performance of an LP, increasing the number of both RBCs and WBCs in the CSF. To distinguish a true increase in CNS WBCs from a traumatic tap-induced rise one may calculate the predicted CSF WBC count from the following formula:

Predicted CSF WBC count/ μL = CSF RBC count x (peripheral blood WBC count ÷ peripheral blood RBC count).

The utility of this approach was illustrated in a report of 720 traumatic LPs in which approximately one-half of the CSF samples obtained from patients without meningitis had more white cells than could be

accounted for by the proportionate number of red cells [11]. A CSF WBC count that was more than 10 times the predicted value had a 48 % positive predictive value for bacterial meningitis, while a value less than 10 times the predicted value had a 99% negative predictive value for meningitis.

Specimen Collection, Handling, and Transport for Routine CSF Analysis

In order to ensure sufficient CSF for microbiology, for protein and glucose measurement, and for spectrophotometric scan the following protocol should be followed:

1. Label three sterile plain universal containers and one fluoride EDTA/oxalate tube with the patient's name, Date of Birth (DOB), Medical Record Number (MRN), the time that the CSF was obtained and the sequence order of the sampling.
2. The first sample should be a minimum of 0.5mL of CSF and be placed in a fluoride EDTA/oxalate tube for glucose estimation (plus lactate if required) and sent to the clinical biochemistry department ASAP.
   The revised guidelines on CSF in suspected subarachnoid haemorrhage [16]recommend using the fluoride EDTA/oxalate tube for protein as well as glucose. However, many laboratories have a preference for a plain container for protein analysis, usually using sample from the microbiology aliquots (once processed rapidly by the Microbiology Department) or from the xanthochromia sample (where such sample is taken).
3. The second and third samples should be a minimum of 2.5mL each, be placed in sterile universal containers labelled 'second' and 'third' and sent to the microbiology department ASAP.
4. If CSF xanthochromia needs to be determined a fourth sample with a minimum of 1mL (labelled 'fourth'), should be placed in sterile universal container and sent to the clinical biochemistry department for spectrophotometric scan. This sample must be protected from light by placing in a thick brown envelope outside the usual plastic specimen bag.

Importantly, a blood specimen should be taken simultaneously for serum bilirubin, total protein and glucose estimation, which are needed to aid interpretation and sent to the clinical biochemistry department as soon as possible.

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Pleural Fluid

Physiology

The pleural cavity is the space between the chest wall and the lungs. It is lined by two membranes and lubrication between these serous membranes is provided by a very thin layer of fluid, usually less than 10mL in each cavity. Pleural fluid is an ultra-filtrate of plasma.

Pathology

A pleural effusion occurs when fluid formation exceeds removal resulting in accumulation of excess fluid in the pleural space. This accumulation can be due to increased fluid production or decreased fluid removal. The etiology of the accumulation is related to the underlying condition e.g. congestive cardiac failure causes increased fluid due to raised hydrostatic pressure gradient whereas in malignancy or infection, the increased production is usually the result of increased permeability of capillary vessels.

Diagnostic Use

The primary use of biochemical analysis of pleural fluid is to differentiate between transudates and exudates (see Biochemical tests performed routinely on pleural fluids), this differentiation being an important pointer in determining the cause of the effusion.

The most common causes of *exudative* pleural effusions are parapneumonic effusions (particularly bacterial pneumonia), and malignancy.

The most common causes of *transudative* pleural effusions are left ventricular failure (very common) and cirrhosis.

Occasionally where there is a less common cause of fluid accumulation, biochemistry tests may point to the origin of the fluid (see Biochemical tests performed in specific clinical circumstances).

Biochemical Tests Performed Routinely on Pleural Fluids

Total Protein

A clearly low total protein (<25g/L) or a clearly high protein (>35g/L) will usually differentiate between transudate (<25g/L) and exudate (>35g/L). However the frequency of borderline results, and also the need for further evaluation to determine the cause of an exudate, means that on most occasions further biochemistry tests are required. It is therefore best practice to take appropriate samples in the first instance for all tests that might be needed.

A protocol should be in place in every laboratory whereby all pleural fluids have samples preserved, in the correct container (see Analytical Factors below), for an agreed list of tests. For most laboratories the tests include

protein and albumin, LDH, pH, and glucose.

Blood plasma/serum levels of protein and albumin, LDH, and glucose should be measured for comparison.

Light's Criteria

Light's criteria, originally published in 1972, and re-issued 2002 [3], are the most frequently used criteria for differentiating exudate from transudate. In this scheme a fluid is deemed exudate if any of the following apply:

- Ratio of fluid protein to serum protein is greater than 0.5
- Ratio of fluid LDH to serum LDH is greater than 0.6
- Pleural fluid LDH is greater than two-thirds of the upper reference limit for plasma LDH.

Note: Light's criteria are highly sensitive in identifying an exudate. However their specificity is low, particularly in patients with heart failure. Studies have shown that up to one third of these patients may fulfill at least one of Light's criteria for an exudate. Patients with false positive results are more likely to meet only one of Light's criteria and to have received intravenous diuretics within 24hours before the pleural tap.

Some studies have questioned the value of the fluid to plasma ratios, proposing instead that fluid levels are diagnostic on their own.[2]

Cholesterol

Though not tested routinely, cholesterol may be helpful if there is uncertainty in the measurement of Light's Criteria. Cholesterol concentration is lower in transudates than in exudates. Cut-offs from 1.6mmol/L down to 1.2 have been suggested as giving improved diagnostic accuracy (i.e. for the cut-off of 1.6 mmol/L, cholesterol less than 1.6 supports transudate). One slight concern is the method reliability at such low levels.

pH

Normal pH of pleural fluid is approximately 7.6. A pH<7.3 is associated with inflammatory states. Some patients with pneumonia and parapneumonic effusion may develop empyema (pus in the fluid). A pH<7.2 has been proposed as indicating need for chest drainage in such patients, but the evidence is not clear-cut.

The guidelines issued by the British Thoracic Society (BTS) [4] recommend that pH should be performed in all non-purulent effusions, and if an effusion is infected a pH of <7.2 indicates the need for tube drainage.

Low pH is also proposed as a general indicator of poor prognosis.

Measurement of pH in fluid is particularly prone to pre-analytical problems. Samples must be collected under anaerobic conditions (in practice into a 'blood-gas' syringe with all air expelled) and analysed promptly.

Biochemical Tests Performed in Specific Clinical Circumstances

Some additional biochemical tests may provide valuable information in response to a specific clinical question.

Query Chylothorax

Measurement of triglycerides and cholesterol can help to confirm chyle in the chest cavity. A triglyceride level greater than the cholesterol level supports chylothorax. One can also check for chylomicrons by ultra-centrifuging the sample or by standing in a refrigerator overnight.

Query TB

Adenosine deaminase has in the past been suggested in many publications and textbooks as a biochemistry test that may aid in diagnosis of TB. In practice this test is not readily available in Ireland or the U.K. and so must be regarded as a research tool only.

Query Malignancy

The value of various tumour markers (e.g. CEA) in pleural fluid is questionable. There is also a major concern over the accuracy of such tests in a fluid that has a different matrix to that covered by the manufacturers' guarantees.

Query Pancreatitis

A raised fluid amylase level indicates possible pancreatitis. The recommendation of the BTS Guidelines to use amylase iso-enzyme determination to help differentiate causes of raised amylase is impractical as this assay is not readily available within a clinically useful time-frame.

Other Laboratory Tests

Tests performed by the Microbiology, Histology / Cytology, and/or Haematology Departments play a very important role in the differential diagnosis of pleural effusion.

Tests used include cytology, differential white cell count, Gram stain / culture & sensitivity, and specific tests for TB.

Analytical Factors

In routine practice one of the biggest problems with fluid analysis is failure to provide the lab with appropriate specimens.

Unless the effusion is small and fluid is in short supply, separate samples should be collected for each test or group of tests needing different preservatives or being analysed in different departments. A protocol should be in place for clinical staff indicating the number and type of bottles of fluid to be collected, what tests are to be requested, and where they should be sent.

- **Proteins, LDH**: MSU bottle, Li Hep plasma or plain bottle (as for serum).

- **pH**: Air-free sample, preferably taken into 'blood-gas' syringe. Measurement of pH is best done on a 'blood gas' analyzer using a clot-catcher.
- **Glucose**: Fluoride Oxalate bottle.

Safety: Pleural fluid samples are high risk, especially in query-TB cases, and should be treated accordingly. Protocols for analyzing these samples should be agreed with the Microbiology Department.

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Pericardial Fluid

Physiology

The pericardial space normally contains 15-50 mL of fluid, which is essentially an ultrafiltrate of plasma. This fluid is thought to originate from the visceral pericardium and serves as lubrication to visceral and parietal layers of the pericardium.

Pathology

Pericardial effusion is an abnormal amount and/or character of fluid in the pericardial space. It can be caused by local or systemic disorders, but in many cases the underlying cause cannot be identified and the effusion is defined as idiopathic. Effusions can be acute or chronic and the time course to development has a major impact on patient symptoms.

Diagnostic Use

Pericardial fluid obtained at pericardiocentesis is often subjected to biochemical, haematological, microbiological, and cytological analysis. Abnormal fluid production is usually secondary to injury to the pericardium (i.e., pericarditis). A transudative effusion results from obstruction of fluid drainage through lymphatic channels. Exudative effusions reflect inflammatory, infectious, malignant, or autoimmune processes within the pericardium. Clinical manifestations of pericardial effusion are highly dependent upon the rate of accumulation of fluid in the pericardial sac

Biochemical Tests

In practice the clinical setting associated with pericardial effusion helps define the underlying pathology and, unlike the situation with pleural effusion, biochemistry is only rarely of value. Tests performed by other laboratory departments are usually more important in the differential diagnosis of the cause of pericardial effusions (see Other Laboratory Tests - below).

Transudate v Exudate

Most effusions are exudates and biochemical differentiation is only rarely needed. Where such differentiation is needed biochemistry tests are often interpreted using criteria borrowed from pleural effusions. The validity of this approach is however uncertain. The composition of physiologic pericardial fluids (obtained at time of open heart surgery) is remarkable for a high LDH and protein content as well as for predominance of lymphocytes. Thus, biochemical criteria useful for diagnosing pleural effusions may not be wholly applicable to differentiating transudative from exudative pericardial effusions, and lymphocytosis should be interpreted with caution.

Routine Tests

The two most common biochemical tests are Lactate Dehydrogenase (LDH) and Total Protein.

Light's criteria have been used to distinguish between exudative and transudative effusions; for exudative pleural effusion:

- Total protein fluid to serum ratio > 0.5
- LDH fluid to serum ratio >0.6
- LDH fluid level exceeds two thirds the upper limit of normal serum level.

Caution is advised in applying Light's criteria to pericardial fluids, particularly in respect of LDH levels.

Other Laboratory Tests

Cytological examination as well as bacteriologic smears and cultures of fluid are the primary laboratory tests used in initial investigations of pericardial effusions of unclear aetiology.

Cell Count

Elevated leucocytes (greater than 10,000/μL) with neutrophil predominance suggests a bacterial or rheumatic cause.

Analytical Factors

Care must be taken to preserve samples correctly for the various tests required. Use the same preservatives / bottles for the fluid as would be used for the same test in plasma / serum.

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Ascitic / Peritoneal Fluid

Physiology

Ascites is the accumulation of free fluid within the peritoneal cavity. Ascites can be either transudative or exudative.

The normal peritoneal fluid volume rarely exceeds 5 ml of transudative fluid (an ultrafiltrate of plasma that seeps across capillary walls and contains less than 30g protein per litre fluid) in men. In women, normal values are usually up to 5-18 ml, depending on the phase of the menstrual cycle.

Transudative ascitic fluid is produced by visceral capillaries and drained via the diaphragmatic lymphatic system. Exudative fluid is rich in protein and cellular debris. It leaks out of blood vessels and is deposited in tissues or tissue surfaces usually as a result of inflammation.

Pathology

Ascitic fluid (peritoneal fluid), is a common clinical finding with a wide range of causes.

Ascites is caused by:

- cirrhosis in 75% of cases
- malignancy in 10%
- cardiac failure in 5%
- various other causes account for the remaining 10%.

Conditions that may be associated with ascites include:

Increased hydrostatic pressure associated with portal hypertension: cirrhosis, alcoholic hepatitis, fulminant hepatic failure, fatty liver of pregnancy, hepatic fibrosis, Budd - Chiari syndrome [clotting of the hepatic vein], constrictive pericarditis, congestive heart failure, veno-occlusive disease

Decreased colloid osmotic pressure secondary to hypoalbuminaemia: end stage liver disease with poor protein synthesis, nephrotic syndrome with protein loss, malnutrition, protein-losing enteropathy,

Increased permeability of peritoneal capillaries: tuberculous peritonitis, bacterial peritonitis, fungal peritonitis, HIV associated perotinitis,

Leakage of fluid into the peritoneal cavity: bile ascites, pancreatic ascites, chylous ascites, urine ascites,

Malignant conditions: peritoneal carcinomatosis (GI cancer that has spread throughout the abdomen), hepatocellular carcinoma, hepatic metastases, pseudomyxoma peritonei (extensive mucus accumulation within the abdomen), mesothelioma, and cancers associated with breast, large bowel, bronchus,

stomach, pancreas, ovary, and endometrium,

Miscellaneous causes:

myxoedema, ovarian disease, Meig's syndrome (condition associated with benign ovarian tumours), chronic haemodialysis.

Diagnostic Use

Although ascites is not intrinsically life threatening, cirrhotic patients with ascites have a two-year mortality rate of 50%. Diagnostic paracentesis (an abdominal tap to obtain a sample of fluid) should be performed routinely in all patients with new onset ascites and in all patients admitted to the hospital with ascites.

Appearance:

Ascitic fluid is generally straw colored or yellow tinged. Cloudiness or opaque appearance is due to the presence of neutrophils. Milky appearing ascites is due to the presence of triglycerides (chylous ascites). Non-traumatic bloody ascites may be associated with tuberculosis or malignancy. Tea-coloured fluid is occasionally seen in pancreatic ascites.

Sample:

Specimens should be collected into a sterile container and sent to the laboratory for analysis. Care must be taken to preserve samples correctly for the various tests required (see also Analytical Factors).

Biochemical Tests

Although several laboratory tests are helpful in distinguishing transudates from exudates in pleural fluids, the criteria for differentiating these fluid types in ascites is not clear-cut. Biochemistry tests include total protein (values greater than 30g/L suggest the fluid is an exudate indicating inflammatory or malignant ascites), amylase (raised in pancreatitis), triglycerides (raised in chylous ascites), pH (less than 7.0 indicates bacterial infection).

Diagnosis of ascites due to portal hypertension is established by measurement of the serum-ascites albumin gradient (SAAG). The SAAG is calculated by subtracting the ascitic fluid albumin concentration from the serum albumin concentration in simultaneously obtained specimens.

- **SAAG > 11g/L**

  Ascites due to portal hypertension (transudative ascites) is characterized by a SAAG of 11 g/L or higher. This cut-off may be used to diagnose portal hypertension with about 97% accuracy. The ascitic fluid total protein concentration is used to differentiate the various causes of ascites in patients with a high SAAG. For example, patients with cirrhosis, alcoholic hepatitis, cardiac failure, or fulminant hepatic failure have a low total protein concentration (<10 g/L); patients with congestive heart failure, Budd-Chiari syndrome,

or constrictive pericarditis in whom hepatic synthetic function is essentially preserved have a relatively high total protein concentration (>20 g/L).

- **SAAG < 11g/L**

  A SAAG less than 11 g/L occurs in tuberculous peritonitis, chylous ascites, peritoneal carcinamotosis, pancreatic or biliary inflammation, nephrotic syndrome and bowel obstruction/infarction.

Other Laboratory Tests

Initial analysis of ascitic fluid should include macroscopic and microscopic examination, gram stain, culture and cytology (important for diagnosing malignancy). A cell count with WBC differential should always be performed. An increase in neutrophils (>250 /μL) is associated with peritonitis (bacterial, tuberculous, pancreatic or malignant). A WCC greater than 1000 /μL is also associated with bacterial or tuberculous peritonitis. A red cell count greater than 50,000 /μL denotes haemorrhagic ascites, usually due to malignancy, tuberculous or trauma.

Analytical Factors

When collecting ascitic fluid by paracentesis collect as much sample as possible into a sterile container. Microbiological tests should be performed first before distributing to other laboratories.

For analysing the SAAG, both serum and fluid albumin should be measured. Bloods should also be analysed for liver function tests (including total protein), renal function tests, and amylase; fluids should be analysed for total protein, amylase, and triglyceride levels, as appropriate to the clinical questions. Some authors recommend measuring adenosine deaminase if tuberculous peritonitis suspected, but in practice this test is not readily available in Ireland or the U.K. and so must be regarded as a research tool only.

Blood and fluid samples should be taken concurrently.

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Sweat

Physiology of Normal Sweat

Sweat is a watery fluid secreted by glands in the skin. The primary purpose of sweating is to regulate body temperature, through the cooling effect of evaporating sweat. Sweat glands are of two types, eccrine and apocrine. About 3 million eccrine sweat glands are distributed all over the body. They are controlled by the hypothalamic thermoregulatory centre via sympathetic cholinergic nerves. The gland consists of a long coiled tube in the dermis with a duct to the surface.

Sweat is produced as an isotonic ultrafiltrate of plasma in the blind end (acinus) of the coil. It contains salt and urea, but very little protein or fatty acids. As this primary secretion passes up the duct, chloride and sodium ions are reabsorbed and the excreted sweat becomes hypotonic, with a sodium and chloride concentration of 5-40 mM. During periods of low sweat production, most of the salt is re-absorbed, but when the sweat flow rate is high, it passes through the duct more rapidly, fewer ions are re-absorbed and a more concentrated sweat is produced.

Apocrine sweat glands are distributed mainly in the axillae and around the genitals, and produce a sweat containing fatty acids and salts.

Pathology of Sweat in Cystic Fibrosis

Cystic fibrosis (CF) is caused by mutations in a gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein, resulting in dysfunctional epithelial chloride channels. Re-absorption of chloride ions is diminished or abolished in the duct of the sweat gland. Re-absorption of sodium is also reduced to maintain electronic equilibrium. The result is the production of a more concentrated sweat with sodium and chloride concentrations of >60 mM.

There are more than 1200 known mutations of the CFTR gene.

The defective CFTR also causes altered secretion of fluids by the pancreas and in the lungs, which is the cause of morbidity and mortality in cystic fibrosis.

Diagnostic Use

The alteration in the composition of sweat provides the basis for a diagnostic test for cystic fibrosis. Pilocarpine (a cholinergic agent) is introduced into a small area of the skin by iontophoresis, producing localised stimulation of sweat glands. Sweat is collected into sodium-chloride-free filter paper pads covered with impervious material sealed to the skin (Gibson-Cooke method), or into capillary tubing (Wescor Macroduct apparatus), and the electrolyte content is quantified.

Collection and analysis of sweat is a highly specialised procedure, requiring special expertise for proper performance. Its use should be confined to specialist centres of excellence with fully-trained experienced personnel. To maintain adequate expertise a minimum of 50 tests per annum should be performed in a centre, and at least 10 collections per annum by each person.

Care must be taken to avoid burns or blistering of the patient's skin during passage of the iontophoretic electric current; a battery-powered apparatus with safety cut-out should be used.

Detailed guidelines for performing the sweat test have been published.[1,2]

Biochemical Tests

Sweat chloride concentration should be determined as it shows better discrimination than sodium or osmolality. Sodium should not be the only or primary analyte determined (historically it often was). Sweat potassium or osmolality measurement is not recommended. Sweat chloride >60mmol/L supports a diagnosis of CF. An intermediate chloride concentration of 40-60 mmol/L is suggestive but not diagnostic of CF. Sweat chloride <40mmol/L is normal, and the probability of CF is low. Sweat sodium should not be interpreted without chloride. Suitable assay methods for sweat chloride are colorimetry, coulometry and ISE; for sodium, flame photometry or ISE are suitable.

Sweat conductivity measurements (Wescor apparatus) show better inter-laboratory precision than chloride or sodium, and two large studies show good discrimination between CF and normal subjects. However, due to lack of data from tertiary and referral centres, conductivity is not recommended as the sole test. Conductivity <60 mmol/L (NaCl equivalents) is unlikely to be associated with CF; values >90 mmol/l support a diagnosis of CF.

The test should be repeated if the result is not in keeping with clinical phenotype or genotype. Non-physiological or discrepant results should be questioned and the test repeated e.g. Cl^- or Na^+ > 150mmol/L, discrepancy of >20mmol/L between Cl^- and Na^+ results, or conductivity > 170 mmol/L.

Analytical Factors

The patient must be suitable for testing:

- Sweat tests can be performed on infants more than 2 weeks of age and weighing over 3 kg.
- Exceptionally, term infants can be tested after 7 days, but may yield insufficient sweat, and sweat sodium and chloride can be high in the first 7 days, and especially the first 2 days.
- Pre-term infants do not sweat in the first 7-14 days.
- It is difficult to get enough sweat in very young infants, especially those under 3 kg.

Sweat electrolytes can be elevated in underweight or dehydrated infants or

if the collection site has active eczema, and lowered in infants on systemic corticosteroids or with oedema. Testing should be postponed in any of these conditions or if the subject is systemically unwell. Sweat electrolytes are not affected by diuretics or i.v. fluids.

The flexor surface of either forearm is the preferred site of sweat collection. Other sites (e.g. upper arm, thigh, back) can be used if both arms are unsuitable (e.g. too small or eczematous). A pilocarpine solution of 2-5g/L should be iontophoresed at 4mA for 3-5 minutes. Sweat should be collected from the stimulated area for between 20 and 30 minutes. A sweat secretion rate of not less than $1g/m^2/min$ is required over the collection period. Collections less than this are unsuitable. It is not valid to pool insufficient collections. It is essential to prevent contamination of the sample or evaporation losses during collection. All sweat produced, including condensate on the waterproof covering, must be transferred back to the filter paper. Sweat should be eluted from filter paper for at least 40 minutes before analysis.

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Amniotic Fluid

Physiology

Amniotic Fluid (AF) is a clear, watery and slightly yellowish liquid that surrounds the foetus during pregnancy and it is contained in the Amniotic Sac. The Amniotic Sac has an inner and outer membrane. The inner membrane, the Amnion, contains the AF and the foetus. The outer membrane, the Chorion, contains the Amnion and is part of the placenta.

AF accomplishes numerous functions for the foetus.

These include:
- cushioning the foetus from injury, from outside sudden movement or blows
- allowing for freedom of foetal movement and permitting symmetrical musculoskeletal development
- helping to maintain constant temperature and permitting proper lung development.

The amniotic fluid is a dynamic medium whose volume and chemical composition, though narrowly controlled, are constantly changing throughout pregnancy. In the early stages, the AF is largely of maternal origin being a complex dialysate of the mother's serum. The fluid is in constant flux, exchanging with placenta, umbilical cord, foetal skin, foetal membranes and lungs. Also the AF is being inhaled and exhaled by the foetus and being added to by foetal urination, which becomes a more prominent source of AF in the latter stages of gestation. The volume of AF increases as the foetus develops, to a maximum of around 800 mL at approximately 34 weeks of gestation. This decreases to around 600 mL at full term of 40 weeks.

An excessive amount of AF is called *polyhydramnios*. This condition may accompany multiple pregnancy (twins or triplets), congenital abnormalities, or gestational diabetes.

An abnormally small amount of AF is known as *oligohydramnios*. This condition may accompany postdates pregnancies, ruptured membranes, placental dysfunction, or foetal abnormalities.

Pathology

Erythroblastosis foetalis is a haemolytic disease of the foetus and the newborn and is caused by maternal antibodies directed against antigens on foetal erythrocytes. Pregnant women who are Rhesus Negative (Rh neg) and whose blood has been exposed to foetal erythrocytes that are Rhesus Positive (Rh pos) are in danger of becoming sensitised and producing anti-D antibodies. This can happen in cases of spontaneous abortion, ectopic pregnancy or with normal delivery when significant volumes of foetal blood may enter the maternal circulation by crossing the placental

barrier. If left untreated this can give rise in subsequent pregnancies, where there is the same Rh neg / Rh pos conflict, to Erythroblastosis foetalis, also known as isoimmune disease or haemolytic disease of the new born (HDN) or simply Rh disease. The anti-D antibodies produced are usually of the smaller IgG class and can readily cross the placental barrier and attack the foetal erythrocytes. The severity of Rh disease depends on the degree of the maternal immune response and the level of anti-D antibodies in circulation. In worst case scenarios where the destruction of foetal red cells is excessive, the resulting anaemia causes a cascade of effects that can lead to congestive heart failure and generalised foetal oedema with ascites as well as pleural and pericardial effusions. This condition is known as hydrops foetalis and is generally fatal. One of the consequences of Rh disease is the increase in bilirubin in AF. It was noted by Liley that there was a direct relationship between gestational age, severity of the disease, and amniotic bilirubin concentration.

Diagnostic Use

Liley developed a chart of changing AF bilirubin levels and gestation, with three zones delineating the severity of *Rh disease.* This chart ranged from 27 to 40 weeks and was found to be clinically useful and became an important tool for assessing *Rh disease* in pregnancy and is widely adopted. Queenan has since published another predictive chart. This chart ranges from 14 to 40 weeks and has four zones of changing AF bilirubin levels and gestation.

New imaging techniques have largely replaced the use of this test but it does remain of value in individual cases.

Biochemical Tests

For the obstetrician, looking at the AF bilirubin is an indirect method for assessing the level of anaemia in the foetus. Normal levels of bilirubin in AF are very low (2.7 to 3.1 µmol/l), peaking at around 19 to 22 weeks. Scanning spectrophotometry is used to measure bilirubin at this concentration level. A spectrophotometric scan of normal AF shows a negative sloping straight line (baseline) from 350nm to 550nm. Bilirubin when present will absorb light maximally at 450nm. The absorption difference at 450nm (delta abs @ 450nm), that is from the peak absorbance and the baseline, drawn as a tangent at 350nm and 550nm on the curve, is the *bilirubin index* for that sample. Knowing the gestational age of the sample and using the Liley or Queenan chart, the result is plotted and depending on what zone it lies in, the severity of the *Rh disease* can be evaluated. Only well-trained staff should do the scan. A very fine tangent line is drawn and the delta absorption is calculated manually to the third decimal place. In practice there are always minute amounts of blood contaminating even good Amniotic samples. This can lead to the presence of oxyhaemoglobin, which absorbs at 410nm. The tailing of this peak adds to the absorbance at 450nm. By subtracting 5% of

the delta abs @ 410nm, from the delta abs @ 450nm, the corrected delta abs @ 450nm can be obtained. Depending on the initial result, a second sample is taken from one to four weeks later.

Usually, consecutive AF samples that show decreasing values are associated with mild disease. Usually no action is taken in those cases and pregnancy is allowed progress to full term. Repeat scans that show stable or rising values, are associated with severe anaemia and indicate the need for clinical intervention either by intrauterine transfusion or by early delivery.

All Rh neg mothers that have Rh pos babies are now routinely given anti-D IgG prophylaxis in an attempt to reduce the incidence of *Rh disease*. This works by destroying any Rh pos foetal erythrocytes circulating in the maternal blood before the maternal immune system becomes sensitised. This has resulted in a dramatic reduction in *Rh disease*.

Analytical Factors

The amniotic sample (10ml approx.) is obtained by amniocentesis under ultrasound guidance. A long thin needle is inserted through the uterus into the amniotic sac, taking care not to contaminate the sample with blood. The AF is put into a brown coloured bottle or sample tube that is totally protected from light as the bilirubin in the sample is very light sensitive.

Samples are taken to the laboratory and centrifuged immediately (2000 x g for 5min)

Note the state of the sample, bloodstained, clear, yellow, etc. Every effort should be made to perform the scan there and then but samples can be frozen and scanned at a later date. Use quartz cuvettes, 1.0 cm light path. Fill the cuvette with undiluted centrifuged AF and place in sample holder. Place a saline-filled cuvette in reference holder. Run the scan from 300nm to 600nm at 0.0 - 0.1 absorbance range. Rerun the scan at greater range if indicated. The *bilirubin index* is then calculated as outlined in the previous section; also see the review references below.

Other Laboratory Tests

Biochemical tests for foetal lung are now obsolete. All premature babies at risk from developing RDS can be treated intratracheally with a nebulized form of exogenous surfactant immediately at birth.

Elevated levels of alpha-fetoprotein (AFP) in amniotic fluid (AF) have long been associated with open neural tube defects and Down's syndrome. Amniocentesis is widely used in the U.S. and in the UK for these conditions but not in this country. Maternal serum may be used with a combination of tests including AFP for screening for neural tube defects and Down's syndrome in this State.

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Saliva

Physiology

Whole saliva is a mixture of oral fluids including salivary gland secretions, cellular material and food debris. Saliva also contains molecules normally found in serum that reach the saliva by several mechanisms: intra-cellular routes include passive diffusion, while extra-cellular routes include ultra-filtration at tight junctions between the cells.

Pathology

Saliva may be affected directly by systemic diseases or may reflect changes in serum concentrations of certain analytes. A reduction in salivary secretions is seen in Sjorgen's Syndrome, while other conditions (malignancy, infection, endocrinopathies) allow bio-markers to be identified and thus aid diagnosis of disease. Exogenous substances such as drugs can also be identified and measured in saliva.

Diagnostic Use

The primary uses of saliva testing are in the areas of toxicology, endocrinology and infection.

Advantages of using saliva include ease of collection (particularly when such collection requires supervision) and storage. Saliva collection is also non-invasive and stress-free which may be of use in paediatrics and in the measurement of stress affected hormones such as cortisol. Disadvantages of saliva analysis include the low levels of analytes present compared to serum, contamination from the oral cavity before collection, and viscosity of the fluid. Collection may also be difficult in dehydrated patients.

Biochemical Tests (Endogenous analytes)

Infectious Disease

Antibody detection includes Helicobacter pylori, Lyme disease, mumps and measles. Viral particles may be measured by PCR. HIV-1 antibodies is particularly suited to salivary testing due to the non-invasive method of sample collection, reducing the risk of infection for healthcare staff.

Hormones

The majority of hormones enter saliva by passive diffusion along a concentration gradient across the acinar cells of the salivary gland. Such hormones are lipid-soluble (i.e. steroids). Salivary levels may represent the free or non-protein-bound hormone levels. Cortisol levels, for example, correlate well with serum levels (except in conditions of increased binding proteins such as pregnancy and during use of the oral contraceptive pill) and may represent 10% of the unbound plasma concentration. However cortisol

may undergo metabolism in the salivary gland to cortisone which may have implications for the specificity of the analytical process. Salivary testosterone also correlates well with serum levels and may be a useful test in research on male hypogonadism or in sports medicine.

Biochemical Tests (Exogenous analytes)

The presence of a drug in saliva is influenced by the physico-chemical characteristics of the drug molecule. Passive diffusion of small non-ionised molecules is the major mechanism by which a drug will appear in saliva. Since binding proteins do not cross the membrane due to their size, only the unbound fraction of the drug in serum is available for diffusion into saliva. However, this is usually the pharmacologically active fraction. The correlation of blood and serum levels with saliva levels differs depending on the structure of the drug. For acidic drugs, the equilibrium favours blood, while basic drugs are found in higher levels in saliva.

Oral fluid has been seen as a non-invasive alternative to blood but also as an alternative to urine when substitution or adulteration is suspected.

Saliva can be used to detect and/or monitor cotinine, cannabinoids, cocaine, opioids, diazepines, amphetamines and ethanol. Ethanol is neither ionised nor protein–bound and, due to its low molecular weight and lipid solubility, rapidly diffuses into saliva. Thus the oral fluid to plasma concentration of ethanol averages about 1.

Analytical Factors

Saliva can be collected with or without stimulation. Saliva samples should be collected after a thorough mouth-rinsing, centrifuged to remove debris and frozen immediately. Commercial saliva collectors exist, containing absorbent pads which are left in the mouth for 2-3 minutes. Stimulation, masticatory or gustatory, may affect the concentration of some constituents and / or the pH of saliva. Since this collection method lends itself to being used in an out-patient setting, it is essential that the collection procedure is standardised and explained to the patient.

The preferred method for handling saliva samples before assay is to freeze them. On thawing and centrifugation, the glycoproteins precipitate out leaving a non-viscous fluid that is easier to pipette. Samples can also be heat-treated to reduce matrix effects.

Conclusion

Although saliva is useful as a non-invasive test for drugs of abuse and alcohol, these assays are not yet routinely available. The measurement of free cortisol is being investigated as a routine assay and may soon be available but will require the establishment of appropriate cut-off points.

The standard immuno-assays may not be sensitive for the low levels of

analytes present. Salivary proteomics using mass spectrometry methods are potentially the future of saliva testing.

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Seminal Fluid

Physiology

Semen is a fluid formed at ejaculation. It is composed of spermatozoa in seminal plasma. Semen therefore is made up of secretions of all the accessory glands of the male genital tract as well as the testicular sperm component.

The testicular contribution to semen volumetrically forms a relatively small portion of the ejaculate. Other secretions are produced mainly by the seminal vesicles and the prostrate. Small contributions to the seminal plasma are also made by other structures such as the epididymis.

Pathology

The seminal plasma functions as a nutrient transport medium for the spermatozoa. Changes in one or more of the secretions that form the semen may have effects not only on the concentration of sperm in the ejaculate but also on sperm function. Reproductive failure may be the result of pathology of one of the accessory glands rather than an abnormality of sperm itself.

Diagnostic Use

Some of the biochemical components of semen are specific to certain accessory glands and their presence or absence in the fluid can be useful diagnostically.

Biochemical Tests

Fructose

Fructose, which is the major source of glycolytic energy in spermatozoa, is produced by the seminal vesicles. A very low fructose level in the semen of an azoospermic man indicates absence of the seminal vesicles and/or vas deferens.

Alpha-glycosidase and Glycerylphosphoryl choline

Cases of obstructive azoospermia due to epididymal obstruction can be distinguished from non-obstructive azoospermia by the very low levels of epididymal derived alpha-glycosidase or glycerylphosphoryl choline.

Acid Phosphatase

Acid phosphatase is used as a marker for the presence of prostatic fluid.

Other Laboratory Tests

The evaluation of new therapeutic procedures (assisted reproductive technology) has ensured that the diagnosis of male fertility disorders remain under the microscope rather than in the test tube. However spermatozoa and seminal plasma remain as a resource material for research into aspects

of the molecular and cellular events in spermatogenesis

Analytical Factors

A fresh sample is required, the sample must be received within 2 hours.

Synovial Fluid

Physiology

Synovial fluid is a colourless to light yellow highly viscous fluid which does not clot. It has the consistency of egg white and is found in joint cavities. It is formed as an ultrafiltrate of plasma across the synovial membrane. Its function is to supply nutrients to cartilage, act as a lubricant to joint surfaces and to carry away waste products.

Pathology

Increased volume of synovial fluid may be the result of a variety of pathological processes. Such synovial fluids are often classified pathologically into four groups:
- Non-inflammatory (e.g. Osteoarthritis, neuroarthropathy)
- Inflammatory (e.g. Rheumatoid arthritis, gout)
- Septic (e.g. Bacterial or fungal infection)
- Haemorrhagic (e.g. Haemophilia, trauma).

Diagnostic Use

Examination of synovial fluid provides important diagnostic information in joint disease. In practice the most common site for collection of synovial fluid is the knee. Normal volume of knee joint fluid is 3-4 ml. Needle aspiration of synovial fluid is known as arthrocentesis.

Synovial fluid analysis is used in differentiating different types of arthritis i.e. infectious, crystal induced, inflammatory, non-inflammatory or haemorrhagic. A number of procedures are utilised to distinguish these conditions.

Tests Based on Physical Characteristics of Fluid

Initially a "string test" can be performed. This is a simple test of viscocity. Normal fluid, when dropped from a syringe, forms a string of greater than 10-15 cm. Inflammatory fluid has low viscocity and drips like water.

The clarity of the fluid can also be examined at this stage. Normal fluid is transparent and is colourless to light yellow. Non-inflammatory fluid is clear and yellow. Inflammatory and septic fluids are cloudy and yellow/green. Haemorrhagic fluid is cloudy and red/red-brown. Presence of crystals gives fluid a yellow to white appearance.

Biochemical Tests

The most commonly requested biochemistry test is glucose. A blood sample should be taken at the same time as the knee aspirate. The synovial fluid glucose concentration is normally no more than 0.6 mmol/l lower than the serum concentration. Significantly decreased synovial fluid glucose

concentration indicates presence of inflammatory and/or septic joint disorders. Other biochemical tests have been suggested e.g. protein and lactate, but these have not been shown to be useful.

Other Laboratory Tests
Other laboratory tests include cell counts (red and white) and differential, microbiological tests (gram stain and culture), and microscopic examination for crystals.

Analytical Factors
Samples should be collected into sterile tubes. Do not use oxalate or lithium heparin.

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