The biochemistry of body fluids



ACBI
Association of
Clinical Biochemists
in Ireland

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Preface

It is a pleasure to introduce the **revised 2025 edition of *The Biochemistry of Body Fluids***. This booklet reflects the collective expertise of members of the Association of Clinical Biochemists in Ireland (ACBI) and our shared commitment to high-quality, patient-centred laboratory medicine.

Body fluid analysis—whether cerebrospinal, pleural, pericardial, peritoneal, synovial, or other specialised specimens—often sits at the junction of urgent decision-making and nuanced interpretation. In revising this edition, our contributors focused on what matters most at the bench and the bedside: clear specimen guidance, sound analytical practice, harmonised interpretation where possible, and concise recommendations for clinical action.

The authors have highlighted when tests add value, identified common pitfalls, established critical thresholds, and described patterns that can change management. For laboratory scientists, the guidance emphasises pre-analytical essentials, method selection and verification, quality control, measurement uncertainty, and interpretive comments that elevate reports from results to recommendations. The booklet contains practical tables, checklists, and summaries designed for rapid use in busy settings.

This update is the result of a generous voluntary effort. I want to thank the chapter leads, authors, reviewers, and the editorial team for their diligence and collegiality; your contributions ensure the guidance is relevant, usable, and aligned with contemporary Irish and international practice and standards.

We offer this edition as a living reflection of best practice. Methods and evidence evolve; so too will our guidance. We welcome constructive feedback and proposals for future updates so that this resource continues to serve patients, clinicians, and laboratories across Ireland and beyond.

On behalf of ACBI, I extend sincere thanks to all authors for their time, expertise, and commitment to this revision. In particular, I wish to acknowledge Dr Peadar McGing for his encouragement and enthusiasm throughout this endeavour.

Warmest regards,

Dr Paula M. O’Shea

President, Association of Clinical Biochemists in Ireland (ACBI),

November 2025

Introduction

The use of blood and urine remain the key interpretative tools in the diagnosis and monitoring of disease. In diagnosis, 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. These are often termed atypical fluids, and that terminology is used in this booklet. Most such 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.

At the time of writing the first edition of this booklet, there were many clinical biochemistry tests performed on atypical fluids, particularly on CSF, pleural fluid or peritoneal / ascitic fluid. Use of these tests was long established, e.g. Light's Criteria in pleural fluid, but in general they were not covered by manufacturers' instructions for use (IFUs). In recent years quality requirements have changed the landscape, and all such applications should be properly validated. To that end a new section has been added covering Test validation in non-standard fluids.

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 intervals / action limits 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 the past decade new EQA schemes for atypical fluids have been established and where possible such schemes should be availed of (e.g. UKNEQAS). Nevertheless, most analyses in atypical fluids are not covered by EQA and in such circumstances it may be necessary to exchange samples with other sites performing similar assays if a diagnostic service is to be offered.

As with the original booklet, this edition of ACBI's guidelines aims to explain the use of biochemical tests for atypical fluids, and how they may help us in the diagnosis of patients and thus facilitate appropriate and effective treatment. Since no discipline in health care operates in isolation some information is included on other Pathology testing operating alongside biochemical testing.

All sections have been expanded and updated to include both new tests and new uses of older tests. A number of protocol changes have been highlighted. The reference section has been expanded and a small list of useful references covering multiple aspects of atypical fluids has been added.

The clinical testing of atypical fluids has potential to increase in importance, particularly as new technologies develop and as our knowledge increases from use of such tests. It will not be easy to validate all tests requested in such fluids, but it is important that laboratory scientists continually evaluate the use of these tests and work to ensure maximum reliability in the testing process, and so add benefit to patient care.

Cerebrospinal Fluid

Authors: Eileen Byrne, Paula M O'Shea, Peadar McGing.

1. Physiology

Cerebrospinal fluid (CSF) is a clear, colourless fluid circulating within the ventricular system of the brain and the subarachnoid space around the brain and spinal cord. It is produced by the choroid plexus (within the lateral, third, and fourth ventricles) and reabsorbed into the venous circulation via arachnoid granulations. In healthy adults, approximately 20mL is produced each hour, and the CSF volume is about 150mL. CSF is renewed approximately three to four times daily, ensuring dynamic clearance of metabolites and maintenance of intracranial homeostasis.

The term blood-brain barrier refers to both the blood-brain and blood-CSF barriers. These barriers prevent the simple diffusion of blood-borne substances from the blood into the CSF, thereby protecting the brain. The effect of this barrier is critical to our use of laboratory markers of health or disease.

Key Functions of CSF

- **Buoyancy and Protection:** Cushions the brain and spinal cord, reducing mechanical trauma.
- **Regulation of CNS Environment:** Maintains a stable chemical environment for neuronal function.
- **Waste Clearance:** Facilitates removal of metabolic waste via the glymphatic system.
- **Immune Surveillance:** Contains immune mediators to detect and respond to infections or inflammatory conditions [1].

2. Pathology

Alterations in CSF composition or flow can signal various neurological disorders:

2.1. Infectious Disorders

Meningitis is an inflammation of the leptomeninges, 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.

- **Bacterial Meningitis:** Elevated neutrophils, high protein, low glucose.
- **Viral Meningitis:** Predominantly lymphocytes, normal/mildly elevated protein, normal glucose.
- **Fungal/Tuberculous (TB) Meningitis:** Significantly elevated protein, lymphocytic predominance, markedly low glucose.
- **Chronic Infections (e.g., Neurosyphilis):** Elevated protein, lymphocytosis, specific serology/ PCR- based identification [2].

2.2. Non-Infectious Disorders

The most common non-infectious disorders which demonstrate CSF changes are listed below. The mechanisms by which these diseases alter CSF vary considerably.

- **Multiple Sclerosis (MS):** Oligoclonal bands indicating intrathecal antibody synthesis. The 2017 edition of the McDonald Criteria includes that in patients with a typical clinically isolated syndrome and clinical or MRI demonstration of dissemination in space, the presence of CSF-specific oligoclonal bands allows a diagnosis of MS. [3]

- **Guillain-Barré Syndrome (GBS):** Albuminocytologic dissociation (high protein, normal cell count).
- **Subarachnoid Haemorrhage (SAH):** Xanthochromia, consistent RBC counts across serial tubes, or bilirubin detection via spectrophotometry.
- **Hydrocephalus:** Elevated intracranial pressure from abnormal CSF accumulation.
- **CNS Malignancy:** Malignant cells on cytology [4].
- **Dementia:** CSF levels of neuronal-specific biomarkers, such as Beta-Amyloid peptides, have in recent years become important contributors to the diagnosis of Alzheimer's Disease, particularly in drug trials.
- **Autoimmune/paraneoplastic encephalitis:** Detection of specific neuronal autoantibodies (e.g. N-methyl-D-aspartate receptor (NMDA-R), Leucine-rich Glioma-Inactivated 1 (LGI1), Contactin-associated protein-like 2 (CASPR2) may support diagnosis when clinical suspicion is high [5,6].

3. Diagnostic Use

Lumbar puncture (LP) is the procedure by which a CSF sample is obtained for testing. Under local anaesthesia, a spinal needle is inserted between the spinous processes of the 3rd and 4th or 4th and 5th lumbar vertebrae [7]. LP and subsequent CSF analysis remain central to diagnosing CNS disorders. According to NICE guidelines:

- **CSF opening pressure [8],** measured with the patient lying on their side, provides key diagnostic information for conditions such as idiopathic intracranial hypertension, meningitis, and normal-pressure hydrocephalus.
- **Suspected Meningitis (NG240):** Perform LP before antibiotics if not contraindicated [9].
- **Suspected SAH (NG228):** If a Computed Tomography (CT) scan is negative or equivocal, consider LP ≥12 hours after symptom onset to detect xanthochromia [10].

Typical Investigations

- **Appearance:** Clear and colourless normally; cloudy suggests infection; yellowish discolouration (xanthochromia) indicates possible haemorrhage.
- **Cell Count & Differential:** Distinguishes infections, inflammation, or haemorrhage.
- **Glucose & Protein:** Low glucose in bacterial/fungal/TB meningitis; elevated protein in inflammation, infection, or Blood Brain Barrier (BBB) dysfunction.
- **Microbiology Tests:** Gram stain, culture, and Polymerase Chain reaction (PCR) tests for specific pathogens.
- **Cytology:** Detection of malignant cells [11].

4. Biochemical Tests (Routine)

- **Albumin Quotient (QAlb):** provides a quantitative assessment of BBB permeability:

$$Q_{Alb} = \frac{\text{CSF Albumin (mg/L)}}{\text{Serum Albumin (g/L)}}$$

- Normal range (adults): $<9 \times 10^{-3}$. Elevated values indicate barrier dysfunction (e.g., infection, inflammation, tumour, or trauma). The EFNS Task Force guideline (now under EAN) explicitly recommends that the albumin CSF/serum ratio (QAlb) be preferred to total protein, with age-related

- upper reference limits, to assess blood–CSF barrier dysfunction [8,12].
- **Protein:** Elevated in infectious, inflammatory, and neoplastic conditions.
- **Glucose: T**ypically, 50–80% of serum glucose; decreased in bacterial/fungal/TB infections.
- **Lactate:** Markedly elevated in bacterial/fungal meningitis; normal or mildly elevated in viral meningitis.

5. Biochemical Tests (Specific Clinical Circumstances)

- **Oligoclonal Bands:** Intrathecal antibody synthesis (MS, chronic inflammatory disorders).
- **Myelin Basic Protein (MBP):** Marker of active demyelination.
- **Beta Trace Protein (BTP):** Confirms CSF leakage. BTP is now the preferred marker for CSF leakage as it can be automated (nephelometry), essentially replacing Beta-2 Transferrin
- **Amyloid Beta & Tau Proteins:** Because of the development of Disease Modifying Therapies (DMTs) for Alzheimer's Disease (AD), measurement of these analytes in CSF is important in drug trials for DMTs. Considerable research is being undertaken to replace CSF assays with blood-based testing.
- **Neopterin:** Elevated in viral CNS infections and neuroinflammatory conditions [13].
- **Neurotransmitter metabolites (monoamine metabolites):** Measurement of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) may be of value in diagnosing and monitoring neurological disorders affecting the dopamine and serotonin pathways, particularly inborn errors of neurotransmitter metabolism.
- **Amino acids:** Certain inborn errors of metabolism, for example, non-ketotic hyperglycinaemia (NKH).

6. Other Laboratory Tests

6.1. Microbiology

- Gram Stain & Culture: Critical for identifying bacterial pathogens [2,11].
- PCR (e.g., Herpes simplex virus (HSV), Enterovirus, Cytomegalovirus (CMV), Epstein-Barr virus (EBV), TB).
- Reflex CSF PCR testing when CSF WBCs >5/μL or protein >1.0 g/L may improve diagnostic yield and turnaround time
- India ink for Cryptococcus.
- Acid-fast bacilli (AFB) stain for TB meningitis.

6.2. Haematology

- Cell Count/Differential: Aids in distinguishing infection and inflammation
- Red Blood Cell (RBC)/White Blood Cell (WBC) differentials (distinguishing traumatic tap vs. SAH) [14].
- Flow cytometry for lymphoma/leukaemia.

6.3. Histology/Cytology

- Detection of malignant cells in leptomeningeal disease [11].

7. Analytical Factors

- **Sample Collection:** Atraumatic LP under sterile conditions, following NICE NG240 & NG228 [9,10].
  CLSI C-49 recommends that CSF is collected into three or four containers, numbered sequentially, with the first for clinical chemistry and serology, the second for microbiology, and the third for cell counts and any other testing.

- **Handling & Transport:** Immediate processing is ideal; refrigerate if delayed. If pneumatic tube delivery of such samples is being considered, it should be appropriately validated [2].
- **Contamination Avoidance:** Aseptic technique is crucial.
- **Reference Interval Standardisation:** While reference intervals should be standardised as much as possible, this is not feasible for most fluid biochemical analytes. In practice, decision limits are more appropriate. For all analytes, the laboratory should provide the best evidence available to help the clinician in interpreting the results [15].
- **Participation in external quality assessment (EQA) schemes,** such as UK NEQAS for CSF spectrophotometry and protein/glucose assays, is recommended to ensure analytical accuracy and comparability across laboratories.

Practical Points

- Use the first collected CSF tube for biochemical analyses. If Xanthochromia testing is required, a fourth tube is recommended.
- Minimum recommended volumes:
  - Biochemistry: 0.5–1.0 mL
  - Microbiology: 1.0–2.0 mL
  - Cytology: 1.0–2.0 mL
  - Xanthochromia test: >1.0 mL.
- **Storage:**
  - Biochemistry: Refrigerate (2–8 °C) if delayed.
  - Microbiology: Keep at room temperature.
  - Cytology: Analyse fresh, unpreserved.
  - Xanthochromia: protect from light.

8. CSF Xanthochromia and Spectrophotometry (NICE NG228)

8.1 Definition and Clinical Significance

Xanthochromia refers to the yellow or pink discolouration of CSF resulting from haemoglobin breakdown products following the lysis of red blood cells (RBCs). This discolouration indicates that blood has been present in the CSF for at least two hours and is critically important in diagnosing subarachnoid haemorrhage (SAH)—especially in patients presenting with acute severe headache when a CT scan is negative or equivocal [10, 16].

8.2 Pathophysiology and Timing

After haemorrhage into the subarachnoid space, RBCs lyse and release haemoglobin, initially forming oxyhaemoglobin (pink) and later bilirubin (yellow) via haem oxygenase activity2. Detectable xanthochromia typically develops within 2 to 4 hours post-haemorrhage and is reliably evident by about 12 hours in up to 90% of SAH cases. This discolouration can persist for 2–4 weeks, thus providing a diagnostic window beyond the acute stage [10, 16].

8.3 Differential Diagnoses of Xanthochromia

While SAH is the primary suspicion in xanthochromia, other causes include:

- **Elevated CSF Protein** >1.5 g/L.
- **Severe Systemic Hyperbilirubinaemia** (serum bilirubin typically >200 μmol/L)
- **Traumatic Lumbar Puncture (LP):** Spectrophotometry is needed to distinguish genuine SAH from a traumatic tap [16].

8.4 Spectrophotometric Analysis

Because visual inspection alone is neither sufficiently sensitive nor specific, spectrophotometric analysis for bilirubin is the gold-standard approach16.

- **Normal Scan:** Minimal absorbance, no identifiable peaks at oxyhaemoglobin (~415 nm) or bilirubin (~455 nm).
- **Traumatic Tap:** May show an oxyhaemoglobin peak with little or no bilirubin peak.
- **SAH:** Shows a clear bilirubin peak (~455 nm), often with or without an accompanying oxyhaemoglobin peak.

A declining RBC count across sequential collection tubes cannot reliably exclude SAH, as RBC reduction may occur in both traumatic taps and genuine SAH. Hence, spectrophotometric detection of bilirubin remains vital [10, 16].

Automated spectrophotometry is becoming the standardised methodology for interpretation of these scans. Interpretation methods provided by two international EQA providers, UK-NEQAS and Sweden's Equalis, were compared in a recent Swedish study [17]. It found that the UK-NEQAS automated method showed significantly higher specificity with equal sensitivity, resulting in fewer false positives. A caveat is that a different study found that if using data from a sample where spectrophotometric analysis was delayed, there was a risk of falsely low values of NOA and NBA [18].

Multi-wavelength automated spectrophotometry (using 340, 415, and 460 nm) has been developed to mathematically correct for haemoglobin and protein interference, though these methods are not yet widely implemented in routine practice [19].

Important Note on Wavelengths: The wavelengths used are typically 415 nm for oxyhaemoglobin and 455 nm for bilirubin absorption peaks, but for reporting, 476 nm is preferred for bilirubin to minimise oxyhaemoglobin interference16. The ratio 415/455, being increasingly used in interpretation metrics, refers to these key absorption peaks, although the UK standard uses 476 nm for final bilirubin quantification.

The UK NEQAS guidelines remain the international reference standard, and automated spectrophotometric analysis using these algorithms is becoming increasingly standardised across laboratories.

8.5 Sample Collection and Handling.

- **Collect the fourth LP tube** for spectrophotometric analysis (≥1 mL).
- **Protect from light** immediately to prevent bilirubin degradation.
- **Avoid pneumatic chute systems** (risk of haemolysis and agitation).
- **Prompt delivery** to the laboratory is essential, as delays compromise the integrity of results.
- **Repeating LP if the CSF sample is insufficient -** there are no absolute contraindications to repeating lumbar puncture 12h post-index headache, provided there are no new clinical contraindications such as signs of raised intracranial pressure, local infection, or coagulopathy [20,21].

8.6 Ferritin Measurement for Equivocal Cases

When spectrophotometric findings are equivocal, but clinical suspicion for SAH remains high, CSF ferritin may help clarify the diagnosis. Elevated CSF ferritin can reflect RBC breakdown and local inflammatory processes, supporting a diagnosis of SAH [22,23].

9. Additional Biochemical Tests

9.1. CSF Lactate

- Normal: ≤2.2 mmol/L.

- Elevated in bacterial (>3.5 mmol/L), tuberculous, or fungal meningitis; aids differentiation from viral meningitis [4,11].
- Elevated CSF lactate relative to blood suggests mitochondrial dysfunction - Respiratory Chain (Mitochondrial) Disorders [24].

9.2. Lactate Dehydrogenase (LDH)

- Normal: <40 IU/L.
- Elevated in CNS malignancies, bacterial meningitis, SAH [2,11].

9.3. Adenosine Deaminase (ADA)

- Levels >10 U/L strongly suggest tuberculous meningitis; it helps initiate early targeted therapy [25].

10. Oligoclonal Band Testing: Procedure and Interpretation

10.1. Clinical Significance

Immunoglobulins (IgG, IgM, IgA) may be elevated due to BBB disruption or intrathecal synthesis (MS, neurosyphilis, autoimmune encephalitis). Oligoclonal bands (OCBs) are discrete bands of immunoglobulins (typically IgG) identified by high-resolution electrophoretic techniques. The absence of OCBs in serum indicates intrathecal immunoglobulin synthesis, reflecting inflammatory processes within the central nervous system (CNS). OCBs are strongly associated with conditions such as multiple sclerosis (MS), autoimmune encephalitis, Neurosyphilis, and chronic CNS infections.

10.2. Sample Collection

To reliably detect intrathecal synthesis, it is essential to collect paired samples of cerebrospinal fluid (CSF) and serum simultaneously:

- **CSF Collection:**
  Obtain 1–2 mL of CSF via lumbar puncture, using sterile technique, ideally from the third or fourth tube to reduce blood contamination.
- **Serum Collection:**
  Concurrently collect a blood sample (approximately 2–4 mL) via venipuncture into a plain or serum separator tube.

10.3. Analytical Technique: Isoelectric Focusing (IEF)

The gold-standard method for detecting OCBs is isoelectric focusing electrophoresis, followed by immunoblotting:

- Samples are electrophoresed on agarose or polyacrylamide gels with a pH gradient applied.
- Immunoglobulins are separated by isoelectric focusing, resulting in discrete band patterns.
- Following electrophoresis, immunoglobulin bands are visualised using immunostaining with specific anti-human IgG antibodies.

10.4. Interpretation of Results

Band patterns from CSF and serum are compared side-by-side:

- **Intrathecal Synthesis:** Defined by two or more discrete oligoclonal IgG bands in CSF that are absent in the paired serum sample. This pattern strongly supports a diagnosis of MS or another inflammatory CNS disease.
- **Systemic Immunoglobulin Passage (BBB Dysfunction):** Bands present in both CSF and serum (mirror pattern) indicate passive leakage of systemic immunoglobulins into the CNS due to blood-brain barrier disruption, rather than local synthesis.

10.5. Additional Calculations: CSF IgG Index

To further clarify intrathecal synthesis, the CSF IgG index is calculated:

- **CSF IgG Index:** $= \dfrac{\text{CSF IgG/Serum IgG}}{\text{CSF Albumin/Serum Albumin}}$
- **Normal CSF IgG Index:** ≤0.66
- **Elevated IgG Index (>0.66):** Suggests intrathecal immunoglobulin synthesis, common in MS (~90% of patients), autoimmune encephalitis, and chronic CNS infections [2,6,7].
- Combining the QAlb indicator of BBB dysfunction (see #4, above) with the CSF IgG Index improves the diagnostic power of the IgG Index. When an elevated CSF IgG Index is found, the QAlb result further helps differentiate between true intrathecal synthesis (e.g., multiple sclerosis) and passive transfer from serum via a leaky BBB.

10.6. Limitations and Practical Considerations

- Accurate interpretation relies on simultaneous serum and CSF collection.
- Blood contamination during lumbar puncture may affect interpretation; careful technique reduces risk.
- Mild elevations in IgG index without oligoclonal bands may require repeat analysis to confirm clinical significance.

10.7. CSF Kappa Free Light Chains (KFLC)

Although detection of CSF-restricted OCBs is the gold standard for detection of intrathecal IgG synthesis, a newer, simpler testing system is emerging. Measurement of CSF KFLC and KFLC Index has been shown to have similar diagnostic accuracy as OCB. KFLC measurement as a diagnostic tool is one of the revisions proposed for the updated McDonald Criteria [26,27].

- **CSF KFLC Index:** $= \dfrac{\text{CSF KFLC/Serum KFLC}}{\text{CSF Albumin/Serum Albumin}}$

[KFLC in mg/L; Albumin in mg/L (CSF) and g/L (serum)]

11. Clinical Utility, Measurement, and Interpretation of CSF Protein and Glucose Levels

11.1 CSF Protein

11.1.1 Clinical Significance

CSF protein concentration reflects blood–brain barrier (BBB) integrity and CNS pathology. In normal circumstances, tight junctions and transport mechanisms restrict the entry of proteins into the CSF; barrier disruption or increased intrathecal protein synthesis raise CSF protein levels.

11.1.2 Measurement & Reference Ranges

- **Assays:** Turbidimetric or colorimetric.
- **Adult Normal Range:** 0.15–0.45 g/L.
- **Neonates/Infants:** Higher (0.2–1.7 g/L).
- **Traumatic Tap Correction**: ~0.01 g/L per 1000 RBCs/μL.
    - Measure protein and cell count on the same sample to correct for blood contamination.

11.1.3 Interpretation of Elevated CSF Protein

- **BBB Dysfunction / GBS:** Markedly elevated protein (>1 g/L) with normal or near-normal cell count (albuminocytologic dissociation).

- **Infections:** Bacterial meningitis often >2.5 g/L, fungal/TB >1 g/L, viral ~<1.5 g/L.
- **Inflammatory (MS, Autoimmune):** Moderate protein rise with oligoclonal bands.
- **CNS Tumours:** Elevation due to leptomeningeal infiltration or BBB compromise.
- **SAH:** Elevated protein from RBC breakdown, often with xanthochromia.

11.1.4 Limitations & Cautions

- **Post-infectious Elevation:** CSF protein may remain high after recovery.
- **Traumatic LP:** RBC count is crucial to rule out overestimation.

11.2 CSF Glucose

11.2.1 Clinical Significance

CSF glucose measurement is essential for diagnosing CNS infections/inflammation. Glucose enters CSF via facilitated diffusion and is consumed by CNS cells/pathogens. Normal CSF glucose is ~60% of serum glucose. The term hypoglycorrhachia is used to describe a low CSF glucose concentration, either absolute or as a ratio to blood glucose.

11.2.2 Measurement & Reference Intervals

- **Assays:** Enzymatic colorimetric.
- **Normal Interval:** ~2.2–4.4 mmol/L (~60% of serum glucose).
- **Paired Serum Glucose:** Required for accurate ratio interpretations.

11.2.3 Interpretation of Hypoglycorrhachia

- **Bacterial Meningitis:** <2.5 mmol/L (often <1.0 mmol/L) or CSF-to-serum ratio <0.4.
- **Tuberculous/Fungal Meningitis:** Markedly low (<2.0 mmol/L), with elevated protein and lymphocytosis.
- **Viral Meningitis:** Usually normal/mildly reduced (>50% of serum).
- **Non-infectious (e.g., malignancy, neurosarcoidosis, SAH):** May also reduce CSF glucose.

11.2.4 Hyperglycaemia Considerations

Even in severe hyperglycaemia, CSF glucose rarely exceeds 16 mmol/L. Thus, the CSF-to-serum ratio is paramount.

11.2.5 Recovery & Monitoring

CSF glucose can normalise relatively quickly with effective therapy, serving as an early — though not exclusive — indicator of treatment response.

11.3 Practical Considerations for CSF Protein and Glucose Testing

- **Volume/Tube:** 0.5–1.0 mL of CSF in fluoride EDTA or plain tube (lab-dependent).
- **Paired Serum Glucose:** Essential to interpret CSF glucose ratio.
- **Storage/Transport:** Refrigerate (2–8 °C) if >30 min delay; avoid >2-hour delays.
- **Traumatic Tap:** RBC count needed to correct protein results.

12. Summary of Reference Intervals & Clinical Implications

Test	Normal Range	Elevated / Altered Levels Suggest
CSF Protein	0.15–0.45 g/L	Infections (bacterial, TB), GBS, SAH, inflammatory/autoimmune, etc.
CSF Glucose	~2.2–4.4 mmol/L	Low (<2.5 mmol/L) → Bacterial/TB/fungal meningitis; ratio essential
CSF IgG Index	≤0.66	MS, autoimmune CNS disorders, chronic infections
CSF Lactate	≤2.2 mmol/L	Elevated in bacterial, TB, fungal meningitis; mitochondrial disease
CSF LDH	<40 IU/L	High in CNS malignancy, acute meningitis, SAH
CSF Oligoclonal Bands	Negative	Positive → MS, chronic infections, autoimmune disorders
Myelin Basic Protein	<4 ng/mL	Active demyelination (e.g., MS)
CSF ADA	<10 U/L	Elevated → Tuberculous meningitis

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Cerebrospinal Fluid Laboratory Interpretation Summary

This summary provides a concise reference for the routine and specialised laboratory assessment of cerebrospinal fluid (CSF). It integrates analytical parameters, reference intervals, clinical interpretations, and practical considerations for routine diagnostic and reflex testing.

Parameter / Test	Reference Interval / Expected Findings	Clinical Interpretation	Key Notes / Reflex Testing
Appearance	Clear, colourless	Cloudy = infection; Xanthochromia = SAH; Turbid = raised WBCs or protein	Visual check before analysis; protect from light if SAH suspected
Opening Pressure	10–20 cmH_2O (adults)	↑ = Idiopathic intracranial hypertension, meningitis; ↓ = CSF leak or hypotension	Record in lateral decubitus position; manometer measurement essential
Protein	0.15–0.45 g/L (adults)	↑ = Infection, inflammation, BBB damage, GBS; ↓ = CSF leak	Correct for blood contamination (0.01 g/L per 1000 RBC/µL)
Glucose	2.2–4.4 mmol/L or ~60% serum glucose	↓ = Bacterial/TB/fungal meningitis; normal in viral	Always compare with simultaneous serum glucose
Lactate	≤2.2 mmol/L	↑ = Bacterial/fungal/ TB meningitis, mitochondrial disorders	Useful in differentiating bacterial vs viral meningitis
Cell Count (WBC/ RBC)	WBC <5/µL (lymphocytes); RBC = none	↑ WBC = infection/ inflammation; ↑ RBC = SAH or traumatic tap	Perform promptly; delay >1h causes cell lysis
Xanthochromia (Spectrophotometry)	No bilirubin / oxyhaemoglobin peaks	Bilirubin peak (455 nm) → SAH	Perform ≥12 h post onset; protect sample from light; ferritin if equivocal
Albumin Quotient (QAlb)	$<9\times10^{-3}$	↑ = BBB dysfunction (infection, tumour, trauma)	Interpret with IgG Index and OCBs
IgG Index	≤0.66	↑ = Intrathecal IgG synthesis (MS, chronic inflammation)	Paired serum required; use with OCBs
Oligoclonal Bands (OCB)	Absent or identical in serum & CSF	CSF-only bands → intrathecal synthesis	Perform via isoelectric focusing; paired serum mandatory
Kappa Free Light Chain Index	≤5	↑ = Intrathecal immunoglobulin synthesis	Useful adjunct to OCB; elevated in early MS

Parameter / Test	Reference Interval / Expected Findings	Clinical Interpretation	Key Notes / Reflex Testing
Adenosine Deaminase (ADA)	<10 U/L	↑ Suggests tuberculous meningitis	Add when lymphocytic pleocytosis and low glucose present
Neopterin	Low/undetectable	↑ = Viral infections, neuroinflammation	Supports diagnosis of viral meningitis / encephalitis
Ferritin (CSF)	Low/undetectable	↑ RBC breakdown → supports SAH	Add if spectrophotometry equivocal
Beta Trace Protein / β_2-Transferrin	Not detected in serum	Detected → confirms CSF leak	Collect fluid for investigation in sterile container

Reporting and Quality Assurance

Reports should include appearance, cell counts, protein, glucose, and any additional analyses performed. Critical results (e.g., CSF glucose <1.0 mmol/L or neutrophils >500/µL) must be communicated immediately. Participation in EQA (e.g. UK NEQAS) and adherence to ISO 15189 quality systems are recommended for all laboratories performing CSF analysis.

Reflex testing may include PCR panels when WBC >5/µL or protein >1.0 g/L, ADA for suspected TB meningitis, and ferritin for equivocal xanthochromia. All results should be interpreted in conjunction with clinical context and imaging findings.

Pleural Fluid

Authors: Peadar McGing, Micheál Ryan.

1. 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.

2. 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.

In cases of pleural effusion, fluid may be removed for therapeutic or diagnostic purposes. This process, termed thoracentesis, involves inserting a suitable needle, under ultrasound guidance, through the posterior intercostal space into the pleural cavity and draining off an appropriate amount of fluid into a syringe.

3. Diagnostic Use

The primary use of biochemical analysis of pleural fluid is to differentiate between transudates and exudates, this differentiation being an important pointer in determining the cause of the effusion. Transudates need only the underlying cause to be treated, whereas exudates require further investigation to identify the local source of inflammation or infection.

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

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

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

4. Biochemical Tests Performed Routinely on Pleural Fluids

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 LDH, glucose, protein and albumin, should be measured for calculation of fluid to plasma / serum ratios.

4.1. Total Protein / Lactate Dehydrogenase (LDH) / Albumin

Fluid Total Protein measurement alone is rarely used to differentiate exudate from transudate effusion. Instead, Light's Criteria or the 'Abbreviated Light's Criteria' are used, backed up on occasions by serum to effusion albumin gradient (SEAG).

4.1.1. Light's Criteria and related tests.

Light's criteria, originally published in 1972, and re-issued in 2002 [1], 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.

A 2023 Best Practice review [2] recommended use of the 'Abbreviated Light's Criteria' originally proposed by Heffner et al [3]. This uses the two ratios only, and does not include an absolute cut-off for fluid 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 fulfil 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.

For heart patients treated with diuretics, the use of the serum to effusion albumin gradient (SEAG) may be useful in evaluating pleural effusions. In this patient cohort. SEAG > 12g/L is consistent with a transudative effusion.

Measurement of NT-proBNP in blood is a more specific indicator of heart failure and is preferred to fluid measurement of natriuretic peptides [4].

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 1.6 mmol/L down to 1.2 mmol/L have been suggested as giving slightly improved diagnostic accuracy; e.g. in a 2022 paper, pleural fluid with a cholesterol level of >1.2 mmol/L (>45 mg/dL) was classified as exudative [5]. One slight concern is the method reliability at such low levels.

4.2. pH and Glucose

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).

The guidelines issued by the British Thoracic Society (BTS) recommend that 'for patients with parapneumonic effusion (PPE) or suspected pleural infection, where diagnostic aspiration does not yield frank pus, immediate pH analysis should be performed', this test having high sensitivity and specificity [4].

If the pleural fluid pH is ≤7.2, this indicates a high risk of complex parapneumonic effusion (CPPE) or pleural infection, in which case an intercostal drain (ICD) should be inserted.

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.

Where pleural fluid pH measurement is not available within an appropriate time frame, pleural fluid glucose <3.3 mmol/L also indicates a high probability of CPPE/pleural infection.

Pleural fluid glucose levels are usually similar to the patient's plasma level. A low fluid glucose is usually defined as ≤3.4 mmol/L (or ≤3.33 mmol/L / 60 mg/dL). This is associated with a select group of causes of exudative effusion, particularly

- Complicated parapneumonic effusion or empyema
- Malignancy

- Rheumatoid pleurisy
- Tuberculosis (TB pleurisy)
- Oesophageal rupture

Very low glucose (usually defined as ≤1.6 mmol/L (or ≤1.67 mmol/L / 30 mg/dL) is often associated with rheumatoid arthritis or empyema [6,7].

5. Biochemical Tests Performed in Specific Clinical Circumstances

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

5.1. Query Chylothorax / Pseudochylothorax

Measurement of triglycerides and cholesterol can help to confirm chyle in the chest cavity. In patients with milky looking fluids these tests help differentiate chylothorax from pseudochylothorax. In general, a triglyceride level greater than the cholesterol level supports chylothorax. Fluid triglyceride level of >1.24 mmol/L (110 mg/dL) with fluid cholesterol <5.2 mmol/L (200 mg/dL) is diagnostic of chylothorax. A raised fluid cholesterol (>5.2 mmol/L) combined with low fluid triglycerides (<0.56 mmol/L [50 mg/dL]) is diagnostic of pseudochylothorax.

One can also check for chylomicrons by ultra-centrifuging the sample or by standing in a refrigerator overnight. This may be useful in equivocal cases.

5.2. Query TB

The main biochemical test in diagnosing tuberculous pleural effusion is fluid adenosine deaminase (ADA). The value of the test is predicated on the local prevalence. In high prevalence areas, an ADA level >40 to 60 units/L strongly supports a diagnosis of tuberculous pleural effusion. Interferon gamma (IFN-gamma) testing can also be considered for diagnosing tuberculous pleural effusion.

In low prevalence populations, pleural fluid ADA is mainly used as a rule-out test for tuberculous pleural effusion. Values <40 U/L make TB unlikely to be the cause of the patient's effusion.

PCR methods (for Mycobacterium tuberculosis) can be particularly helpful, when available, though there are issues with sensitivity.

ADA testing is often not routinely available, and laboratories should evaluate with clinicians whether likely turnaround time warrants referral to a provider laboratory.

5.3. Query Malignancy

The value of various tumour markers (e.g. CEA or CA125) 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 manufacturer's guarantees.

5.4 Query Pancreatitis

Routine measurement of amylase in pleural fluid is not recommended, and if performed, it should be interpreted in conjunction with the serum amylase. Amylase-rich pleural effusions are commonly associated with pancreatitis, oesophageal rupture, malignancy, pneumonia, and liver cirrhosis.

Iso-enzyme analysis is rarely performed, but if available salivary amylase points to oesophageal rupture or malignancy, while pancreatitis is associated with the pancreatic isoenzyme.

6. 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.

Gross appearance of the fluid at time of sampling can give important clues as to the aetiology of the effusion, can warn of potential pre-analytical issues, and can help point to particular tests [2,7]. Such features include:

Colour of fluid – e.g. brown indicates likely parapneumonic effusion; red (bloody) can point to a variety of conditions including malignancy or pulmonary infarction; milky colour points to chylothorax or pseudochylothorax, but can be due to infection or presence of cellular debris.

Other macroscopic features of fluid – e.g. purulent pints to infectious cause or empyema; viscous sample indicates mesothelioma; presence of debris points to mesothelioma; smell of ammonia implies urinothorax.

7. Analytical Factors

7.1. Correct Samples.

In routine practice one of the biggest problems with fluid analysis is failure to provide the lab with appropriate specimens. Avoiding pre-analytical errors is extremely important given that the sample is usually a one-off that cannot be repeated [8].

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. Typical sample bottles include

- Proteins, LDH: MSU bottle, Li Hep plasma or plain bottle (as for plasma / serum analysis).
- pH: Air-free sample, preferably taken into 'blood-gas' syringe. Measurement of pH is best done on a 'blood gas' analyser using a clot-catcher.
- Glucose: Fluoride Oxalate bottle.

7.2. Safety:

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

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Pericardial Fluid

Authors: Mark Kilbane, Peadar McGing.

1. Physiology

The pericardial space surrounding the heart normally contains 10-50 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.

2. 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.

Pericardial fluid may be removed for therapeutic or diagnostic purposes by pericardiocentesis, preferably performed under radiological guidance.

3. 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 and increased systemic venous pressure, e.g. in congestive heart failure or pulmonary hypertension. Exudative effusions reflect increased fluid formation through 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.

4. 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 laboratory departments other than Clinical Biochemistry are usually more important in the differential diagnosis of the cause of pericardial effusions (see Other Laboratory Tests – below).

4.1. Transudate v Exudate

Most pericardial effusions are exudates, and biochemical differentiation is only rarely needed. Where such differentiation is needed biochemistry tests were in the past often interpreted using criteria borrowed from pleural effusions. The validity of this approach is however increasingly questioned (see below). 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.

4.2. Routine Tests

The two most common biochemical tests are Lactate Dehydrogenase (LDH) and Total Protein. Light's criteria have in the past been used to distinguish between exudative and transudative effusions, as for pleural

effusions, but are no longer recommended.

The 2015 European Society of Cardiology guidelines state that fluids containing high values of protein (>30 g/L; fluid/serum ratio >0.5) and LDH (>200 IU/L; fluid/serum ratio >0.6) are commonly interpreted as exudate but add that this has not been validated for pericardial fluid. More recent reviews have concluded that testing of protein and LDH is unlikely to add diagnostic value.

5. Other Laboratory Tests

Cytological examination plus cultures, cell count, and PCR of fluid, are the primary laboratory tests used in initial investigations of pericardial effusions of unclear aetiology. Malignancy and infection are the leading causes of pericardial effusion in the developed world, and tuberculosis (TB) the main cause in less developed areas.

6. 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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Peritoneal / Ascitic Fluid

Authors: Martin Healy, Peadar McGing.

1. Physiology

The peritoneum comprises a double layer of serous membrane [1]. The parietal peritoneum lines the walls of the abdomen; the visceral peritoneumcovers the surface of the abdominal organs. Normally the space between the layers contains a thin film of peritoneal fluid which reduces friction as a result of organ movement. An abnormally increased volume of serous fluid in the peritoneal cavity is known as ascites. It can be either transudative or exudative.

The normal peritoneal fluid volume rarely exceeds 10 mL of transudative fluid (an ultrafiltrate of plasma that seeps across capillary walls and contains less than 30g protein per litre fluid) in men and post-menopausal women. In pre-menopausal women, normal fluid volumes can be up to 5-18 mL, depending on the phase of the menstrual cycle [1,2].

2. Pathology

Pathological accumulation of fluid in the abdominal cavity is usually referred to as ascites (from the Greek askos, meaning 'bag'). Ascitic fluid (peritoneal fluid), is a common clinical finding with a wide range of causes. In Western populations the most common causes of ascites are:

- cirrhosis in about 60 to 80% of cases
- malignancy in about 10 to 25%
- cardiac failure in about 5%
- various other causes account for the remaining 10%.

The lower figures for cirrhosis and malignancy are derived from the 2020 British Society of Gastroenterology guidelines [3]. Figures vary between studies, and between different populations [1,4,5].

Transudative ascitic fluid, watery and low in protein, is produced by visceral capillaries and drained via the diaphragmatic lymphatic system. Transudative ascites occurs when fluid formation rates are greater than fluid absorption due to increased hydrostatic pressure and decreased oncotic pressure

Exudative fluid is rich in protein and cellular debris. It leaks out of blood vessels and is deposited in tissues or tissue surfaces. Causes can include inflammatory processes triggered by cancers, tuberculosis, pancreatitis and bacterial infections.

The list of conditions that may be associated with ascites is long, and includes:
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.

In cirrhosis, the most common cause of ascites in the Western world, splanchnic vasodilation causes a reduction in arterial volume triggering activation of the renin-angiotensin system resulting in increased sodium and water reabsorption in the kidneys. The resulting increase in body fluid causes leakage of fluid into the peritoneal space.

3. Diagnostic Use

The process of obtaining peritoneal fluid is termed paracentesis. Percutaneous aspiration, via needle and usually under ultrasound guidance, is used to remove fluid for diagnostic or therapeutic purposes

Although ascites is not intrinsically life threatening, cirrhotic patients with ascites have a two-year mortality rate of 50%. Diagnostic paracentesis 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) [6,7]. Transfer to the appropriate preservative should take place as soon as possible.

4. Biochemical Tests

Although several laboratory tests are helpful in distinguishing transudates from exudates in pleural fluids, and the pathologies behind them, the criteria for differentiating the primary diseases associated with ascites is not clear-cut. The clinical separation between transudate and exudate is not as clear as in pleural fluid. Biochemistry tests which have been used traditionally 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), LDH, and tumour markers . It should be noted, however, that the specificities of these biochemical tests can vary significantly.

4.1. Serum to Ascites Albumin Gradient (SAAG).

Serum-ascites albumin gradient (SAAG) was first proposed by Hoefs et al. in 1981 [8], but it was a multicenter, prospective study by Runyon et al. in 1991 that pushed this test to the top of the list [9]. That demonstrated that SAAG measurement in portal and nonportal hypertension was superior to the exudate versus transudate concept in the differential diagnosis of ascites.

Measurement of the serum-ascites albumin gradient (SAAG) has become the main front-line biochemical

test, particularly indetermining the cause of new-onset ascites [3-5,10].

The SAAG is calculated by subtracting the ascitic fluid albumin concentration from the serum albumin concentration in simultaneously obtained specimens. Although termed 'serum' gradient, plasma can equally be used where this is the laboratory's usual sample type; The SAAG test name is retained even when plasma to ascites gradient is what is actually measured.

$$SAAG = [Serum\ Albumin\ (g/L)] - [Ascitic\ Fluid\ Albumin\ (g/L)]$$

The diagnostic cut-off of 11 g/L (using S.I. units) proposed by Runyon [9] has been adopted for interpretation of this test, and is discussed in more detail below. In countries which do not use SI units, albumin is measured in g/dL and the cut off is 1.1 g/dL.

The laboratory information system (LIS) should be set up to include calculated SAAG as a specific requestable test, and the LIS should be able to automatically retrieve fluid and serum / plasma albumin results and perform the calculation. Where fluid albumin levels below the reporting limit of the albumin assay are obtained, it is valuable to report an SAAG as 'less than' (<[Ser Alb] – [lower limit of fluid Alb assay]); this is usually sufficient for diagnostic purposes.

- **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 [9,10].

The ascitic fluid total protein concentration is used to differentiate the various causes of ascites in patients with a high SAAG [3-5,10]. For example, patients with cirrhosis, alcoholic hepatitis, cardiac failure, or fulminant hepatic failure have a low total protein concentration (<15 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 (>25 g/L).

- **SAAG<11g/L**

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

Significant hypoalbuminaemia (due to malnutrition, for example), hypergammaglobinaemia, hypotension or chylous ascites can result in falsely low SAAG values. Falsely high values can be seen in patients with hepatic malignant metastases linked to portal hypertension.

4.2. Glucose:

Ascitic fluid glucose may have a role in countries with high tuberculosis (TB) prevalence, but in Western populations the US guidelines only recommend testing when there is suspicion of secondary bacterial peritonitis [10].

4.3. LDH:

Measurement of LDH is non-contributory and is not recommended [7].

4.4. Adenosine Deaminase (ADA).

Fluid ADA may be helpful in diagnosis of TB in areas of high prevalence. However, it is not routinely available in most laboratories and in many Western countries newer molecular tests, with quicker turnaround times, are superceding traditional tests.

4.5. Lipids.

When further investigating fluid with a milky appearance, measurement of cholesterol and triglycerides in peritoneal / ascitic fluid helps to differentiate between chylous and pseudochylous effusions [11]. A milky-

coloured fluid is likely to be chylous if fluid triglycerides are greater than 2.2 mmol/L (200 mg/dL) and / or greater than the serum level. The effusion is more likely to be pseudochylous if the triglyceride is low.

Cholesterol's value is dismissed by some, e.g. the US (AASLD) guidelines [10], whereas others strongly advocate its usefulness, e.g. Du et al [5]. An ascitic fluid cholesterol of greater than 1.2 mmol/L (45 mg/dL) supports pseudochylous ascites.

5. Other Laboratory Tests

Initial analysis of ascitic fluid should include macroscopic and microscopic examination, gram stain, culture and cytology (important for diagnosing malignancy) [3,4,10]. A cell count with WBC differential should always be performed and is a key test in algoritms for the evaluation of ascites of unknown origin.

- An increase in neutrophils (>250 /μL) is associated with peritonitis (bacterial, tuberculous, pancreatic or malignant). It is the gold standard for diagnosis of spontaneous bacterial peritonitis (SBP)3.
- A WCC greater than 1000 /μL is also associated with bacterial or tuberculous peritonitis. A lower threshhold of 500 /μL is advocated by some due to a greater risk of underdiagnosing BP with the higher threshhold [3].
- A red cell count greater than 10,000 /μL denotes haemorrhagic ascites, usually due to malignancy, tuberculous or trauma [12]. The white blood cell and neutrophil counts need to be corrected in patients with bloody samples. In patients with bloody ascitic fluid samples a "correction" is usually applied to the white blood cell and neutrophil counts4.

Cytology is particularly important if there is any suspicion of malignancy, either from clinical findings or from preliminary asctic fluid testing (particularly SAAG plus cell count and differential.

6. Analytical Factors

- When collecting ascitic fluid by diagnostic paracentesis collect as much sample as possible into a sterile container. For therapeutic paracentesis ensure arrangements are in place that adequate volume sample is reserved for the laboratory; it is unsatisfactory when only a small aliqout is put aside for laboratory testing and the remainder of the large volume collection is disposed of.
- Microbiological tests should be performed first before distributing to other laboratories.
- For analysing the SAAG, both serum (or plasma) and fluid albumin should be sampled at about the same time. The laboratory information system (LIS) should be set up to include calculated SAAG as a specific requestable test, and the LIS should be able to automatically retrieve fluid and serum / plasma albumin results and perform the calculation. Where fluid albumin llevels below the reporting limit of the albumin assay are obtained, it is valuable to report an SAAG as 'less than' (<[Ser Alb] – [lower limit of fluid Alb assay]); this is usually sufficient for diagnostic purposes.
- Fluids should also be analysed for total protein; other biochemical tests are only analysed as appropriate to the clinical questions.
- Blood and fluid samples should be taken concurrently.

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Sweat

Authors: Karen Heverin, Jennifer Brady.

1. 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.

2. 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 2,000 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.

3. Diagnostic Use

The alteration in the composition of sweat provides the basis for a diagnostic test for cystic fibrosis [1-4]. In newborn screening programmes for cystic fibrosis, screen positive infants are referred for diagnostic sweat testing following initial screening with a combination of IRT (immunoreactive trypsinogen) and genetic analysis of the CFTR gene (programme dependent).

Pilocarpine (a cholinergic agent) is introduced into a small area of the skin by iontophoresis, producing localised stimulation of sweat glands. 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.

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 testing must be performed on a sufficient number of patients by well trained personnel [4].

Performance of sweat testing should be reviewed regularly to include criteria such as insufficient collections,

analytical failure rate and EQA performance. Failed sweat collections due to insufficient weight or volume should be monitored. An annual target of < 5% for patients older than 3 months of age, and of < 10% in patients younger than 3 months of age, is recommended3. With bilateral collection, the test is considered insufficient only when an adequate sweat specimen is not obtained from either site.
Detailed guidelines for performing and ensuring the quality of the sweat test have been published [1-4].

4. Biochemical Tests

Sweat chloride concentration is the analyte of choice. Sweat sodium, potassium or osmolality measurements are not recommended. In both children and adults, the diagnostic thresholds listed below are used. These have been recommended by the Cystic Fibrosis Foundation Consensus Guidelines (2017) and endorsed by the European Cystic Fibrosis Society and The Laboratory Services Reform Programme by the HSE [4-6].

- Sweat chloride < 30 mmol/L makes a diagnosis of CF unlikely.
- Sweat chloride in the range of 30-59 mmol/L is an intermediate result, which requires further assessment.
- Sweat chloride ≥60 mmol/L supports a diagnosis of CF.

Suitable assay methods for sweat chloride are colorimetry, coulometry and ISE; where possible duplicate analysis is recommended.
Sweat conductivity measurement (Wescor Sweat-Chek apparatus) is not recommended as a diagnostic test. In particular, conductivity should not be used as a follow-up test for infants with a positive newborn screening result [3].
The sweat test should be repeated if the result is not in keeping with clinical phenotype or genotype. Non-physiological or discrepant results should be questioned, not reported and the test should be repeated e.g. Cl > 150 mmol/L, or conductivity > 170 mmol/L.
Assay precision: The target CV at < 30 mmol/L should be < 7%, and < 5% at other medical decision concentrations according to the CLSI recommendations [3]; the older UKNEQAS guideline stated methods for sweat chloride should be capable of producing a between batch CV of <5% for chloride (at a concentration of 40-50 mmol/L).
IQC at two concentrations (normal and intermediate/abnormal) should be performed and the laboratory must participate in a suitable EQA scheme.

5. 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 2kg at the time of testing, who are normally hydrated and without systemic illness. Exceptionally, term infants can be tested after 7 days, but may yield insufficient sweat, and sweat chloride can be high in the first 7 days, and especially the first 2 days. It is difficult to get enough sweat in very young infants, especially those under 2 kg.
Sweat electrolytes can be elevated in underweight infants, if the collection site has active eczema or in patients on topiramate treatment. It may be altered in dehydrated infants, those 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, i.v. fluids or flucloxacillin [1,4].
The flexor surface of either forearm is the preferred site of sweat collection. Other sites (e.g. upper arm, thigh) can be used if both arms are unsuitable (e.g. too small or eczematous).
An aqueous solution or Wescor gel discs containing 2-5g/L pilocarpine nitrate should be iontophoresed at a maximum of 4mA for 5 minutes. Sweat should be collected from the stimulated area between 20 to 30 minutes. A sweat secretion rate of not less than 1g/m2/min is required over the collection period. Collections less than this are unsuitable and should not be analysed.
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 greater than 1 minute and less than 3 hours, ensuring homogeneity and thorough mixing before analysis. Sweat collected using the Wescor system should be carefully expelled and mixed thoroughly before analysis.
If storage is necessary before analysis, sweat collections on paper pads should be stored at 4°C for a maximum of 72hrs in an appropriately sized, air-tight container. Liquid collections can be stored in sealed Macroduct tubing or capped PCR tubes at 4°C for a maximum of 72hrs. Sweat collections may take place at remote sites and be transported to the laboratory for analysis, providing strict attention is paid to storage requirements [1].

6. Key take home points:

- 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.
- 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.
- Sweat chloride concentration is the analyte of choice.
- Sweat chloride < 30 mmol/L makes a diagnosis of CF unlikely.
- Sweat chloride in the range of 30-59 mmol/L is an intermediate result, which requires further assessment.
- Sweat chloride ≥60 mmol/L supports a diagnosis of CF.
- Suitable assay methods for sweat chloride are colorimetry, coulometry and ISE and where possible duplicate analysis is recommended.
- A number of factors or conditions may result in false positive or negative sweat test results; careful consideration must be made when deciding to proceed with sweat testing in these circumstances.
- A sweat secretion rate of not less than 1g/m2/min is required over the collection period.
- If storage is necessary before analysis, sweat collections should be stored at 4°C for a maximum of 72hrs in an appropriately sized, air-tight container.

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Amniotic Fluid

Author: Carl Talbot.

1. 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 800 - 1000 mL at approximately 34 weeks of gestation. This decreases to 600 -800 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.

2. 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.

Prelabour Rupture Of Membranes (PROM) occurs when the amniotic sac breaks before the initiation of labour. If this occurs prior to 37 weeks of gestation it is referred to as Preterm Prelabour Rupture Of Membranes (PPROM). PPROM can results in significant risks such as sepsis, prematurity, chorioamnionitis and placental abruption. The Rupture of membranes and subsequent release of amniotic fluids is the basis for some the biochemical tests mentioned below.

Preterm labour is when labour initiates prior to 37 weeks of gestation. Spontaneous preterm labour where the membranes remain intact has substantially higher rates of neonatal morbidity and mortality.

3. Historical Diagnostic Uses

The use of AF bilirubin levels for the identification of Rh disease in pregnancy was an indirect measure of assessing anaemia. Amniotic bilirubin levels are measured spectrophotometrically with elevated levels indicating haemolysis. The use of AF bilirubin for identification of Rh disease in pregnancy has been superseded by Doppler ultrasound techniques.

Likewise, the Lecithin:Sphinglomyelin (L/S) ratio used for evaluating the foetal lung maturity in respiratory distress of the newborn. L/S ratio analysis relied on Thin-Layer Chromatography (TLC) with ratios between 2.0 and 2.5 indicating normal lung development and ratios below 2.0 indicating foetal lung immaturity.

4. Biochemical Tests

4.1 Foetal Fibronectin (FFN). FFN is a protein found between the foetal sac and uterine lining. Detection of foetal fibronectin in cervicovaginal secretions between 24 and 34 weeks gestation is associated with preterm delivery in symptomatic and asymptomatic women. The manufacturer of FFN assay to the Irish and United Kingdom market has discontinued the FFN testing kits as of July 2024.
4.2. Phosphorylated IGFBB3. Phosphorylated Insulin-like Growth Factor Binding Protein 3 (phIGFBP3) is made by the decidualized endometrial stromal cells of the uterus. Detection of phIGFBP3 in cervical secretions after week 22 of pregnancy allows for identifying women at high risk of preterm delivery. Phosphorylated IGFBP3 is typically provided as an Immunochromatographical lateral flow near-patient device.
4.3. Alpha Macroglobulin. Placental alpha macroglobulin-1 (PAMG1) is a protein found in very high concentrations in amniotic fluid and in low concentrations in both blood and cervicovaginal fluids. Detection of PAMG-1 by immunochromatographical techniques has been shown to have clinical utility in the investigation of Preterm Pre-labour Rupture Of Membranes (P-PROM).

5. Other Laboratory Tests

Biochemical tests for foetal lung are now obsolete. All premature babies at risk from developing RDS (Respiratory Distress Syndrome) 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 an invasive technique used for the collection of amniotic fluid transabdominally and guided by ultrasound. Pregnant women may be offered amniocentesis for a multitude of reasons including identification of foetal structural abnormalities on ultrasound, for patients with known high risk of inherited genetic disease or based on the findings of Non-Invasive Prenatal Testing (NIPT). It is recommended that amniocentesis be performed after 15 weeks gestation. Use of karyotyping and genetic testing can allow for prenatal testing of genetic abnormalities.

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Saliva

Authors: Dr. Mark Kilbane, Dr. Peadar McGing.

1. Physiology

Saliva is a complex biological fluid secreted by major and minor salivary glands, mixed with gingival crevicular fluid, epithelial cells, food debris and oral microbiota. Saliva production is normally about 500 to 1500 mL per day in healthy adults. It contains water, electrolytes, proteins (most notably enzymes alpha-amylase and lysozyme, and immunoglobulins), nucleic acids, hormones, metabolites, and microbial components. These compounds are primarily serum constituents, but can manifest in saliva: intracellular routes include passive diffusion, while extracellular mechanisms include ultra-filtration at tight junctions between cells. Saliva reflects both local (oral cavity) and systemic physiology. Saliva composition varies with flow rate, circadian rhythm, diet, medications, and collection methods.

2. Pathology

Saliva composition can be affected directly by systemic diseases that may reflect changes in serum concentrations of certain analytes or salivary biomarker profiles which can be useful for early detection, for disease monitoring or prognosis.

- Saliva is particularly useful for infectious disease testing (SARS-CoV-2).
- Cancer: saliva analysis can be used as a substrate to detect changes in expression of tumour antigens, miRNAs (e.g. overexpression of miR-31, reduction of miR-125a, miR-200a, particularly in in oral squamous cell carcinoma), or the presence of somatic mutations or HPV in oral cancers.
- Cardiovascular disease: biomarkers like CRP, myoglobin, myeloperoxidase detectable in saliva have potential to aid early diagnosis.
- Metabolic disease: Changes in salivary glucose, α-2-macroglobulin, melatonin, hormone levels (e.g. free steroids) are a feature of some metabolic and endocrine disorders.
- Inflammation: Elevated cytokines (e.g. IL-1β, IL-6, TNF-α,), acute phase proteins, changes in IgA and other immune-related components in saliva can correlate with immune or inflammatory disease.

Pathologies with high mortality or morbidity benefit most from early salivary biomarker detection. Many pathological states induce overlapping changes (e.g. inflammation in both diabetes and infections), so specificity of biomarkers is essential. Oral health status (periodontitis, oral infections) may confound systemic biomarker signals.

3. Diagnostic Use

Interest in use of saliva as a test sample has increased in recent years, particularly as method sensitivity has increased, allowing more effective analyte measurement.

The advantages of using saliva include the fact that it is non-invasive, painless, cost-effective, and suitable for frequent monitoring. Sample collection is stress-free which may be of use in paediatrics and in the measurement of stress affected hormones such as cortisol. It may also be of value in collecting samples in out-patients at particular times of day, such as at bedtime (hormones).

Disadvantages of use include low levels of analyte present compared to serum, contamination from the oral cavity before collection, and viscosity of the fluid. Collection may also be difficult in dehydrated patients.

Validated saliva applications in use include infectious disease testing (COVID-19), oral health assessment,

hormone measurement, and drugs of abuse testing. Emerging areas include cancer screening, cardiovascular profiling, diabetes monitoring, and microbiome-based diagnostics.

4. Biochemical Tests (Endogenous analytes)

4.1. Infectious Disease

Saliva can be used to detect and diagnose a variety of infectious diseases, including HIV, hepatitis (A, B, and C), cytomegalovirus (CMV), Zika virus, Epstein-Barr virus (EBV), Human Papillomavirus (HPV), and COVID-19. This is possible because saliva contains viral DNA and RNA fragments, as well as host microRNAs and antibodies, which are shed by infected individuals.

4.2. Proteins

Saliva may be collected unstimulated or stimulated, while avoiding contamination by food or blood. Protein analysis can serve as a non-invasive medium to assess cardiovascular risk and inflammatory diseases.

Key proteins include **C-reactive protein (CRP), fibrinogen, lipocalin-2**, and **apolipoproteins**, which reflect systemic inflammation and atherosclerosis. Cytokines such as IL-6 and TNF-α can also be measured as indicators of cardiovascular stress.
Salivary amylase has in recent years emerged as a useful marker in research studies of stress response and has potential for routine clinical use. Salivary amylase testing is a marker of autonomic nervous system stress, and can be used in conjunction with salivary cortisol (a marker of autonomic nervous system).

Detection methods include **ELISA, multiplex bead assays,** and **mass spectrometry**. Marker levels are normalized to total protein or salivary flow for accuracy. Samples should be stored at −80°C to maintain stability. Salivary protein measurement can form the basis of a simple, repeatable tool to assess and monitor cardiovascular and inflammatory risks.

4.3. 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 can accurately reflect the free or non-protein-bound hormone levels making it a suitable matrix for clinical testing of steroid hormones, including cortisol, oestrogens (oestradiol, estriol, estrone), progesterone, androgens (such as testosterone and DHEA), and melatonin.

Cortisol levels in particular correlate well with serum in the presence of normal binding protein concentrations and may represent 10% of the unbound plasma concentration. Using LC-MS/MS measurement of salivary cortisol facilitates concurrent measurement of salivary cortisone which may improve the test's diagnostic performance. While salivary cortisol measurement (late night) is well-established and validated in the investigation of Cushing's syndrome, the testing of other hormones like DHEA is considered experimental or investigational.

4.4. Stones

Salivary stones (Sialoliths) are a common salivary gland disorder. Pathogenesis is not fully understood and is likely multifactorial. Factors include salivary pH and viscosity, anatomical factors, and salivary calcium concentration.

Sialolithiasis is a clinical diagnosis, and imaging may be helpful. Sialoliths may be analysed, as for renal stones, using infrared spectroscopy or X-ray diffraction. Salivary stones are usually composed of calcium phosphate, but other constituents have been described such as struvite, oxalate, carbonate as well as other organic and inorganic components. However, stone analysis is not required for diagnosis or management, and so is not part of routine patient care.

5. 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-ionized 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 pharmacological active fraction.

The correlation of blood and serum levels with saliva levels varies, depending on the structure of the drug. For acidic drugs, the equilibrium favours blood, while basic drugs are found in higher levels in saliva. Ethanol, due to its rapid diffusion into saliva, has approximately equal concentrations in saliva and blood.

Saliva drug tests can detect a wide variety of substances, including common drugs of abuse like alcohol, amphetamines, cocaine, marijuana (THC), and opioids, as well as benzodiazepines and barbiturates. They can also be used to monitor therapeutic drugs like carbamazepine and lamotrigine, The detection window and the specific drugs detectable depend on the individual substance, the test's sensitivity, and the drug concentration.

6. Analytical Factors

6.1. Sample:

Sample collection should prioritize unstimulated whole saliva under standardized conditions.
avoid recent eating, smoking, or oral hygiene activities.
Stabilization of sample (cooling, inhibitors) is critical for nucleic acid integrity.
Measured biomarkers should exhibit
(a) biological plausibility,
(b) reproducible measurement in saliva, and
(c) correlation with clinical gold standards (e.g., blood, imaging, histology).
Panels of complementary analytes should be considered rather than single markers.

6.2. Methods:

Analytical platforms include mass spectrometry (MS), proteomics, quantitative PCR, electrochemical biosensors, lateral flow assays, microfluidics, ELISA and lab-on-chip devices. Validation requires large prospective trials, cross-laboratory standardization, and regulatory compliance. Engagement with regulators and development of user-friendly CE-IVD validated testing methods and devices will be key to innovation and future adoption

7. Conclusion

Saliva-based diagnostics represent a transformative approach for non-invasive, decentralized healthcare. Advances in sensor technology and omics profiling show strong potential, but translation is limited by biological variability, sensitivity constraints, lack of standardization, and regulatory hurdles. Focusing on validated use cases, while building testing infrastructure, will help unlock saliva's full diagnostic potential.

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Seminal Fluid

Authors: Peadar McGing, Micheál Ryan.

1. 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.

2. 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.

3. Diagnostic Use

In conjunction with clinical assessment, semen analysis is the key laboratory test in the assessment of the male partner of a sub-fertile couple. The evaluation of semen parameters is currently based on the standards defined in the sixth edition (2021) of the WHO laboratory manual for the examination and processing of human semen [1].

The primary benefits of semen analysis rest with microscopic examination [2].

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, though these tests are rarely used in routine practice.

4. Biochemical Tests

Poor-quality semen may result from abnormal accessory gland secretions.

Secretions from accessory glands can be measured to assess gland function but are rarely of value clinically and so are not usually part of a routine service.

4.1. pH

The pH at ejaculation is the result of acidic prostatic secretion and alkaline seminal vesicular secretion. The WHO guide states that for normal samples, pH test strips in the range 6.0–10.0 should be used. A pH value under 7.2 points to a lack of alkaline seminal vesicular fluid, but can be caused by urine contamination.

Although included in some test recommendations, seminal fluid pH is generally not considered of clinical value.

4.2. 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. This has probably been the most commonly requested biochemical test in seminal fluid but is now not used routinely.

4.3. Other Biochemical Tests

Many other biochemical tests are available but are not applied to routine clinical investigations.

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 has long been used as a marker for the presence of prostatic fluid. Poor-quality semen may be due to abnormal secretions form the prostate, in which case measurement of citric acid or zinc, along with acid phosphatase can be informative.

5. Other Laboratory Tests

In practice ‘seminal fluid’ analysis focuses mostly on microscopic examinations, particularly of sperm concentration / number, morphology and motility. Other investigations include viscosity, volume, leucocyte count, immature germ cells, sperm antibodies, and presence of debris.

6. Analytical Factors

A fresh sample is required; the sample must be received within 1 hour. WHO recommends that ideally investigations should commence within 30 minutes after collection, but at least within 60 minutes.

For diagnostic purposes WHO recommends that decision limits are more accurate than reference ranges and reference limits.

The WHO document contains detailed method guidance and full instructions for all the tests listed here, some of which may also be available commercially.

EQA is available for some commonly measured parameters, and all laboratories should avail of this.

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Synovial Fluid

Authors: Peadar McGing, Karen Heverin.

1. Physiology

Synovial fluid [1-5] is a colourless to light yellow, highly viscous fluid which is acellular and does not clot. It is found in joint cavities (synovial cavities). It is formed as an ultrafiltrate of plasma across the synovial membrane, combined with hyaluronic acid and lubricin secreted by synoviocytes.

The primary functions of synovial fluid are to act as a lubricant to joint surfaces and supply nutrients to various parts of the joint. It also acts to carry away waste products, acts as shock-absorber, and helps wound healing.

2. 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 [3,4].

- Non-inflammatory (e.g. Osteoarthritis, neuroarthropathy)
- Inflammatory (e.g. Rheumatoid arthritis, gout)
- Septic (e.g. Bacterial or fungal infection)
- Haemorrhagic (e.g. Haemophilia, trauma)

3. 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 from a joint is known as arthrocentesis. It may be performed with ultrasound guidance and pain is minimised by anaesthetising the needle track [2,6].

Synovial fluid analysis is used in differentiating different types of arthritis i.e. infectious, crystal induced, inflammatory, non-inflammatory or haemorrhagic [3,7,8]. Table 1 summarises the most important tests used in the classification of synovial fluid.

A rise in the number of knee and hip replacement surgeries (arthroplasties) has led to an increased demand for synovial fluid analyses [2]. One of the more serious complications of these procedures is prosthetic joint infection and fluid analysis can help distinguish between aseptic failure of an arthroplasty versus acute or chronic infection [3].

4. Tests Based on Physical Characteristics of Fluid

Initially a **“string test”** can be performed. This is a simple test of viscosity. Normal fluid, when dropped from a syringe, forms a string of greater than 10-15 cm. Inflammatory fluid has low viscosity and drips like water [9].

A modification of the string test that is practical for laboratories to use to screen specimens for increased viscosity, a **“drop test”**. Using a plastic disposable pipette held perpendicular to the opening of the sample tube, a volume of the fluid is drawn up and then slowly expelled in a dropwise manner back into the tube. Non-viscous fluids produce uniform spherical drops with relatively short tails, while viscous fluids have longer tails and less spherical shapes [10].

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 [1-4].

5. Biochemical Tests

Biochemical testing is not a routine part of synovial fluid testing algorithms [10], often is non-contributory, and adds little to cell counts and culture results which are the most important tests. Tests sometimes are carried out on similar basis to pleural fluid analysis, but without the same clinical utility.

In general, traditional biochemical tests lack desired sensitivity and specificity in synovial fluid, are very difficult to validate (e.g. high viscosity), and are not covered by fluids EQA schemes. They rarely add clinical value to the non-biochemical tests outlined below.

Biochemistry tests may include

- Glucose – decrease associated with inflammatory or septic conditions.
- Historically, the most commonly requested biochemistry test was glucose. The synovial fluid glucose concentration is normally no more than 0.6 mmol/l lower than the serum concentration; therefore, a blood sample should be taken at the same time as the knee aspirate. Significantly decreased synovial fluid glucose concentration indicates presence of inflammatory and/or septic joint disorders. Usually, this test does not add to diagnostic information from cell counts and differential, but its rapid availability can sometimes help, including possible use in Point of Care devices11. An important caveat to note is potential problems with viscous samples, especially in 'blood gas analysers'
- Lactate – may help distinguish septic from non-septic arthritis [2,11].
- pH – quoted in some publications but samples often too viscous. A low synovial fluid pH is a good indicator of inflammation but not of sepsis.
- Uric acid – may help if MSU crystals suspected but not detected. As with glucose, serum / plasma levels must be sampled at the same time. Levels in synovial fluid and in serum / plasma are normally very similar and elevated levels of fluid urate may be due to raised serum / plasma levels (including in renal failure). A 2023 review listed uric acid in synovial fluid under 'Body test fluids with declining utility [12]. The authors affirmed that there is minimal evidence to support uric acid testing beyond serum.
- Protein – not recommended as issues re sensitivity and specificity.
- LDH (lactate dehydrogenase) − Synovial fluid LDH may be elevated due to inflammatory causes of joint effusion. It is important to measure serum / plasma LDH at the same time and fluid LDH should be interpreted in conjunction with this and with clinical findings.
- CRP – some indication it may be of value (but further work needed)
- Chromium and Cobalt – especially for problems post metal on metal joint replacement.
- Leucocyte esterase – this is a relatively new addition to the diagnostic repertoire but is proving quite useful in differentiating infected from non-infected joint effusions, and its use at point of care can help in faster diagnosis [2,11,13].

6. Other Laboratory Tests

6.1. Cell counts, differential, gram stain & culture.

The primary value of laboratory tests in synovial fluid lies with measurement of cell counts (red and white) and cell differential, plus microbiological tests (gram stain and culture) [2-4,7,10].

These tests help to categorize the fluid as non-inflammatory, inflammatory, septic, or haemorrhagic, in conjunction with clinical findings. Table 1 illustrates typical test values used in this differential. Many clinical algorithms begin with application of these basic tests (e.g. ref. 7).

6.2. Crystal identification.

Microscopic examination for crystals is a less common but extremely important test in synovial fluid [2,7,14-16]. Gout and calcium pyrophosphate deposition disease (CPPD; 'pseudogout') are the two main arthrides caused by crystal deposition. The presence of these crystals causes inflammation, swelling, and pain within the joint.

Gout is caused by the presence of monosodium urate (MSU) crystals, whereas CPPD is the result of calcium pyrophosphate crystals. The finding of either of these types of crystals is pathognomonic for the disease. Clinically it is very important to differentiate MSU from CPPD crystals, though caution is advised that some patients' fluids may contain both MSU and CPPD crystals.

Light microscopy may enable tentative diagnosis of MSU crystals. MSU crystals are needle-shaped crystals whereas CPPD crystals have a rhomboid shape. However, CPPD crystals are smaller and difficult to see using light microscopy. For accurate diagnosis compensated polarised light microscopy is required (gold standard), incorporating a "red compensator" to determine the direction of birefringence.

MSU crystals are strongly birefringent, showing negative birefringence (rotate beam of polarised light clockwise); with compensator the crystals appear bright yellow.

In contrast CPPD crystals are weakly birefringent, showing positive birefringence (rotate beam of polarised light anti-clockwise); with compensator the crystals appear blue.

7. Analytical Factors

Samples should be collected into sterile tubes.

Synovial fluid samples can be highly viscous and thus unsuitable for laboratory mainframe analysers or point of care analysers. Procedures to facilitate reduction in viscosity, such as hyaluronidase or freeze-thaw cycles, must be included in validation procedures if they are to be used in routine analysis.

For crystal identification a service should be available within Pathology, with use of an appropriate microscope (as described above). Microscopes may be either standard use microscopes with manual adaptations for polarised light studies, or may be specific polarising microscopes.

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Table 1. Classification of synovial fluid based on fluid characteristics

	Normal	Non-Inflammatory	Inflammatory	Septic	Haemorrhagic
Colour	Clear or pale yellow	Yellow (straw-like yellow)	Yellow	Yellow / Green	Red
Clarity	Translucent	Translucent	Opaque / Cloudy	Opaque / Cloudy	Cloudy / Bloody
Culture	Negative	Negative	Negative	Often Positive	Negative
WCC (/mm^3)	<200	200 – 2,000*	1,000 – 50,000*	>50,000*	Affected by amount of blood
PMN leukocyte %	<25	<25	>50	>80*	As for WCC
Glucose	90-100% of plasma glucose	Normal	Usually decreased	Decreased	Normal
Crystals	Negative	Negative	May be positive	Negative	Negative

*Figures shown represent average of published guidelines. These limits can vary between different publications.
This table compiled by Dr Peadar McGing.

Pancreatic Cyst Fluid

Author: Peadar McGing.

1. Prevalence

Pancreatic cysts are not ‘normal’components of the pancreas. Nevertheless, two recent population studies detected pancreatic cysts in 49% and 56% of individuals investigated [1,2]. Pancreatic cysts are generally reported as incidental findings on radiology and, in practice, are reported in 1 to 2% of abdominal CT or MRI scans.

2. Pathology

Pancreatic cysts can be either benign (most commonly retention cysts and pseudocysts) or neoplastic (malignant or cystic tumours). Pseudocysts are typically associated with pancreatitis or trauma. The primary clinical and diagnostic focus concerns cystic neoplasms. These may be further classified as serous, mucinous, intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs) [3].

3. Diagnostic Use

Management must balance three facts: cysts are detected frequently; only a minority progress to pancreatic cancer; and pancreatic surgery carries substantial risk. The principal laboratory role is to help distinguish cysts with high malignant potential from those with lower risk—in particular, to differentiate mucinous from nonmucinous lesions [3-5].

4. Biochemical Tests

CEA: Measurement of Carcinoembryonic Antigen (CEA) in pancreatic cyst fluid is well established. In the Cooperative Pancreatic Cyst Study, a large multicenter investigation (n=112), a cystfluid CEA cutoff of 192 µg/L (≈192 µg/L) achieved 79% accuracy for discriminating mucinous from nonmucinous lesions, outperforming endoscopic ultrasound (EUS, 51%) and cytology (59%). No test combination in that study surpassed CEA alone.[6] A subsequent meta-analysis (12 studies) confirmed the diagnostic value of a CEA threshold of>192 µg/L [7].

Glucose:
Recently, cyst fluid glucose has emerged as a simple and inexpensive discriminator of mucinous versus nonmucinous cysts. Using a cut-off of <2.8 mmol/L (50 mg/dL), glucose may be superior to CEA.[8] Multiple studies—and two meta-analyses published in 2021—report high sensitivity and specificity for low glucose in identifying mucinous lesions (low glucose supports mucinous). [9,10] Reported diagnostic accuracy was 94% for glucose versus 85% for CEA alone; combining glucose and CEA did not materially improve performance over glucose alone (97% vs 94%).

5. Other Laboratory Tests

Molecular markers are increasingly important in cystfluid assessment, particularly KRAS and GNAS mutations. A 2023 meta-analysis found that detecting KRAS and/or GNAS mutations identified mucinous cysts with 79% sensitivity and 98% specificity.[11] This area is evolving, including investigations of microRNA (miRNA) panels.

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Drain Fluids

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1. What are ‘Drain’ Fluids?

Technically, any fluid being drained from the body could be termed a drain fluid. The laboratory may receive fluid marked ‘drain fluid’ from a patient with a large pleural or peritoneal effusion being drained for therapeutic purposes. In most cases, it is evident from other clinical details or from tests requested what the correct designation of the fluid is; appropriate pre-analytical procedures can prevent this confusion.

By convention, the term ‘drain fluid’ is most often applied to fluid of unknown or uncertain origin or draining from a wound or surgical site.

2. Diagnostic Use

The primary value in analysing drain fluids is to help determine the origin of fluids which have accumulated in a body space, but the origin of which is unclear or unknown [1].

The laboratory will often receive fluids marked with the description of the catheter name (e.g., Jackson-Pratt drain or Robinson drain). This refers to the surgical drain used therapeutically, and testing should be guided by the clinical question being asked. In most cases, clinical biochemistry tests are not helpful. For example, a recent systematic review of drain fluid analysis for detecting anastomotic leaks after colorectal surgery did not recommend measurement of any biomarkers [2].

3. Biochemical Tests

Creatinine and Urea: The measurement of creatinine, as well as urea, can help diagnose urinary leakage resulting from trauma, surgery, or bladder perforation. Concentrations higher than those in a concurrent blood sample are suggestive of the presence of urine, though in practice, fluid levels are often considerably higher [3,4].

Amylase and Lipase: The pancreatic enzymes may help in the identification of pancreatic fistulas. Fluid enzyme concentrations that are many times higher than in serum/plasma are strongly suggestive of the presence of pancreatic fluid in the drained body cavity [5]. A recent review of drain fluid analysis following genitourinary surgery identified amylase levels greater than 5,000 IU/L as predictive of pancreatic fistula [6].

Bilirubin: Measuring bilirubin levels in a drain fluid can aid in the diagnosis of bile leaks in patients. In a study of Jackson-Pratt (JP) drain fluid, a fluid-to-serum bilirubin concentration ratio greater than 5 was reported to be highly sensitive and specific for detecting a bile leak [7]. This study also investigated the discriminatory value of fluid colour and noted that a green or brown fluid colour was specific to bile leaks.

4. Other Laboratory Tests

Cell count, Gram stain, and culture are the main non-biochemical tests performed, particularly where there is suspicion of infection [6].

5. Analytical Factors

Care must be taken to preserve samples correctly for the various tests required. Use the same preservatives/ bottles for the fluid collection as for the equivalent serum/plasma tests.

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Peritoneal Equilibration Test

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1. Diagnostic Use

Peritoneal dialysis (PD) is a form of renal replacement therapy that utilizes the patient's peritoneal membrane to extract solutes from the uraemic blood into a dialysate medium. In PD the dialysate is temporarily inserted into the patient's peritoneal cavity, allowed to equilibrate with the blood for a period, and then removed.The transport characteristics of the peritoneal membrane varies between individuals, which can have clinical implications for optimising a patient's dialysis therapy prescription. It was for this purpose that the Peritoneal Equilibration Test (PET) was developed in 1987 as a standardized procedure to measure the permeability and transport efficiency of a patient's peritoneal membrane, and use this information to optimize dialysis effectiveness [1].

PET results are used to determine a characteristic transport rate category for the patient on PD. Transport rates are typically defined as slow, slow average, fast average, or fast and indicates which type of PD is most efficient for that patient. Using PET in this way achieves more efficient fluid removal which is associated with better outcomes [2].

2. Biochemical Tests

Overview: For details of the calculation and interpretation of the PET as used in an individual institution, readers are advised to speak to the staff in their hospital's Peritoneal Dialysis Unit, and view the specific literature from the company providing the dialysis fluid. That company will normally provide a software package specific to their company's fluids, one which facilitates input of the biochemistry test results. Calculation of PET is not a function of the Laboratory Information System (LIS).

A brief description of the process and the calculations are given in a number of publications [3,4].
Analytes measured: The biochemical tests carried out for the PET are glucose, creatinine, and urea.
Test Samples: Fluid samples are taken at four time points – after overnight exchange, pre-PET, 2 hour PET Dwell, and at 4 hours (end of PET). A blood sample is taken at the same time as the 2 hour PET Dwell fluid sample.

3. Analytical Factors

Creatinine assay: Traditionally, measuring creatinine with the Jaffe method caused problems for the PET due to the very high glucose level in many of the fluid samples, which interfered with the creatinine assay. Under those circumstances a correction factor needed to be applied. Following the conversion of most laboratories to use of enzymatic creatinine this correction factor is no longer needed where enzymatic creatinine is provided [5]. Laboratories should ensure that staff in units employing PD and testing PET are aware of the lab's assay, so a correction factor is not applied inappropriately.

Laboratory – Clinical Interface: The Peritoneal Equilibration Test is one that requires particularly strong collaboration between laboratory scientists and clinical users of the service. Procedures must be in place to ensure that all samples are taken at the right time and are appropriately and uniquely labelled, including the time taken. Procedures in the lab must include that all samples receive a quantitative result, avoiding any 'greater than' results, with particular attention to very high glucose levels in some fluid samples.

The PET test involves a lot of work for nurses and for laboratory staff. The protocol must be followed exactly on the ward to obtain the correct samples, labelled appropriately, and delivered promptly. The

laboratory must provide accurate, timely, fully quantitative results. All results are needed and collaboration is vital.

The end-product determines the patient's PD treatment for months. It is imperative to get it right.

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Faecal Water

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1. Diagnostic Use

Testing of faeces is an important part of diagnosis for a number of clinical conditions but rarely involves the Biochemistry Laboratory. In the case of watery diarrhoea, diagnosis is usually made through non-biochemical testing, particularly microbiology. For chronic watery diarrhoea, testing of faecal water can be helpful [1].

2. Biochemical Tests

Osmotic Gap: Calculation of faecal osmotic gap (often referred to as stool osmotic gap) can be a very important measurement in the diagnostic workup of idiopathic causes of chronic diarrhoea [2,3].
Osmotic Gap
= 290 (mmol/kg or mOsm/kg) – {2x ([Faecal Water Sodium]+[Faecal Water Potassium])}.
Note: Do not analyse the faecal water for osmolality; an assumed normal osmolality of 290 is used for the calculation.
A gap of greater than 50 mOsm/kg (50 mmol/kg) is consistent with an osmotic aetiology, whereas a gap of less than 50 mOsm/kg is often associated with a secretory etiology [4].
The measurement of faecal osmolality may also be helpful in the diagnosis of factitious diarrhoea, such as when a patient adds water to the faecal sample to simulate diarrhoea.
Electrolytes: In some individual cases measurement of Mg, Na, and K can be helpful [3]. Examples include magnesium-induced osmotic diarrhoea (due to excessive magnesium salt ingestion) [5] or investigation of unexplained electrolyte loss in a patient on a colostomy bag (via timed collection of colostomy bag contents and concomitant timed urine collection).

3. Analytical Factors – Sample Preparation

The faecal sample, or colostomy bag contents if appropriate, should be mixed well in an airtight container and an aliquot taken into a suitable sample bottle. The aliquot should be centrifuged as per blood bottles and a further aliquot of water removed for analysis. All of the preparation, except for the centrifugation, should be carried out in a fume cupboard. The final analysis-ready preparation of fecal water can be treated like any other biological specimen.
Faecal water tests are very rare, but labs should have a procedure in place in case of such a request.

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Test validation in non-standard fluids

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1. Validation or Verification.

ISO 9000 (2015) [1] defines **verification** as the confirmation, through the provision of objective evidence, that specified requirements have been fulfilled. In practice, for clinical laboratories, that means the process of confirming that a pre-validated method, such as a commercial kit, performs correctly in the specific laboratory environment where it is being used, according to the manufacturer's instructions.

ISO 9000 (2015) defines **validation** as the confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled. For clinical laboratories, that means the process of confirming that a newly developed or modified test method is suitable for its intended purpose, proving it meets the required performance criteria.

Asays used for testing atypical fluids are not covered by the manufacturer's own validation, and are not listed in the intended use section of manufacturer's instructions for use (IFU). Utility of an assay for nonstandard fluids (including extravascular body fluids) is considered a modification of a CE marked method, is 'off-label' / 'out of scope' and, therefore, requires validation [2].

It is very important to realise that, apart from a very small number of assays, such as CSF glucose, the more extensive and in-depth process of validation is required for fluid assays even though the simpler verification process may be used for the same analytes in serum / plasma.

2. Test validation in non-standard fluids – General principles

Historically, some tests, such as pleural fluid protein, had been assayed using serum assays. However, that practice can no longer be applied. It should not be presumed that an assay will behave similarly in nonstandard fluids as it does in more typical fluids [3,4]. Routine assays used to measure analytes in serum / plasma, as well as urine and/or CSF in specific cases, will already be verified for use in an ISO15189-accredited laboratory.

For nonstandard fluid validation:

- Tests should be validated by fluid type rather than generically e.g. pleural fluid total protein rather than fluid total protein [2,5].
- The most prevalent fluids and tests with established, evidence-based clinical utility should be considered first [6].
- All testing performed in nonstandard fluids, ultimately, require validation.
- The approach taken should be risk-based with a balance of pragmatism [5].

For such validations, the manufacturer application, for serum, plasma, urine or CSF, that best fits the body fluid characteristics, should be utilised [5]. The assay is tested unadjusted with unmodified calibration curves.

3. Limitations of working with body fluids for test validation

Body fluids used for validation studies are typically remnants of patient samples received for testing [7].

To ensure accurate representation of fluid heterogeneity it is ideal to have at least ten samples of individual fluid types. However, with fewer requests for fluid testing, this may not always be achievable. Limited sample volume may demand pooling within specimen types [7]. Similarly, samples with innate, clinically

relevant analyte concentrations may not always be available, such that spiking becomes necessary [7]. Commutable material with high analyte concentrations suitable for spiking could include routine serum/ urine specimens, assay calibrators or commercially prepared solutions. In all cases, diluting the fluid by more than 10% may adulterate the matrix and is best avoided [5].

Validation plans will require customisation depending on the body fluid type and sample volume available.

While partial or in-process validation may support interim use of an assay in a particular atypical fluid, until full validation is achieved all such test results must be issued with an appropriate disclaimer.

4. Performance characteristics necessary for test validation in non-standard fluids

4.1.Test stability assessment in fluid

This is an important parameter for the laboratory to understand both for routine testing and add-on requests, as well as the validation process, which can occur over several days [6]. The stability of the measurand in the fluid, from collection through to analysis and beyond, should be known [3,4].

Specific fluids from similar sites of origin, with different analyte concentrations across the test measuring range and at clinically relevant concentrations are required for stability testing [5].

- The test analyte is measured in a series of specimens.
- Samples are aliquoted to sufficient number to allow various storage temperatures to be tested over a number of days, e.g. ambient, 4oC / refrigeration, and -20oC / freezer, on days 1, 3 and 7.
- Aliquots are stored as per selected test temperatures and experiment days and measured as appropriate.
- Percentage difference is determined from baseline, which ideally is as close to the collection time as possible (Day 0) and compared to predetermined acceptance criteria [5].

  Percentage difference =

$$\frac{[(\text{result on Day X}) - (\text{result on Day 0})] \times 100}{(\text{result on Day 0})}$$

4.2. Accuracy of test measurement in fluid

Assessment of accuracy or trueness confirms that the analyte can be measured in a nonstandard fluid without undue matrix effects. Ideally, a reference method for each test, which is free from any influence of the matrix, is used for a comprehensive method comparison. However, as few routine tests will have a reference method readily available a series of spiking experiments can instead be used [2,5]. Such experiments highlight if the matrix affects recovery of the measurand. Acceptance criteria are established in advance and are typically based on performance achieved on in-house verification of the test in typical fluids. When available, participation in External Quality Control / Proficiency Testing programmes are encouraged to allow peer-to-peer comparison of fluid analytes [6]. However, such materials should not be used to validate accuracy of measurement [2,5].

4.2.1. Spiked recovery of the test analyte in fluid

A minimum of three specimens, of the same fluid type, with analyte concentrations at or near the assay's lower limit, are required and measured [5]. Subsequently, each sample is divided into aliquots (minimum n = 3) and spiked to achieve clinically relevant analyte concentrations spanning the assay's measuring range [5]. Suitable material for spiking includes serum / plasma with a known concentration of analyte, calibration verification material, or a standard solution [7]. The material used for spiking must be less than 10% by volume.

The expected analyte concentration in each aliquot is calculated. Each aliquot is measured in duplicate and averaged.

Percentage recovery =

$$\frac{[(\text{spiked sample concentration}) - (\text{initial sample concentration})] \times 100}{[\text{concentration of analyte added}]}$$

Results obtained should meet the predefined acceptance criteria for the assay under investigation [2].

4.2.2. Mixed recovery of the test analyte in fluid

This study doubles up for assessment of assay linearity in fluid2. Paired fluids of the same type (minimum n = 3), one with an analyte concentration at the low end of the assay's measuring range and another with an analyte concentration at the upper end of the assay's measuring range are needed5. Such paired samples are blended together to create a set of, at minimum, five samples with proportionate analyte concentrations, e.g. 100%, 75%, 50%, 25% and 0% [5]. The analyte concentration expected in each sample is calculated. The samples are measured in duplicate, and averaged. Percent recovery from each pairing is determined, and should meet predefined acceptance criteria.

Percent recovery =

$$\frac{[\text{measured concentration}] \times 100}{[\text{expected concentration}]}$$

4.2.3 Dilution recovery of the test analyte in fluid

Fluid dilution and determination of analyte recovery can identify if the fluid matrix affects testing accuracy [5]. The simplest diluent to use for any given analyte is the one suggested on the method IFU. At minimum three body fluids of the same type are obtained with a concentration of test analyte at the upper end of the measuring range [5].

Leaving enough sample for a control measurement, serial dilutions are prepared. The number of dilutions made will depend on the expected measuring range of the assay and the volume available, e.g. 1:2, 1:4, 1:8, 1:10 etc. The expected concentration of the analyte in each sample is calculated. The samples are measured in duplicate and results averaged.

Percent recovery from each pairing is determined, and should meet predefined acceptance criteria.

Percent recovery =

$$\frac{[\text{measured concentration}] \times 100}{[\text{expected concentration}]}\ .$$

4.3. Test precision

To assess the reproducibility of a method to measure the analyte in a nonstandard fluid, a minimum of two fluid-specific specimens is required [5]. Analyte concentrations should be near or at critical decision limits for the analyte in fluid. Fluids are aliquoted to allow sufficient volume for five repeats with aliquots stored under conditions where the test analyte is most stable [5]. Each aliquot is tested five times per day over the course of five days (5x5 design). Repeatability and within-laboratory imprecision, i.e. standard deviations and percent coefficient of variations, are calculated and compared against predetermined acceptance criteria [8]. Acceptance criteria may be established using manufacturer performance criteria for the assay in serum, plasma, urine or internal quality control (IQC) material.

To ensure that the assay is suitably precise for fluid measurement in day-to-day practice, IQC typically used with the method application, i.e. serum, urine or CSF IQC, is analysed. IQC concentrations used should be clinically relevant for the analyte in the particular fluid. In using this IQC material, we assume no difference between the IQC matrix and the fluid. Where medical decision limit concentrations are not met using an off-the-shelf IQC product, use of calibrators or diluted IQC material may prove useful [2].

4.4. Lower limit of the assay

Accuracy and precision at the assay's lower limits become relevant only when clinically important decisions are made at very low fluid analyte concentrations [5]. To assess the assay's lower limit of quantitation (LoQ), for each relevant fluid type, fluid with an analyte concentration at or near this LoQ is required. The sample is divided into five aliquots and stored where considered most stable. Over five consecutive days an aliquot should be removed from storage and analysed four times. Means, standard deviations and coefficients of variation are used to determine acceptability [2]. Precision in atypical fluids can be compared to that obtained in more typical fluids (usually serum / plasma).

4.5. Linearity and assay measuring range of the test in fluid

It must not be assumed that the measuring range of any analyte in fluid will mirror that of the analyte measured in a more typical fluid [5]. Commercially available linearity material for fluids can prove useful for assessment of assay linear range across a wide range of concentrations. Otherwise, data generated in the accuracy studies can be re-purposed. The measured (x-axis) and expected (y-axis) analyte concentrations from the recovery experiments can be graphed [2]. Results should meet predefined acceptance criteria for linear regression (slope, intercept and correlation coefficient). Whether dilutions are required for measurement of fluid tests, with concentrations beyond the assay's linear range, will depend on the clinical requirements for that test; a 'greater than' result may be adequate [2].

4.6. Testing Endogenous interference – haemolysis, icterus and lipaemia

Serum/plasma samples are routinely screened for haemolysis (H), icterus (I) and lipaemia (L) on large automated instruments. Manufacturers provide H, I and L index cut-offs for analytes in serum/plasma. When these cut-offs are exceeded, test results are considered inaccurate and are generally withheld. Given non-standard fluids are often not listed as the intended matrix for analyte measurement on IFUs, the effect of H, I and L on such test results has not been studied by assay manufacturers.

Rule In / Out of Interference:

To quickly determine the consequences of these endogenous interferents on a test, an initial screen can be performed in a fluid containing the analyte in question at a clinically relevant concentration where a high concentration of interferent is added [5,9]. A stock solution of the interferent (haemoglobin, bilirubin or lipid mix) approximately 20 times greater than the final concentration needed should be prepared.

- **'Test':** This stock is diluted into the atypical fluid (1 in 20) and is mixed well.
- **'Control':** The diluent used to make the interferent stock is diluted in the same proportion (1 in 20) into a separate aliquot of the fluid and mixed well.
- The analyte in the Test (spiked) and control samples is repeatedly measured, e.g. n=5, and the averages of each obtained [5].

The percent difference is calculated (spiked – control / control x 100).
If the interferent had no effect the percent difference should be less than the predetermined medically significant difference. No further action is required.

Determination of interference dose-response:
If a medically significant difference is observed an interference dose-response protocol should be followed. The concentration of interferent required to illicit a significant effect can be determined using a series of interferent concentrations5.

As before, a stock solution of the interferent is used. At least two fluid samples are required with test analyte concentrations at or near clinically relevant decision limits.

- **High-test solutions:** The stock interferent solution is diluted into the fluids (1 in 20) and is mixed well.

- **Low-test samples:** As controls, the liquid used to make the interferent stock solution is diluted with the fluids (1 in 20) and mixed well.
- High- and low-test sample pairs are mixed into each other in blended ratios to create a set of five samples with proportionate concentrations; 100%, 75%, 50%, 25% and 0% for each fluid [2].
- Each sample series is repeatedly measured, e.g. n=5, in sequence starting from low to high, followed by high to low, back to low to high and so on5. The average of each sample in the series is determined.

The percent difference between the low-test sample and each of the others in the series is calculated (test – low / low x 100).

Data produced is graphed; absolute difference or percent difference versus interferent concentration for each test analyte concentration tested. The concentration of the interferent where a medically significant difference is observed is considered the threshold above which the test result should be rejected [5].

4.7. Sample pre-treatment measures.

Occasionally samples may be deemed unsuitable for analysis in the neat state, but potentially suitable if treated. The most common examples of this are treatment of viscous samples (e.g. synovial fluid with hyaluronidase or freeze-thaw cycles), and high-speed centrifugation to clear lipids.

Where any such treatments are to be applied in routine practice, the process must be included in the validation protocol for that assay in that fluid.

4.8. Published examples of fluid validations.

The increasing accreditation demands for validation of assays used in atypical fluids are likely to result in more such validation activities being published. Such studies can help to guide laboratories through the process and also provide support for use of the assays under the conditions investigated in those papers. Two such studies are listed below [10,11].

5. Interpreting fluid test results

For many of fluids discussed in this guideline, particularly pleural and peritoneal, the presence of sufficient volume to collect immediately implies “pathology”. Such fluids are not present in healthy persons, such that their collection for derivation of reference intervals is not possible. Instead, interpretative comments, reflecting on paired fluid and plasma/serum test results, may be more appropriate [2,5].

In other fluids, such as CSF, it will not be possible to ethically collect samples from healthy donors for the purposes of deriving reference intervals. Samples taken for other medical purposes have allowed some reference intervals to be proposed, but these are limited.

In providing an analytical service for any measurand in a fluid that is not covered by the assay IFU, laboratory personnel must provide the best evidence available to interpret results of validated assays [5].

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Safety Considerations

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1. Safety Overview

All biological fluids must be treated as potentially infectious. Safe handling requires strict adherence to standard laboratory safety practices and to containment measures specified by the Health and Safety Authority (HSA, Ireland), the Health and Safety Executive (HSE, UK) and its Advisory Committee on Dangerous Pathogens (ACDP), the EU Directive 2000/54/EC (as amended by Directive (EU) 2019/1833 and Directive (EU) 2020/739) and international standards (ISO 35001) on the management of biological risks in the workplace. Each laboratory must conduct a local risk assessment and ensure compliance with applicable legislation, local biosafety policies, and accreditation standards [1-6].

1.1. Standard Safety Precautions

Standard safety precautions apply to all laboratory activities involving biological specimens, regardless of type or perceived infection risk [1-4,6-7].

The following measures must be observed to ensure safe handling and to minimise the risk of exposure:

- Wear appropriate personal protective equipment at all times, including gloves, laboratory coat, eye protection and where applicable certified face masks.
- Perform aerosol or splash-generating procedures within a certified Biological Safety Cabinet (BSC) wherever possible.
- Use mechanical or automated pipetting systems and minimise open handling by using sealed containers or automated systems.
- Decontaminate work surfaces before and after each procedure using approved disinfectants appropriate to the biological agent risk level.
- Dispose of contaminated materials by autoclaving or through an approved clinical-waste stream.
- Use certified packaging for the safe transport of biological specimens.
- Report any spills or exposure incidents immediately and follow local emergency response protocols.

2. Risk Group and Containment Classification

According to European and national legislation, biological agents are classified into four Hazard or Risk Groups (RG1 to RG4) based on their potential to cause infection and disease in healthy workers [1,4,5]. The Containment Level (CL1 to CL4), BSC class (Class I, II or III) and CE marked PPE, compliant with EN / EN ISO standards, required for safe handling are determined by this classification and by local risk assessment [6].

2.1 Risk Group 1

Biological agents classified as Risk Group 1 (RG1) are unlikely to cause human disease and present minimal risk to laboratory personnel (e.g. Escherichia coli K12). Procedures may be conducted at Containment Level 1 (CL1) following standard laboratory safety practices. BSCs are not required, and standard PPE provides adequate protection [1-4].

2.2 Risk Group 2

Biological agents classified as Risk Group 2 (RG2) can cause human disease but are unlikely to spread in the community (e.g. Salmonella, Staphylococcus aureus). Work must be performed at CL2, with aerosol or splash generating procedures carried out in a certified Class II BSC. In certain situations (e.g. extensive

aerosol generation), additional containment measures exceeding standard CL2 practices may be required, as determined by local risk assessment. PPE should include gloves (EN ISO 374-5 / EN ISO 374-2), protective clothing (EN 14126), eye protection (EN ISO 16321-1), and fit-tested FFP2 or FFP3 respirators (EN 149) whenever aerosol risk exists [4-7].

2.3 Risk Group 3

Biological agents classified as Risk Group 3 (RG3) cause serious or potentially fatal disease and may spread to the community (e.g. Mycobacterium tuberculosis, Brucella spp.). Analytical procedures must be undertaken at CL3 in a controlled-access facility with negative air pressure and HEPA-filtered exhaust. Manipulations should be conducted in Class II BSC within the CL3 facility. Class III BSC may be required where there is a high risk of aerosol generation or as determined by local risk assessment. PPE includes double gloves (EN ISO 374-5 / EN ISO 374-2), protective clothing (EN 14126), eye protection (EN ISO 16321-1) and fit-tested FFP2 or FFP3 respirators (EN 149) whenever aerosol risk exists [4-7].

2.4 Risk Group 4

Biological agents classified as Risk Group 4 (RG4) cause severe or fatal disease, pose a high transmission risk, and have no effective treatment or prophylaxis (e.g. Ebola virus). All procedures must be undertaken in a CL4 laboratory, using sealed isolators or positive-pressure suits providing full respiratory, eye, and skin protection. Protective clothing (EN 14126) is mandatory, and all materials and waste must undergo validated decontamination before removal [1,2,4,5].

3. Handling of Non-standard Biological Fluids

Non-standard biological fluids (e.g. cerebrospinal, peritoneal, pleural, and pericardial fluids), may pose additional risks due to the potential for high pathogen titre. The same general principles outlined in the proceeding sections also apply, but additional containment and procedural controls described below must also be observed [1,4,8].

3.1 Risks

Procedures such as centrifugation, pipetting, or de-capping can generate aerosols, increasing the risk of airborne transmission. These specimens must therefore be treated as potentially infectious regardless of known diagnosis [4,5].

3.2 Control Measures and Containment

Class II BSCs should be used for open handling, with strict adherence to PPE requirements and spill/exposure response plans. In situations where Class II BSCs are unavailable, laboratories must implement equivalent engineering and procedural controls in accordance with HSA (Ireland), HSE (UK), and EU Directive 2000/54/EC. Minimum PPE requirements include the use of fit-tested FFP2 or FFP3 respirators (EN 149) whenever aerosol risk exists, protective clothing (EN 14126), eye protection (EN ISO 16321-1), and gloves (EN ISO 374). Open handling must be minimised, with sealed systems or splash barriers used wherever possible to maintain operator and environmental safety. A documented local risk assessment must confirm that these alternative measures provide an equivalent level of protection for personnel and the environment [1-3,5-7].

3.2.1 Containment Level Requirements for High-Risk Pathogens

When high-risk pathogens are known or suspected, work must be performed at the corresponding containment level outlined in Section 2, as specified by national and European biosafety legislation, including HSA Code of Practice, ACDP guidance and EU Directive 2000/54/EC (as amended). Additional containment measures, including restricted laboratory access, validated negative air pressure, and HEPA-filtered exhaust systems, must be implemented as determined by risk assessment in accordance with national biosafety standards [1,4-6].

3.3 Decontamination and Disposal

Surfaces must be decontaminated before and after use with appropriate disinfectants. Contaminated materials should be autoclaved or disposed of as clinical waste using the appropriate colour-coded bins (e.g. UN 3291 Category B infectious healthcare risk waste) in line with local disposal policies [1,2,4,9].

3.4 Incident Management

All spills or exposure incidents must be reported immediately to the designated Safety Officer or Laboratory Manager. Staff sustaining potential exposure (e.g. splash, sharps injury, or aerosol inhalation incident) must seek immediate medical attention via the Emergency Department or Occupational Health services. Once the area has been declared safe, decontamination should be undertaken in accordance with the local spill response policy. Laboratories must maintain and routinely review the spill response and sharps handling protocols, and verify that all staff are trained in emergency response [1,2,4,8].

3.5 Training, Documentation, and Audit

Each laboratory must maintain up-to-date risk assessments, provide regular staff training in exposure prevention, emergency response, and biosafety, and conduct internal audits to verify compliance with local, national, and international biosafety requirements [6].

4. Disclaimer

This safety guidance does not constitute legal advice. Each laboratory must conduct its own risk assessments and ensure adherence to local, national, and international biosafety regulations.

5. References

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Appendix 1

Biosafety Classification and Handling Guidelines for Biological Fluids

Fluid Type	Risk Group	Containment Level	Safety Cabinet Class	Potential Pathogens	Key Risks	Recommended Controls
Sweat and non-infectious specimens	RG1	CL1	Not required	Non-infectious / minimal hazard	Minimal infection risk	Standard laboratory CE marked PPE; good laboratory hygiene.
Saliva, semen, amniotic fluid	RG2	CL2	Class II (if splash/aerosol risk)	Herpes simplex virus, Cytomegalovirus, Human papillomavirus	Potential exposure via splash or aerosol	Standard precautions; Class II BSC for splash or aerosol risk; use CE marked protective clothing, gloves, and eye/face protection.
Peritoneal (ascitic) fluid	RG2	CL2	Class II preferred	Escherichia coli, Klebsiella spp., Mycobacterium tuberculosis	Potential pathogen exposure via splash or aerosol during manipulation	Handle in Class II BSC; use CE marked gloves, gown, and eye/face protection; sealed transport.
Pleural fluid	RG2–RG3 (agent-dependent)	CL2–CL3	Class II	Streptococcus pneumoniae, Mycobacterium tuberculosis, fungi	Aerosol generation; splash risk	Class II BSC; CE marked fluid-resistant gown, gloves, and eye/face protection; FFP2 or FFP3 respirator as determined by risk assessment.
Pericardial fluid	RG2–RG3 (agent-dependent)	CL2–CL3	Class II	Coxsackievirus, Salmonella typhi, Staphylococcus aureus	Aerosol or splash exposure; potential bloodborne infection	Class II BSC; CE marked fluid-resistant PPE; vaccination (e.g. HBV) where appropriate.
Cerebrospinal fluid (CSF)	RG2–RG3 (agent-dependent)	CL2; CL3 if RG3 pathogen suspected/ confirmed	Class II	Neisseria meningitidis, Enteroviruses, Mycobacterium tuberculosis	Aerosol generation; CNS infection	Class II BSC within CL2 (routine) or CL3 if RG3 pathogen suspected; CE marked PPE; sealed centrifuge rotors; FFP2/FFP3 respirator based on risk assessment.
High-risk fluids (e.g. CSF with suspected Ebola)	RG4	CL4	Class III or sealed isolator system	Ebola virus, Marburg virus	Severe/fatal infection; high transmission risk	Work in CL4 facility; Class III BSC or isolator system; full-body CE marked PPE; HEPA-filtered exhaust; validated waste decontamination.

Note: “CE marked PPE” refers to protective equipment meeting relevant European and international standards, including but not limited to EN 14126 (protective clothing), EN 374 (gloves), EN 149 (respiratory protection), and EN 16321-1:2022 (eye/face protection).

Appendix 2 – General Overview References

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