Bilirubin (CSF)

1 Name and description of analyte

1.1 Name of analyte
Bilirubin

1.2 Alternative names
CSF xanthochromia

1.3 Heading not used

1.4. Function(s) of analyte
Bilirubin is the end product in the breakdown of the haem moiety of molecules such as haemoglobin, myoglobin and cytochromes. It is produced *in vivo* from red cell breakdown after haemorrhage into the CSF.

2 Sample requirements and precautions

2.1 Medium in which measured
CSF

2.2 Precautions re sampling, handling etc.
Samples should be collected from patients with a history suggestive of subarachnoid haemorrhage (SAH) and with a negative or equivocal CT result. Lumbar puncture should be performed a minimum of 12 h post-event, and within 10 days of suspected SAH. The time of sampling relative to possible haemorrhage should be recorded. The specimen should be centrifuged at >2000 rpm for 5 minutes within 1 h of collection. Samples must be protected from light as bilirubin is photosensitive. Samples must not be transported by pneumatic tube.

Analysis should be performed on the least bloodstained CSF sample collected (usually the last) to minimise contamination with blood.
A paired serum sample should be analysed for total protein and serum bilirubin, which may be needed for interpretation.

3 Summary of clinical uses and limitations of measurements

3.1 Uses
Bilirubin is measured in CSF to aid in the diagnosis of subarachnoid haemorrhage in patients who have had a negative or equivocal CT scan result.
SAH releases erythrocytes into the CSF, which then lyse and liberate oxyhaemoglobin. Oxyhaemoglobin is converted into bilirubin *in vivo*, in a time-dependent manner. Oxyhaemoglobin may also be released into the CSF during sampling via lumbar puncture (traumatic tap), but due to the time taken for bilirubin to be produced, its measurement in CSF can distinguish between SAH and a traumatic tap.

3.2 Limitations
CSF bilirubin is not specific for SAH and may occur in conditions such as meningitis or spontaneous intracranial hypotension.

Repeat samples collected after a previous traumatic tap may give false positive results due to the iatrogenic introduction of oxyhaemoglobin into the CSF. Samples collected less than 12 hours post-suspected SAH may give false negative results as the conversion of oxyhaemoglobin to bilirubin is time-dependent. Samples collected more than 10 days post-suspected SAH may give false negative results due to *in vivo* clearance of bilirubin from the CSF.

4 Analytical considerations

4.1 Analytical methods

A. Zero-order spectrophotometry

A scan is performed between 350 and 600 nm on a centrifuged, undiluted specimen. A predicted baseline (tangent) is drawn and the absorbance above this is measured to identify the presence of pigments in the sample and to give a net absorbance value for each.

Oxyhaemoglobin has an absorbance maximum between 410–418 nm

Bilirubin appears as a broad peak between 450–460 nm, or a shoulder adjacent to the oxyhaemoglobin peak; net bilirubin absorbance is measured at 476 nm to enable separation from the oxyhaemoglobin peak

Methaemoglobin occurs as a broad peak between 403–410 nm but is rarely present

Derivative spectrophotometry is an acceptable alternative but is not in widespread use due to the level of expertise required for interpretation.

Visual inspection of CSF for yellow coloration is not a reliable method.

Analysis of CSF bilirubin using adaptation of serum bilirubin methods has been reported but is not currently recommended owing to a lack of validation information.

4.2 Reference method

Zero-order spectrophotometry

4.3 Reference materials

None available

4.4 Interfering substances

- Oxyhaemoglobin – if present in sufficient concentration the oxyhaemoglobin peak may obscure the bilirubin peak; similarly, methaemoglobin may do this but this pigment is observed very rarely in CSF.
- Turbidity of the sample may obscure the bilirubin peak.

4.5 Sources of error

- Timing of sample collection relative to suspected SAH
- Previous traumatic tap
- Exposure of sample to light

5 Reference intervals and variance

5.1.1 Reference interval (adults)

Net bilirubin absorbance ≤0.007 AU

Net oxyhaemoglobin absorbance ≤0.02 AU

5.1.2 Reference intervals (others)
Not applicable

5.1.3 Extent of variation

5.1.3.1 Interindividual CV
Not available – not reported as numerical data

5.1.3.2 Intraindividual CV
Not available – not reported as numerical data

5.1.3.3 Index of individuality
Not available – not reported as numerical data

5.1.3.4 CV of method
Not available – not reported as numerical data

5.1.3.5 Critical difference
Not available – not reported as numerical data

5.1.4 Sources of variation
CSF bilirubin concentration varies dependent on the magnitude of the haemorrhage and the time of sampling relative to the event.

6 Clinical uses of measurement and interpretation of results

6.1 Indications and interpretation
The main use of CSF bilirubin measurement is in the diagnosis or exclusion of SAH in patients with a normal CT scan, or in whom CT scans are contra-indicated.
A net bilirubin absorption >0.007 AU is usually suggestive of SAH; however, there are several confounding factors that should be considered when interpreting results (see 6.2).

6.2 Confounding factors

- Timing of the sample relative to onset of symptoms: samples collected <12 h post-event may give false negative results due owing to the time taken for bilirubin to accumulate, whereas samples >10 d post-event may give negative results due to clearance of pigments from the CSF.
- A repeat sample after a previous traumatic tap may give a false positive result.
- Oxyhaemoglobin may obscure the bilirubin absorbance peak if present in sufficient quantity (NOA >0.1); therefore, SAH cannot be excluded.
- An increased white cell count may cause the *in vitro* conversion of oxyhaemoglobin to bilirubin if analysis is delayed.
- Hypercarotenaemia, iodine contamination and treatment with rifampicin may cause CSF to appear xanthochromic in the absence of SAH.

7 Causes of abnormal results

7.1 High values

7.1.1 Causes

- Subarachnoid haemorrhage
- Elevated serum bilirubin: bilirubin may cross the blood-brain barrier in patients with elevated serum [bilirubin] (>20 μmol/L), potentially resulting in a false positive measurement.
- Increased CSF [total protein] may aid the transfer of bilirubin across the blood-brain barrier, leading to an increased CSF bilirubin in the absence of SAH.
- Meningitis or other infectious pathologies
- Spontaneous intracranial hypotension

7.1.2 Investigation

Ensure results are interpreted according to the clinical context.
A paired serum sample should be analysed for bilirubin. The adjusted net bilirubin absorbance (NBA) may be calculated if the serum bilirubin is >20 μmol/L, to determine whether the increased CSF [bilirubin] is due to SAH or secondary to increased serum bilirubin, using the following formulae:

$$PA = \frac{\text{CSF total protein (g/L)}}{\text{Serum [total protein] (g/L)}} \times \text{serum [bilirubin] (μmol/L)} \times 0.042 \text{ AU}$$

(PA: predicted absorbance at 476 nm)
Adjusted NBA = measured NBA-PA

This approach is not recommended where CSF [bilirubin] is increased owing to increased CSF [total protein] (>1.0 g/L), due to lack of validation. CSF [total protein] should be measured. NBA results >0.007 AU from patients with CSF [total protein] >1.0 g/L must be interpreted with caution.
CSF glucose and cell count should be measured to aid diagnosis.

7.2 Low values

7.2.1 Causes

No clinical significance.

7.2.2 Investigation

Not indicated.

7.3 Notes

Not applicable.

8 Performance

8.1 Performance is dependent on the timing of the sample after subarachnoid haemorrhage. A systematic review by Carpenter *et al*. (2016) found a sensitivity of 100% and a specificity of 95% for spectrophotometric bilirubin.

9 Systematic reviews and guidelines

9.1 Systematic reviews

Spontaneous subarachnoid hemorrhage: a systematic review and meta-analysis describing the diagnostic accuracy of history, physical exam, imaging, and lumbar puncture with an exploration of test thresholds Carpenter, CR, Hussain, AM, Ward, MJ *et al*. Acad Emerg Med 2016; 23: 963–1003.

Cerebrospinal fluid analyses for the diagnosis of subarachnoid haemorrhage and experience from a Swedish study. What method is preferable when diagnosing a subarachnoid haemorrhage?
Nagy, K, Skagervik, I, Tumanj, H *et al*. Clin Chem Lab Med 2013; 51: 2073–86

9.2 Guidelines

Revised national guidelines for analysis of cerebrospinal fluid for bilirubin in suspected subarachnoid haemorrhage (UK NEQAS Specialist Advisory Group for EQA of CSF Proteins)
Cruickshank, A, Auld, P, Beetham, R *et al.* Ann Clin Biochem 2008; 45:238–244

SIGN 107: Diagnosis and management of headache in adults
Published November 2008; Scottish Intercollegiate Guidelines Network

9.3 Recommendations

None published

10 Links

10.1 Related analytes

10.2 Related tests

CSF ferritin has been suggested as an alternative test to CSF bilirubin, particularly for late-presenting patients. However, there are doubts as to the adequacy of its specificity for routine use.

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