

Spectrophotometry Of Cerebrospinal Fluid (CSF)

Test: For investigation of suspected intracranial bleeding, particularly subarachnoid haemorrhage (SAH).

CT scan usually confirms a bleed in the acute stage (90% sensitivity), but detection by CT scan decreases with time, from 50% at one week to almost nil at 3 weeks. LP and spectrophotometry is the most sensitive procedure for patients who present late (1-2 weeks post-bleed).

CSF is scanned for pigments spectrophotometrically between wavelengths of 350 and 650nm. If present, the characteristic spectra of oxyhaemoglobin, bilirubin or methaemoglobin can be demonstrated. **Bilirubin is the key pigment** in SAH and can be quantified using the spectrophotometric data (preferred method).

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Timing of lumbar puncture - CSF collection

- It is advisable to delay lumbar puncture to allow bilirubin formation (at least 12 hours post-headache).
- Bilirubin may then persist for 2-4 weeks or longer (CT scan is usually negative at 3 weeks post-bleed).

Pigments in the CSF and calculating bilirubin

CSF pigments (absorption maxima):

Oxyhaemoglobin: 416, 540, 578 nm.

Bilirubin: either a broad peak in the range of 450-460 or a shoulder adjacent to oxyhaemoglobin.

Methaemoglobin: absorption maxima 412, 540, 578nm. The latter two peaks have characteristically different shapes from the corresponding peaks of oxyhaemoglobin.

Calculations of bilirubin: Draw predicted baseline from about 360nm to a point where this will form a tangent to the scan between 440-530nm and then measure the absorbance of the scan above this baseline at 476nm to yield net bilirubin absorbance (see typical examples referred to the section on reporting results).

The concentration of CSF bilirubin (if present) is given by:-

$$(A_{475} - A \text{ at baseline drawn above}) \times 23 = (\text{bilirubin}) \mu\text{mol per litre}$$

Net bilirubin absorbance greater than 0.007 is considered abnormal and therefore should be calculated using the above formula. In case of oxyhaemoglobin, absorbance values greater than 0.02 is considered reportable.

Other sources of pigments

NOTES:

The present of bilirubin in the CSF can occur in several ways: -

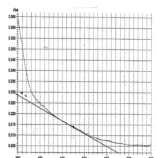
- Contamination by the entry of blood, pathological or accidental ('traumatic tap').
- Leakage of serum protein by transudation through the blood-CSF barrier.
- Hyperbilirubinaemia, serum levels exceeding 20 μmol per litre.
- Conversion of oxyhaemoglobin to bilirubin, following a bleed into the intracranial space.

Methaemoglobin is rarely seen, but is said to be characteristic of encapsulated subdural haematomas and old loculated intracerebral haemorrhages. It may be found in the CSF if the encapsulations are large enough.

In cases of suspected intracranial bleeding, typical examples of spectrophotometric scans seen are given below:-

Typical scans and reporting of Results

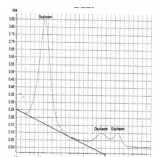
'No pigments detected'.



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"No evidence of SAH"

'A spectrum of oxyhaemoglobin. No bilirubin detected.'

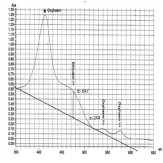


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"Oxyhaemoglobin can mask bilirubin presence therefore SAH cannot be excluded"

'A mixed spectrum of oxyhaemoglobin and bilirubin.'



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"Bilirubin present. Supports a diagnosis of previous bleeding into the CSF"

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