

Division of Laboratory Medicine

Biochemistry

Bilirubin (conjugated/direct)

General Information

Note this test is **NOT** included in the standard liver function profile.

Collection container:

Adults - serum (with gel separator, 4.9mL Sarstedt brown top).
 Paediatrics - lithium heparin plasma (1.2mL Sarstedt orange top tube)
 Neonates – capillary samples are acceptable

Type and volume of sample:

Serum or lithium heparin plasma, minimum 1 ml whole blood required (200 µl separated serum/plasma).

Specimen Transport/Special Precautions:

No special precautions required.

Laboratory Information

Method principle:

Vanadate oxidation method (Wako). In the presence of a detergent, conjugated bilirubin is oxidised to biliverdin by vanadate. The absorbance at 450 nm decreases and is inversely proportional to the concentration of conjugated bilirubin in the sample.

Biological reference range or cut off:

Neonates	1-13 µmol/L
Older children (up to 18 years)	1-8 µmol/L
Adult	<5 µmol/L

A 'normal' level in a very young baby could be an artefact of poor conjugating ability and be falsely reassuring. Please repeat when >10 days old (>14d if preterm).

Turnaround times:

Results are available within 4 hours (routine) or 2 hours (urgent - phone lab in advance of sampling).

Clinical Information

This assay measures the conjugated/direct fraction of bilirubin only and should be requested when the cause of jaundice or a raised total bilirubin is unknown.

Bilirubin is the breakdown product of the haem component of haemoglobin (and other haem-containing proteins). It undergoes conjugation with glucuronic acid in the liver in order to increase its solubility and allow excretion from the body via the biliary system. Conjugated hyperbilirubinaemia (typically defined as

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>20 $\mu\text{mol/L}$ or >20% of total bilirubin) occurs when excretion is impaired, typically as a result of cholestasis and/or hepatocellular injury.

Conjugated bilirubin should be measured in any baby who remains jaundiced after two weeks of life (3 weeks for preterm infants). Note that in a very young baby, conjugated bilirubin levels can be falsely normal due to the poor conjugating ability of the immature liver (and therefore falsely reassuring). Measurement should be repeated after 10 days of life (14 days if preterm).

Conjugated hyperbilirubinaemia in neonates is never physiological and always requires further investigation. Refer to British Society of Paediatric Gastroenterology, Hepatology and Nutrition (BSPGHAN) guideline "Investigation of Neonatal Conjugated Hyperbilirubinaemia", as well as MFT local guideline available on the intranet.

Factors known to significantly affect the results:

Bilirubin is unstable when not protected from light. Add-on requests cannot therefore be accepted if the sample is older than 12 hours.

Clinical decision points:

For neonates, BSPGHAN recommends:

- If conjugated bilirubin level is > 25 $\mu\text{mol/L}$ and/or >25% of the total bilirubin, the infant should be investigated promptly for possible underlying liver disease.

References:

- 1) Investigation of Neonatal Conjugated Hyperbilirubinaemia. BSPGHAN (updated Dec 2016)
- 2) Investigation of Neonatal Conjugated Hyperbilirubinaemia. MFT local guideline (Version 3, Dec 2018)
- 3) Marshall, W. Bilirubin. Association of Clinical Biochemistry: Analyte Monographs alongside the National Laboratory Medicine Catalogue (Aug 2012)

(Last updated November 2019)