

# Chemistry Case Study: Conjugated Bilirubin in Neonatal Jaundice

## Case History

Patient was a 1-week-old infant in the level 2 NICU born at 37 weeks. This infant was initially born with indirect hyperbilirubinemia but now also has increasingly elevated level of direct bilirubin (see measurements in table below). Neonatologist requested conjugated and unconjugated bilirubin test due to increasing elevated level of direct bilirubin. Conjugated bilirubin test is not routinely performed in our hospital laboratory and needs to be send out.

Question: What's the difference between conjugated bilirubin and direct bilirubin? When does conjugated bilirubin need to be assessed?

|                            | Ref Range       | 3/6/18 | 3/7/18 | 3/9/18 | 3/10/18 | 3/12/18 |
|----------------------------|-----------------|--------|--------|--------|---------|---------|
| Bilirubin total, neonatal  | 1.0-10.5 mg/dL  | 9.2    | 8.7    | 10.8   | 10.2    | 8.6     |
| Bilirubin direct, neonatal | 0.0 – 0.6 mg/dL | 0.5    | 0.7    | 1.8    | 1.8     | 2.1     |

## Discussion

Neonatal jaundice is commonly seen in newborns in the first few days of life, mainly due to increased bilirubin formation from break down of red blood cells and limited conjugation of bilirubin. Total bilirubin normally peaks at day 2-3 and should decline by day 4-5. Sample is collected via heelstick in green top tube and protected from light. Measurement of total bilirubin is interpreted based on the Bhutani Nomogram to assess risk of hyperbilirubinemia. Most often, unconjugated bilirubin is elevated in neonatal jaundice owing to hemolytic causes. In cases with prolonged jaundice, conjugated bilirubin needs to be determined to rule out cholestasis.

Conjugated bilirubin refers to bilirubin conjugated with one or two glucuronic acid, and this term “conjugated bilirubin” is often used interchangeably with direct bilirubin. Direct

bilirubin refers to bilirubin fractions that can directly react with diazo reagent without the addition of accelerator, such as methanol or ethanol. This fraction usually includes conjugated bilirubin and delta bilirubin. Delta bilirubin is formed by covalent bonding between conjugated bilirubin and albumin, and has a similar half-life as albumin, 21 days. Therefore, direct bilirubin measurement overestimate conjugated bilirubin and in cases with persist or atypical jaundice, clear differentiation between conjugated and direct bilirubin is important. Clinician should know what the laboratory is measuring when interpreting the bilirubin fraction results.

In laboratories, conjugated bilirubin can be assessed by the VITROS BuBc dry slide, which simultaneously measures unconjugated (Bu) and conjugated (Bc) bilirubin by use of a mordant. In the presence of the mordant, the visible spectra of conjugated and unconjugated bilirubin are different, allowing measurement of both species from a single slide. Fractions of bilirubin can also be separated by HPLC, but this is not practical to use in a routine clinical laboratory. In this case, conjugated bilirubin was measured by VITROS BuBc slide test, and result came back elevated at 1.0 mg/dL (ref range: < 0.3 mg/dL).



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Reference - <https://labmedicineblog.com/category/chemistry/page/3/>