

## Unbound Bilirubin determination in newborns : development of an automated assay on the DxC 800 of Beckman-Coulter.

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The unbound bilirubin (UBB) concentration is probably the most critical parameter in establishing the risk for bilirubin encephalopathy in neonates. This parameter takes in consideration 3 biological risk factors for kernicterus in newborns: hyperbilirubinaemia, hypoalbuminaemia and competitors of the bilirubin-albumin bond. It thereby allows to identify risk situations of bilirubin encephalopathy which cannot be detected with individual testing of either bilirubin and albumin. In our laboratory, the UBB analysis has been routinely performed since 1987 on a dedicated instrument, the UB Analyser (Arrows, Co, Ltd.Osaka, Japan, non-automated assay) with peroxidase method. The principle of this assay is a rapid deterioration of the UBB into a leuco-derived compound by the action of a peroxidase in the presence of hydrogen peroxide. The UBB concentration is calculated from the oxidation kinetics. The aim of our work is the transfer of the UBB assay to open biochemistry systems: the DxC 800 and CX4-CE of Beckman-Coulter. Validation of this transfer was conducted by reducing the volumes of reaction (6 µl of plasma instead of 25 µl, 200 µl of phosphate buffer instead of 1 ml, 6 µl of peroxydase instead of 25 µl). We have chosen a Cinet1 decreasing mode as reaction type with a primary wavelength at 470 nm and a secondary wavelength at 650 nm. We have adjusted the parameters of reading in order to calculate the initial kinetic of the reaction: incubation time of buffer with sample =48sec, blank reading =40sec, first reading after peroxydase addition = 10sec and reading time =30sec. Calibration has been done with two points (first point : distilled water, second point : UBB Calibrator prepared in our laboratory with a Target value for UBB: 0.70 µg/dL or Bilirubin/Albumin = 0,75). We have verified analytical performances of this test (Within run imprecision: CV of 2.78% for low level, 1.90 % for high level, between run imprecision: CV of 3.19% for low level, 2.15% for high level). Inter-instrument correlation has been made with a hundred clinical samples (CX4/UB Analyzer,  $r_2 = 0.92$ , linear regression,  $y = 0.98 x + 0,001$ ) (CX4/DxC:  $r_2 = 0.98$ , linear regression  $y=1.071x+0,015$ ). Beyond the interest of having an automated assay for UBB dosage (lower sample volumes, better reliability of assays, data export...), this work may contribute to larger diffusion of UBB determination among laboratories equipped with DxC800 or CX4-CE. Diffusion of this method would be of great help for pediatricians in order to assess severity of jaundice especially in newborns who have risk factors for bilirubin toxicity (hemolysis, acidosis, dehydration and prematurity).