Introduction

In 2012 the Roche Direct Bilirubin (diazoe dye-based) assay (BILD2) was introduced on the Cobas c501 and c702 platforms. Our Laboratory subsequently received many complaints from the Neonatal and Paediatrics Specialists across the area due to poor performance of this method by interference from haemolysis above 10 mg/dL. This Roche method BILD2 was later revised in June 2012 to have no significant interference up to an H Index of 25 mg/dL (15.5 umol/L). This minor increase in the H Index allowed only a small number of additional Direct Bilirubin results to be released. We still found that 81% of the Direct Bilirubin results from neonatal patients were being withheld due to the H Index and reported as ‘haemolysed’. Of course was due to the collection procedure – blood drawn from a heel prick is usually haemolysed and above the H Index of 25 mg/dL. New automated methods brought into the laboratory do not necessarily provide improved performance. A major problem with diazo dye-based bilirubin assays is haemoglobin interference. Because of this the laboratory has assessed and introduced an alternative oxidation method manufactured by Randox which claims not to suffer from interference up to a haemoglobin concentration of 5.0 g/L or 310 umol/L.

Method

The Randox Direct Bilirubin Vanadate Oxidation Method (RDBIL) - Cat No. BR9765 was evaluated at 2 Laboratory sites, on the Cobas c501 and Cobas c702 analysers in parallel with the current Roche Direct Bilirubin (BILD2) Method - Cat No.05168384190. Patient correlations without any significant haemolysis were analysed by both methods to see if there was a method difference. We also tested 12 levels of haemolysis (Haem Index range 0–750 mg/dL) at 6 levels of conjugated bilirubin range 5.8–106 umol/L for each method on both Cobas analysers. Interference was judged to be unacceptable if the direct bilirubin recovery was less than 80% of the unhaemolysed specimen at that bilirubin concentration. The data was correlated using Passing-Bablock linear regression analysis.

Results

Good correlation between the Roche BILD2 and Randox RDBIL methods was shown for patient samples using the same Calibrators (Lot No 163 945-01) for both methods. Linear regression analysis revealed the following equation for the Direct Bilirubin range 2–350 umol/L on the Cobas c702 as: RDBIL = 0.92*BILD2 –0.16; R2 = 0.99 and RDBIL = 1.00*BILD2 – 1.0 on the Cobas c501 for range 2 – 90 umol/L (see Fig 1 and 2). For the haemolysis studies we considered the point of interference to be the concentration of haemoglobin that causes results to deviate by greater than 10% from the target. The target is the value obtained when no haemolysis is present. For Direct Bilirubin values ≤15 umol/L we used the RCPA-AACB Allowable Limits of Performance (ALP) ± 3 umol/L to determine the haemolysis interference cut-off levels.

![Figure 1: Scatter Plot with Passing & Bablok Fit for Direct Bilirubin Methods: Randox RDBIL vs Roche BILD2. Routine patient samples March 2013.](image1)

![Figure 2: Scatter Plot with Passing & Bablok Fit for Direct Bilirubin Methods: Randox RDBIL vs Roche BILD2. Routine patient samples March 2013.](image2)

![Figure 4: Showing POOL 3 with DBIL = 20.9 umol/L and %Recovery with increasing Haemolysis Index (umol/L).](image4)

Conclusions

We have developed an alternative method for Direct Bilirubin using the Randox Vanadate Oxidation method. This Randox method was implemented simultaneously across five laboratories, on the Cobas c501 and c702 platforms in May 2013 with a good interaction and favourable responses from Neonatologists and Paediatricians; 97.6% of Direct Bilirubin results are now being released. Oxidation methods such as the Randox Vanadate Oxidation Direct Bilirubin method have been demonstrated not to suffer from interference up to haemoglobin concentrations of 500 mg/dL (5 g/L) or 310 umol/L. The Liverpool Clinical Chemistry Laboratory has performed in-house studies to confirm this. Laboratory assessment of this Randox Vanadate method has shown to be 20 fold better than the current Roche method. Direct Bilirubin concentration levels were studied from 5 to 106 umol/L at Haemolysis Indices of 0 to 750 mg/dL (7.5 g/L or 465 umol/L). In conclusion we have found the Randox Vanadate Oxidation Direct Bilirubin method to be acceptable for Haemoglobin Interference up to 7.5 g/L or 465 umol/L.