

# INTERFERENCE VERIFICATION OF THREE CREATININE METHODS



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## Introduction

Creatinine method standardisation and its impact on eGFR estimation has been a topic of extensive discussion in the clinical literature, but non-specificity of creatinine measurements attracts somewhat less attention and awareness by clinical end-users of results. Interferences with creatinine measurements may result in inaccurate estimations of the glomerular filtration rate, and thus potential mismanagement particularly in children and neonates. Therefore we investigated the impact of common interferences on three creatinine methods in order to design the best autovalidation rules and diagnostic algorithms for assessing renal function in our adult and paediatric patient population.

## Study Aims

- To verify the Haemolysis (H), Icterus (I) and Lipaemia (L) indices of the Roche Cobas 6000 analyser for the Roche compensated, rate-blanked kinetic Jaffe and enzymatic creatinine methods and to compare our findings to the manufacturer's recommendations.
- To investigate how both Roche creatinine methods compare to the Radiometer ABL 827 enzymatic creatinine method in the presence of various interferences.

## Methods

Pooled serum samples at three creatinine concentrations representative of different age groups and renal status were spiked with HbA, HbF, conjugated and unconjugated bilirubin and Intralipid®. Samples were measured in duplicates with Roche's compensated, rate-blanked, kinetic Jaffe and enzymatic creatinine methods and on Radiometer's ABL827 analyser which also uses an enzymatic creatinine method. Results for the ABL827 were corrected for serum samples using a correction formula ( $cCrea_{[corrected\ for\ plasma/serum]} \mu mol/L = 0.950 \times cCrea_{[whole\ blood]} - 0.4$ ). Absolute and relative differences between spiked and unspiked specimens were compared to interference indices recommended by the manufacturer. Our interference criterion was  $\pm 8\%$  recovery of unspiked samples (see dotted lines on Figures), based on the allowable limits of performance set by the external quality assurance program of the Royal College of Pathologists of Australasia.

For spiking with HbA and HbF, K-EDTA anticoagulated adult and cord blood (with measured HbF proportion of 82.8%) were used when preparing the haemolysates. For mimicking unconjugated hyperbilirubinaemia, bilirubin powder was dissolved to give a final stock concentration of 10mmol/L; for conjugated hyperbilirubinaemia, ditaurobilirubin powder was dissolved to a final stock concentration of 10mmol/L. Intralipid® was used in the lipaemia interference study.

## Results

### Compensated rate-blanked kinetic Jaffe method (Roche)

#### Haemolysis:

Roche recommends a single haemolysis index (HI) cut-off of 1000<sup>1</sup> (i.e. 10g/L Haemoglobin A). Our study shows that HbA positively interferes with the assay to differing degrees, depending on the actual creatinine concentration of the sample (Figure 1a). When creatinine is <100  $\mu mol/L$ , the HbA interference criterion (i.e.  $\pm 8\%$ ) is met at lower indices at around 500. In agreement with findings in the literature<sup>2</sup>, the assay seriously underestimates creatinine in the presence of HbF, thus is unsuitable for neonatal specimens (Figure 2a).

#### Lipaemia:

No significant interference was observed up to a LI of 1125 (Figure 3).

#### Icterus:

Unconjugated bilirubin (e.g. in neonatal specimens) has a clinically non-significant interference with the method up to 700  $\mu mol/L$  (Figure 4). The Roche unconjugated I index for the method is 171. This index can be increased to 700. This is confirmed by Owen and Keevil when creatinine was measured by LC-MS/MS as the reference method<sup>3</sup>. Conjugated bilirubin was found to interfere with the assay at 50  $\mu mol/L$  (Figure 5), but the Roche index reading program cannot distinguish between the different types of bilirubin so the lowest value needs to be used for autovalidation rules. This would, however, invalidate the release of the majority of neonatal creatinine results unnecessarily, unless specific reflex testing and manual validation protocols overrule it.

### Enzymatic Creatinine method (Roche)

#### Haemolysis:

For the method Roche recommends a HI cut-off of 800<sup>1</sup> (i.e. 8g/L Haemoglobin A) which has been confirmed in our experiments (Figure 1b). This assay also underestimates creatinine in the presence of HbF, particularly at lower creatinine concentrations in the range of 40-50  $\mu mol/L$  (Figure 2b). Therefore the Roche enzymatic creatinine method is not the best alternative for haemolysed neonatal specimens, but performs better in children and adults than the Jaffe test even if there is some degree of haemolysis<sup>3,4</sup>.

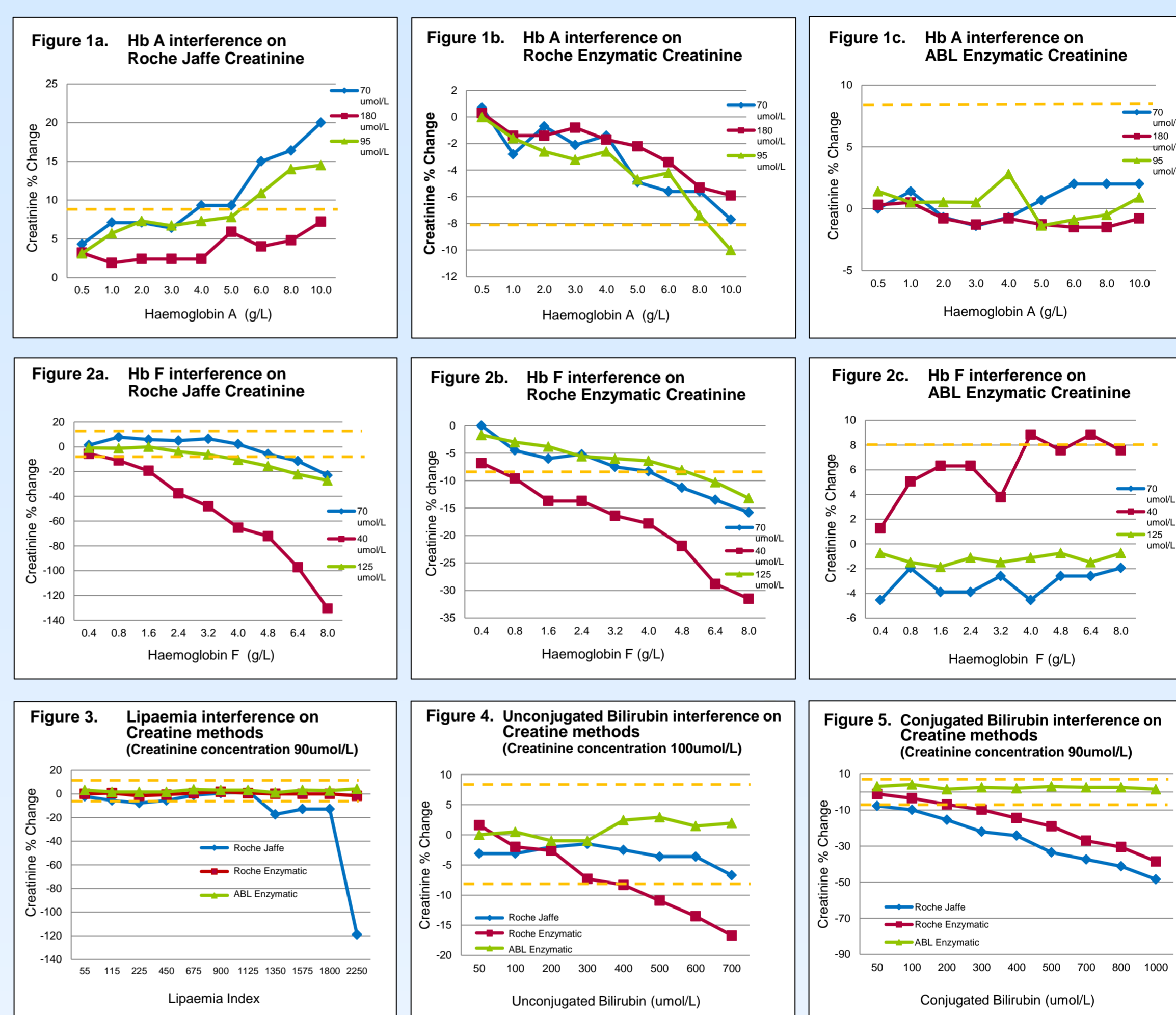
**Lipaemia:** does not interfere with the enzymatic creatinine method (Figure 3).

**Icterus:** Unconjugated bilirubin has clinically significant interference with the method. The Roche I rule of 428 results in an approx. 10% underestimation of creatinine, which exceeds our criterion of  $\pm 8\%$  (Figure 4). This interference is confirmed in the literature and may be attributed to the consumption of peroxide in the initial reaction mixture as suggested by others<sup>3</sup>. Conjugated bilirubin also interfered with the assay at 200  $\mu mol/L$  (Roche recommendation is 257  $\mu mol/L$ ) (Figure 5).

### ABL Enzymatic creatinine method (Radiometer)

The ABL enzymatic creatinine method is not affected by adult or foetal Haemoglobin, lipaemia or any forms of bilirubin (Figures 1c, 2c, 3-5).

## Interference Studies



## Conclusions

- Haemolysis interferes with the Roche creatinine assays to differing degrees, depending on the creatinine concentration of the sample. The direction and magnitude of interference is also different for HbA and HbF. This means that one single HI cut-off cannot be automatically applied to all types of specimens and various patient populations.
- Conjugated and unconjugated bilirubin interfere with the assays to different degrees. Roche instrumentation does not have the capability of distinguishing between the two and so the lowest cut off can only be used by the laboratory's autovalidation system which could unnecessarily invalidate or delay the release of results.
- Our results highlight the importance of appropriate interference and index verification studies across several concentration ranges of both the analyte and interfering substance in question, and in different patient populations<sup>4</sup>.
- The Roche Enzymatic assay underestimates creatinine in the presence of HbF, particularly at low creatinine concentrations, and thus it is not the best alternative for haemolysed neonatal specimens.
- The Radiometer ABL827 enzymatic creatinine method is not affected by these common interfering substances, and thus the ABL blood gas machine seems to be the best alternative at any level of haemolysis, icterus or lipaemia for measuring creatinine in adult or paediatric and neonatal samples. The day-to-day imprecision of the instrument is excellent even at low creatinine concentrations which makes it suitable for handling any types of specimens. The only disadvantage in paediatric patient population can be the additional sample volume that the instrument requires as well as the additional processing time and the relatively lower through-put of the machine in routine application.

## References

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