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Does the Performance of Routine Bile Acid Methods Meet the Requirement for Risk Management of Intrahepatic Cholestasis of Pregnancy?

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Introduction

Diagnosis and management of intrahepatic cholestasis of pregnancy (ICP), indicating a risk of stillbirth in pregnant women, relies in part on measured levels of circulating total bile acids (TBA). Guidelines issued by the Royal College of Obstetrics and Gynaecologists (RCOG) in the UK indicate a risk-based approach for grading based on the presence of itching (pruritus) and defined peak TBA measurement ranges (19–39 µmol/L [mild ICP], 40–99 µmol/L [moderate ICP], 100 µmol/L or more [severe ICP]).

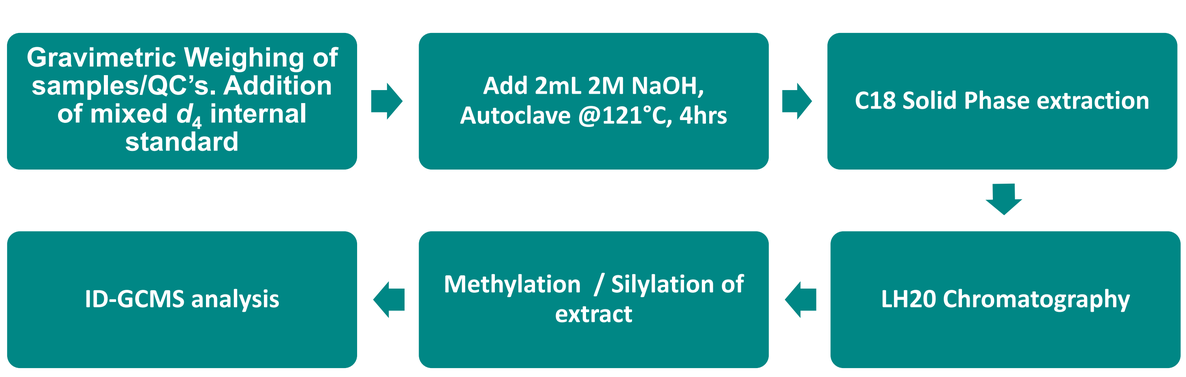
TBA are routinely measured by non-specific enzymatic methods resulting in measurement differences between methods. The most common methods use 3α-hydroxysteroid dehydrogenase to convert bile acids to 3-ketosteroids, with monitoring of the formation of NADH. An ID-GCMS method for bile acids previously developed has been used to compare participant returns for TBA within the Weqas EQA programme.

Method

The preferred comparison method of returned EQA results is to the SI unit utilising a reference target, ensuring the transfer of accuracy from higher order reference measurement methods to routine methods. The previously published ID-GCMS method[1] has been redeveloped using an alkaline hydrolysis stage at high temperature using an autoclave[2] . The method provides a traceable value for each of the main bile acids (cholic acid, chenodeoxycholic acid and deoxycholic acid) with a TBA value represented as the sum of these three.

Linear serum pools containing cholic acid and deoxycholic acid, reflecting levels observed in ICP were distributed to participants. The ID-GCMS method for bile acids within the Weqas Reference Measurement laboratory was used to compare the returned data for TBA within the Weqas EQA programme.

Figure 1 ID-GCMS Method Flow Diagram



Bile acids were measured in all samples using exact matching isotope dilution according to the method detailed in figure 1. Quantitation involved bracketed standard curves using the purest available bile acids (table 1).

Table 1 ID-GCMS Bile Acid Traceability

Measurand	Purity of standard	Control Material
Chenodeoxycholic Acid	Sigma (98%)	**In House:** Gravimetric material prepared from charcoal stripped serum (none available commercially)
Deoxycholic Acid	Sigma (99%)	
Cholic Acid	Sigma (99%)	

Results

Observation of EQA data, when compared to the ID-GCMS method, showed reasonable agreement between the enzyme-formazan Sentinel methods and the thio-NADH methods for both Sentinel and Randox. Between the various other thio-NADH methods, there is a variation of bias across the different manufacturer methods, from 15-20% across the measurement range. Where bias was observed, this is more evident at the cut point level between mild and moderate ICP. The spread of this data could indicate calibration issues across the different methods. Results from samples distributed on multiple occasions also showed some poor within method precision.

Figure 2 shows the relative participant numbers for each of the method groups, where users within the UK favour the Sentinel Enz-Formazan and ENZ-ThioNADH methods and the Randox Enz-Thio-NADH methods. Target values were assigned to the EQA material utilising the ID-GCMS method. Deviations from the ID-GCMS result for main analytical groups were plotted in the form of a correlation for the main method groups (figure 3) and bias plots (Bland–Altman plots, figure 4).

Figure 2 Total Bile Acid Method groups

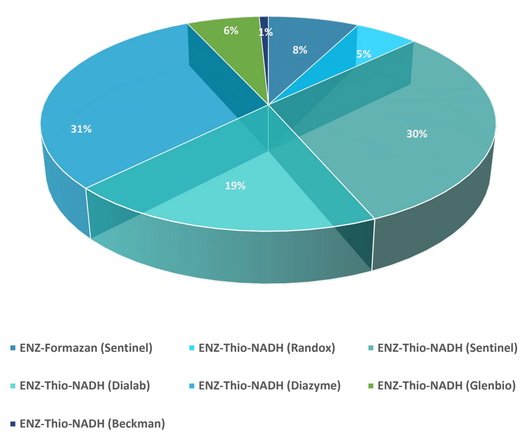


Figure 3 Total Bile Acid Method group correlation with ID-GCMS values

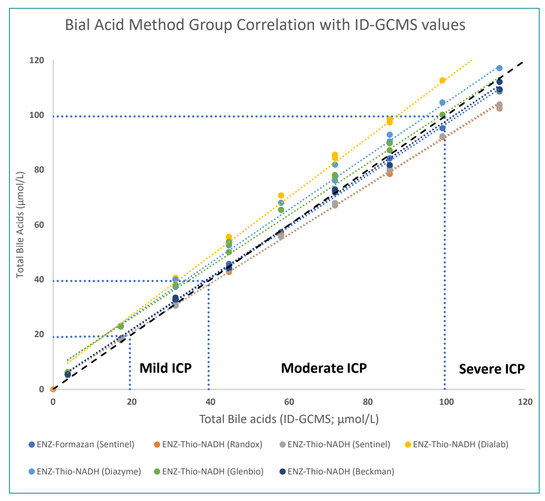
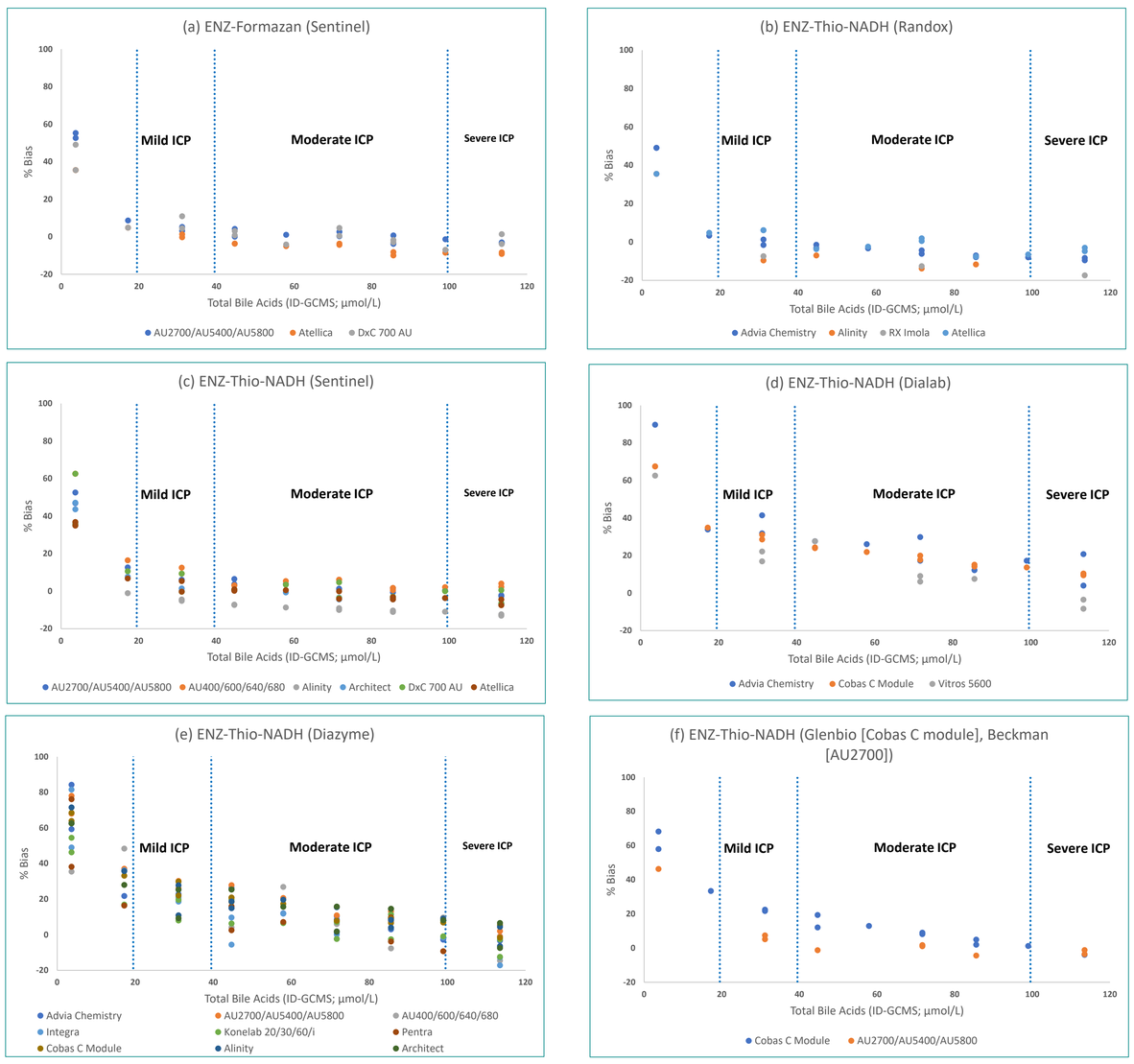


Figure 4 Bland-Altman Plots for Total Bile Acid Method groups



Discussion

The ID-GCMS target measurement values have been used to assess the performance of total bile acid methods within the Weqas EQA programme. Comparing all of the current methods, proportional errors between 2.5 and -17% and constant errors between 3-5 µmol/L (figure 3) were observed. This spread of data would influence the final risk classification based on the RCOG guidelines.

Within each method group, variation can also be observed depending on the instrument used for the method, as seen in the Bland-Altman plots. For the Enz formazan Sentinel methods (figure 4a), little difference across the instruments was observed with reasonable agreement at concentrations above 17 µmol/L. For the Randox Enz-Thio-NADH method group (figure 4b), a small negative bias was observed for the Alinity and RX Imola. The Advia Chemistry and Atellica instruments show good agreement for this method. Within the Sentinel Enz-Thio-NADH method group (figure 4c), the Alinity data was in good agreement with the ID-GCMS target value at 17 µmol/L but trended towards negative values above this level. Data for the other instruments in this method group showed a similar pattern but with a slight positive bias. The Dialab Enz-Thio-NADH instruments (figure 4d) generally had a positive bias across the measurement range observed. This was more pronounced in the Advia Chemistry and Cobas C Module. Again, a trend towards a positive bias at lower concentrations of TBA is observed in the Diazyme method group (figure 4e) with variation across the instrument groups. The Glenbio Enz-Thio-NADH method on the Cobas C Module (figure 4f) showed a marked positive bias at lower concentrations, whereas the Beckman Enz-Thio-NADH method showed good agreement across the range

Conclusion

The various TBA methods show a range of bias values both within each of the method groups and across the various instrument platforms, spanning the measurement range. Assignment of risk of the pregnancy outcome, as defined by the RCOG guidelines, is based on TBA values. There is therefore a potential for misclassification of risk dependent on the TBA method or instrument platform used.

References
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[2]Yamaga, N *et al*., An Examination of Alkaline Hydrolysing Conditions of Conjugated Bile Acids with Carbonyl Groups Yonago Acta Medica 1997; 40:73–77