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Performance of serum total bile acid methods in comparison to ID-GCMS measurement target values

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Introduction

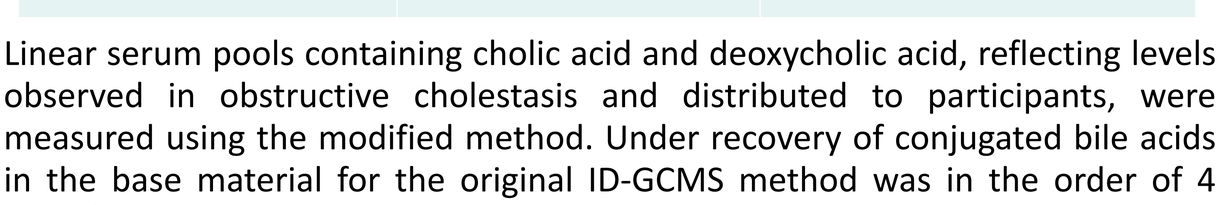
Total bile acids are routinely measured by non-specific enzymatic methods resulting in measurement differences between methods. The most common methods use 3α-hydroxysteroid dehydrogenase to convert bile acids to 3-ketosteroids, with monitoring of the formation of NADH. The overall mean is an inappropriate target value for this analyte, being greatly influenced by the predominant method (Thio-NADH). The preferred comparison method of returned EQA results is to the SI unit utilising a reference target, ensuring the transfer of accuracy from gold standard methods to routine methods.

An ID-GCMS method for bile acids previously developed has been used to compare participant returns for total bile acids within the Weqas EQA programme. The method provides a traceable value for each of the main bile acids (cholic acid, chenodeoxycholic acid and deoxycholic acid) with a total bile acid value represented as the sum of these three. This original method was found to underestimate the amount of conjugated bile acids in the base material and the method was improved and used to compare returned EQA returns for total bile acids.

Methods

The previously published method[1] used a hydrolysis step with sodium hydroxide at room temperature to release conjugated bile acids. This hydrolysis step was found to be inefficient and not all conjugated bile acids were released. The method was therefore redeveloped using an alkaline hydrolysis stage at high temperature using an autoclave[2].

Figure 1 Method Flow Diagram



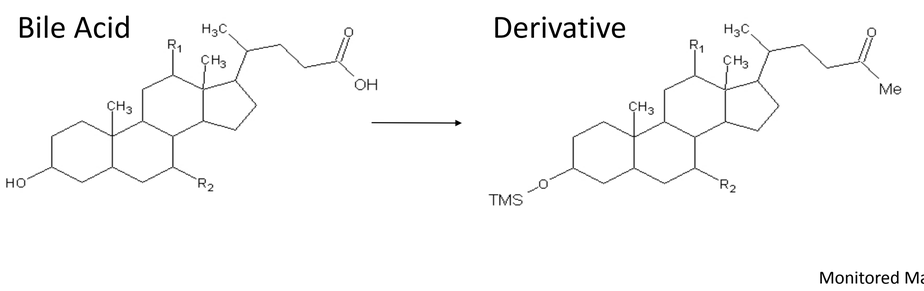
Bile acids were measured in all samples using exact matching isotope dilution according to the method detailed in figure 1. Quantitation involved bracketed standard curves using the purest available bile acids (table 1). The masses monitored are detailed in figure 2.

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%)	

Linear serum pools containing cholic acid and deoxycholic acid, reflecting levels observed in obstructive cholestasis and distributed to participants, were measured using the modified method. Under recovery of conjugated bile acids in the base material for the original ID-GCMS method was in the order of 4 µmol/L when compared to the modified method.

Figure 2 Methyltrimethylsilyl Ether Derivatives (Me TMS)



			Monitored Mass (m/z)
Cholic Acid:	R1 = OH, R2 = OH	R1 = O-TMS, R2 = O-TMS	623/627 (M-15)
Chenodeoxycholic Acid:	R1 = H, R2 = OH	R1 = H, R2 = O-TMS	370/372 (fragment ion)
Deoxycholic Acid:	R1 = OH, R2 = H	R1 = O-TMS, R2 = H	535/539 (M-15)

Target values were assigned to the EQA material utilising the previously published ID-GCMS method, with the addition of a heat treatment stage to release conjugated bile acids in the serum. Figure 3 shows the relative participant numbers for each of the method groups. Deviations from the ID-GCMS result for main analytical groups were plotted in the form of bias plots (Bland–Altman plots, figure 4).

Figure 3 Total Bile Acid Method groups

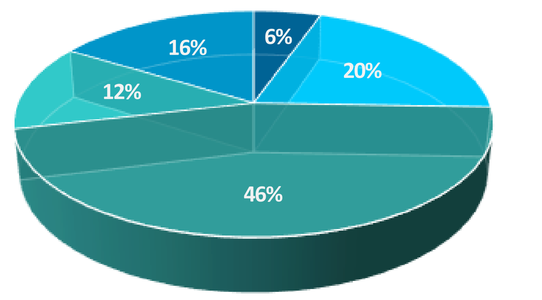
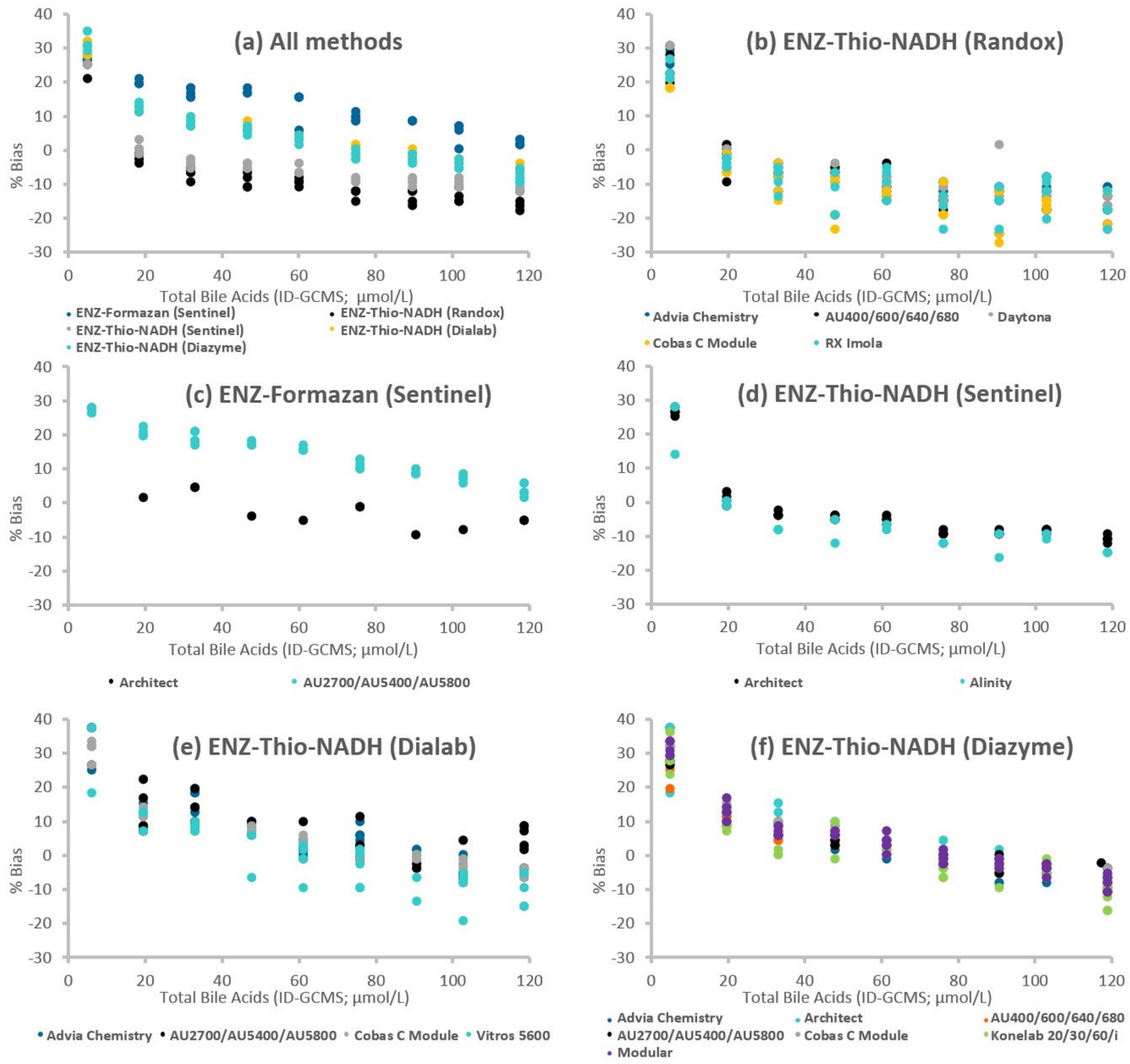


Figure 4 Bias Plots (a) All Methods; (b) ENZ-Thio-NADH (Randox); (c) ENZ-Formazan (Sentinel); (d) ENZ-Thio-NADH (Sentinel); (e) ENZ-Thio-NADH (Dialab); (f) ENZ-Thio-NADH (Diazyme).



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 4) were observed. Here the spread of data could indicate calibration issues across the different methods.

A marked difference was observed between the enzyme-formazan (Sentinel) methods and the thio-NADH methods, with the former showing a positive bias across the measurement range. The Architect enzyme-formazan (Sentinel) method does show better agreement with the ID-GCMS values, with the AU methods showing a positive bias. Between the various thio-NADH methods, there is a variation of bias across the different manufacturer methods. This method variation ranges from 15-20% across the measurement range. Observation of the Dialab method again showed a spread of data between the various manufacturers. The predominant Diazyme method shows relatively good agreement, in particular at higher concentrations. Results from samples distributed on multiple occasions also showed some poor within method precision.

Conclusions

The use of ID-GCMS target measurement results provides a common comparison for returned results in EQA schemes, highlighting any method differences. This can then aid in between method harmonisation of results and traceability of routine methods observed for total bile acids and highlight any calibration issues. The various total bile acid methods show a range of values both within each of the method groups and across the various instrument platforms.

References

[1]Ducroq, DH *et al*., Analysis of serum bile acids by isotope dilution-mass spectrometry to assess the performance of routine total bile acid methods Annal Clin Biochem 2010, 47: 535-540

[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