Weqas
Unit 6, Parc Tŷ Glas
Llanishen, Cardiff, CF14 5DU

Tel: 02920 314750
Email: contact@weqas.com

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EXTERNAL QUALITY ASSESSMENT

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REFERENCE MEASUREMENT SERVICES

EDUCATION & TRAINING

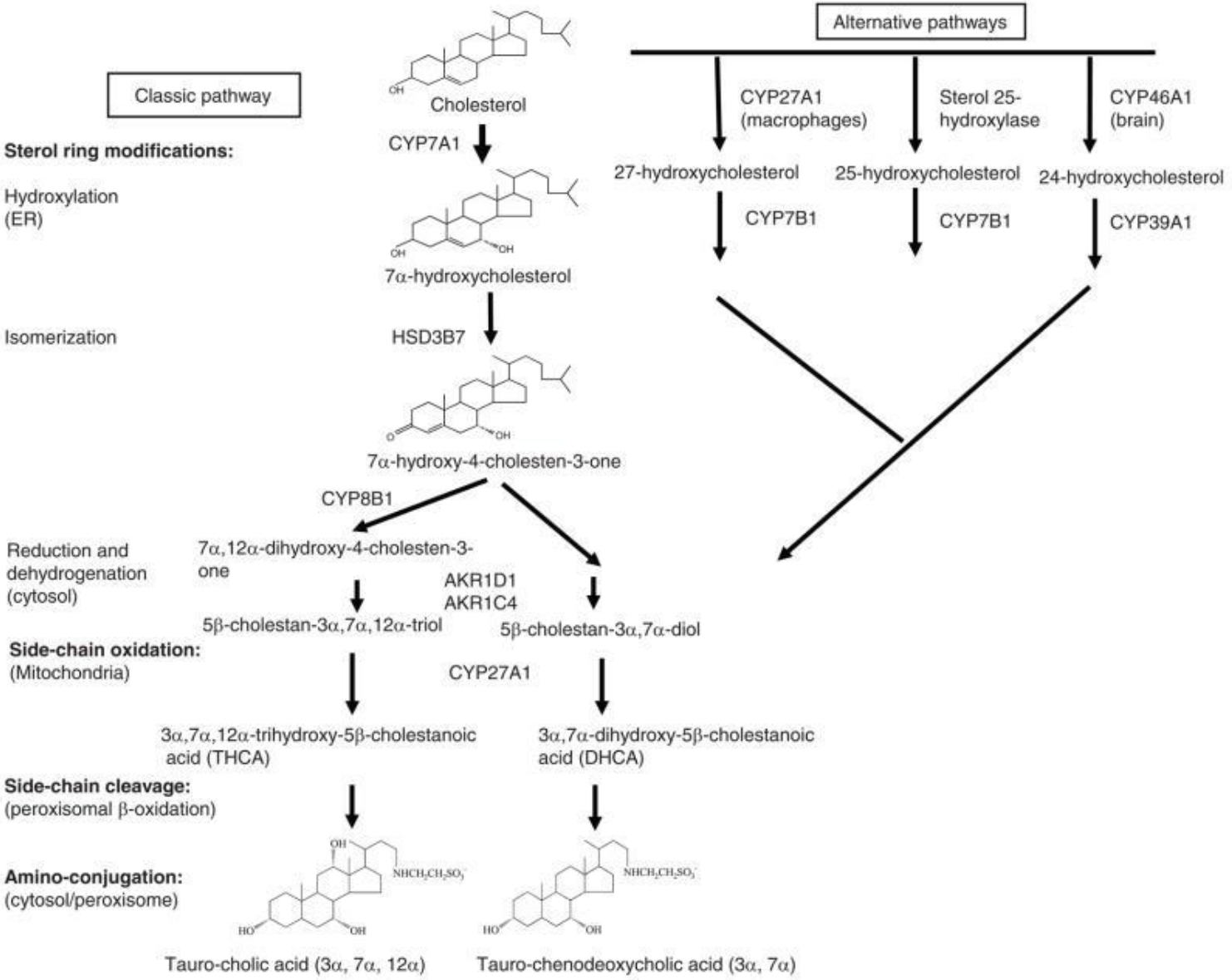
Bile acid performance - is it good enough to use global targets?

David Ducroq
Head of Reference Measurement Laboratory
Weqas

Overview of Presentation

- Biochemistry of Bile acids, what are they?
- Brief overview of Intrahepatic Cholestasis of Pregnancy (ICP)
- Guidelines for grading ICP
- EQA and Target assignment: ID-GCMS method description
- Current Performance of Bile Acid methods – EQA data
- Summary

Bile acids – What are they?



Bile acids – What do they do?

- Traditionally considered as digestive components helping in the emulsion and absorption of dietary fats and liposoluble lipids.
- Recent findings show participation in energy expenditure, intestinal motility, bacterial growth and inflammation.
- Associated with development of degenerative liver and intestinal diseases, chronic inflammation, gut barrier dysfunctions, **CHOLESTASIS** and colon cancer.

Intrahepatic Cholestasis of Pregnancy

- Intrahepatic cholestasis of pregnancy (ICP) is a potentially serious liver disorder that can develop in pregnancy
- Normally, bile acids flow from your liver to your gut to help you digest food. In ICP, the bile acids do not flow properly and build up in your body instead
- Some studies have found that babies whose mothers have ICP have a higher chance of being born prematurely or stillborn
- If you have ICP, you will probably be advised to give birth in hospital under a consultant-led maternity team
- UK Prevalence:
  - 0.7% pregnancies in multi-ethnic populations
  - 1.2 – 1.5% of women of Indian/Pakistani Asian origin

Intrahepatic Cholestasis of Pregnancy: Symptoms

The main symptom is itching (pruritus), usually without a rash. The itching is often:

- More noticeable on the hands and feet, but can be all over the body
- Worse at night

Other symptoms can include:

- Dark urine
- Pale stool
- Jaundice, but this is less common

Symptoms of ICP are most common in the 3rd trimester, but it is possible to develop the condition earlier.

ICP Investigation

ICP is defined by:

- Gestational pruritus (can occur in isolation in more than 20% of pregnancies)
- Elevated total serum bile acids (TSBA)

Pruritus and TSBA levels should return to normal after birth

Until Recently

- Diagnostic thresholds in National / International Guidelines varied
- No consensus on fasting or postprandial sampling
- RCOG guidelines recommended imaging investigations to exclude other causes

Royal College of Obstetrics and Gynaecologists (RCOG) Guidelines

Published Online 9 August 2022

DOI: 10.1111/1471-0528.17206

BJOG An International Journal of Obstetrics and Gynaecology

RCOG GREEN-TOP GUIDELINES

Intrahepatic cholestasis of pregnancy

Green-top Guideline No. 43 June 2022

Joanna Girling | Caroline L. Knight | Lucy Chappell | on behalf of the Royal College of Obstetricians and Gynaecologists

Society of Obstetric Medicine of Australia and New Zealand (SOMANZ): Executive summary

SOMANZ

SOCIETY OF OBSTETRIC MEDICINE OF AUSTRALIA AND NEW ZEALAND

Intrahepatic Cholestasis of Pregnancy – Diagnosis and Management:

A Consensus Statement of the Society of Obstetric Medicine of Australia and New Zealand (SOMANZ)

Aust N Z J Obstet Gynaecol 2023 Oct;63(5):656-665

Diagnosis and thresholds of TSBA for the diagnosis, classification and management of ICP

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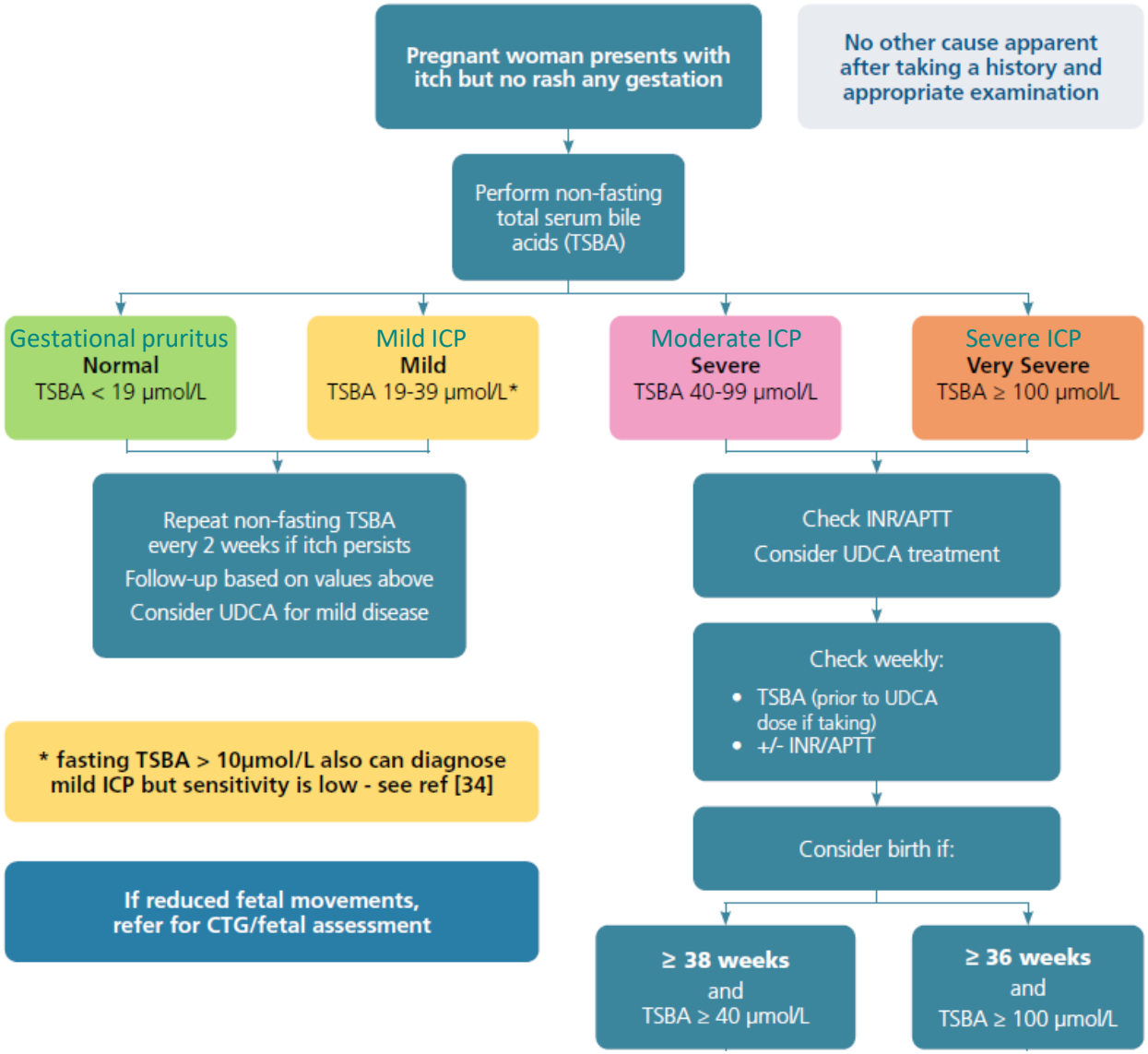
Diagnosis and Classification		Non -fasting TSBA concentration
RCOG	**SOMANZ**	
Gestational pruritus	Normal	Itching and peak bile acid concentrations **<19 µmol/L** *(Threshold is conditional recommendation by SOMANZ)*
Mild ICP	Mild ICP	Itching and raised peak bile acid concentrations **19–39 µmol/L**
Moderate ICP	Severe ICP	Itching and raised peak bile acid concentrations **40–99 µmol/L**
Severe ICP	Very Severe ICP	Itching and raised peak bile acid concentrations **≥100 µmol/L**

Threshold targets were based on the performance of the Diazyme kit

Flow Chart for Diagnosis and Management of Intrahepatic Cholestasis of Pregnancy (ICP)



RCOG classification

Treatment and implications

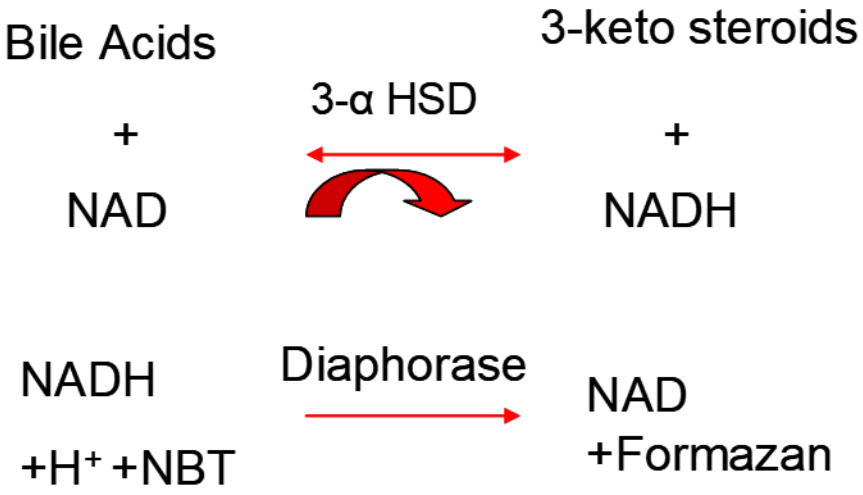
- Ursodeoxycholic acid (UDCA), a dihydroxycholic BA is often used in the UK as a therapeutic option and is recommended by SOMANZ for treatment in women remote from term.
- UDCA replaces the more toxic hydrophobic BAs (such as cholic acid) in the circulating BA pool.
- Be aware of the sensitivity and specificity of each of the methods to the different BAs , and UDCA in particular.

Lab Performance: Can it meet the guidelines?

Routine Field Methods – Total Bile Acids

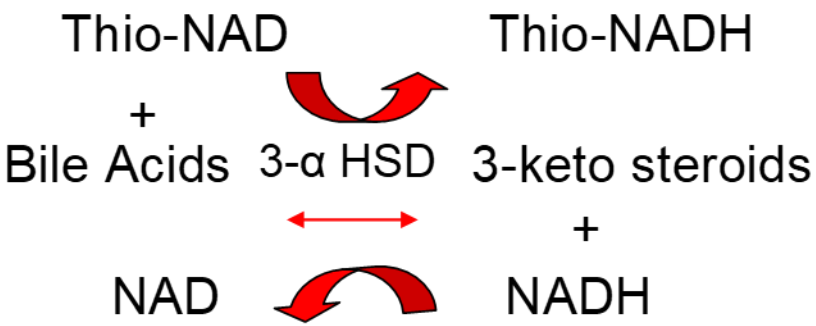
Enzymatic

Formazan



Read formation of formazan @ 540nm

Thio-NADH



Monitor Rate of formation of Thio-NADH @ 405nm

Sentinel Method

EQA Design

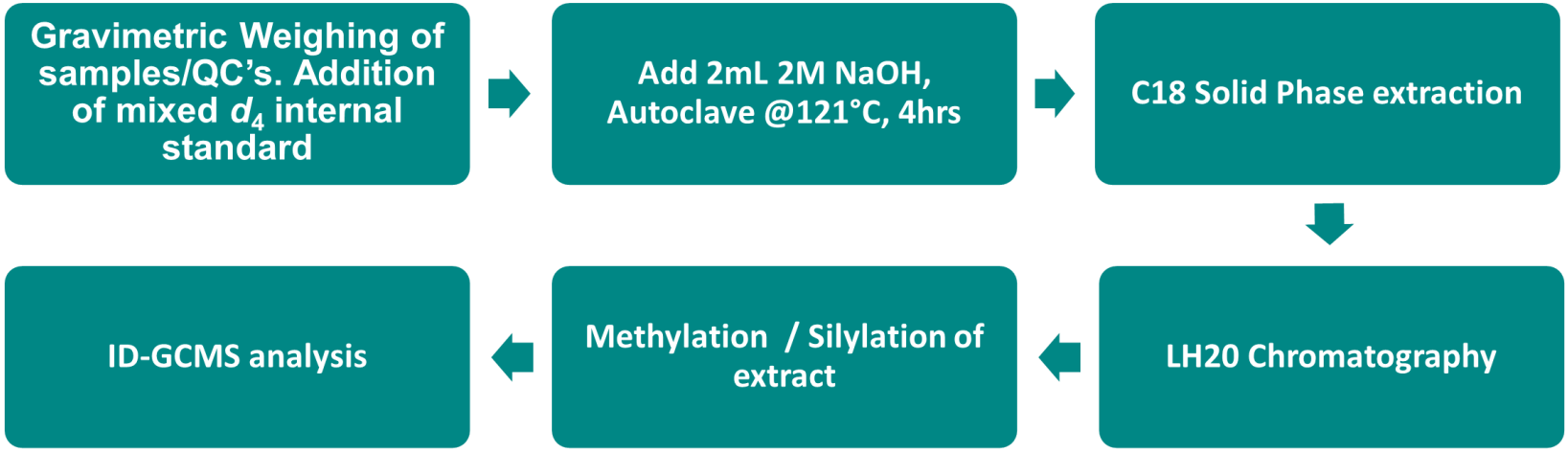
The programme is designed to assess laboratory and method performance, including bias, within and between batch imprecision, **linearity, trueness, sensitivity and specificity** of TSBA

Analyte	Approx. Range Covered
Total Bile Acids	5-108 µmol/L
Cholic Acid	0-83 µmol/L
Deoxycholic Acid	0-25 µmol/L

Challenging samples are also distributed periodically to assess specificity of the methods including those containing **UDCA, Chenodeoxycholic acid, glycocholic acid, glycochenodeoxycholic acid, taurocholic acid, and taurochenodeoxycholic acid**

EQA Target value assignment

ID-GCMS Method – Cholic acid, Chenodeoxycholic acid and Deoxycholic acid



All samples, standards and QC material prepared using exact matching isotope dilution

Currently in the final stages of validating the method for Ursodeoxycholic acid

ID-GCMS Method (2) – Cholic acid, Chenodeoxycholic acid and Deoxycholic acid

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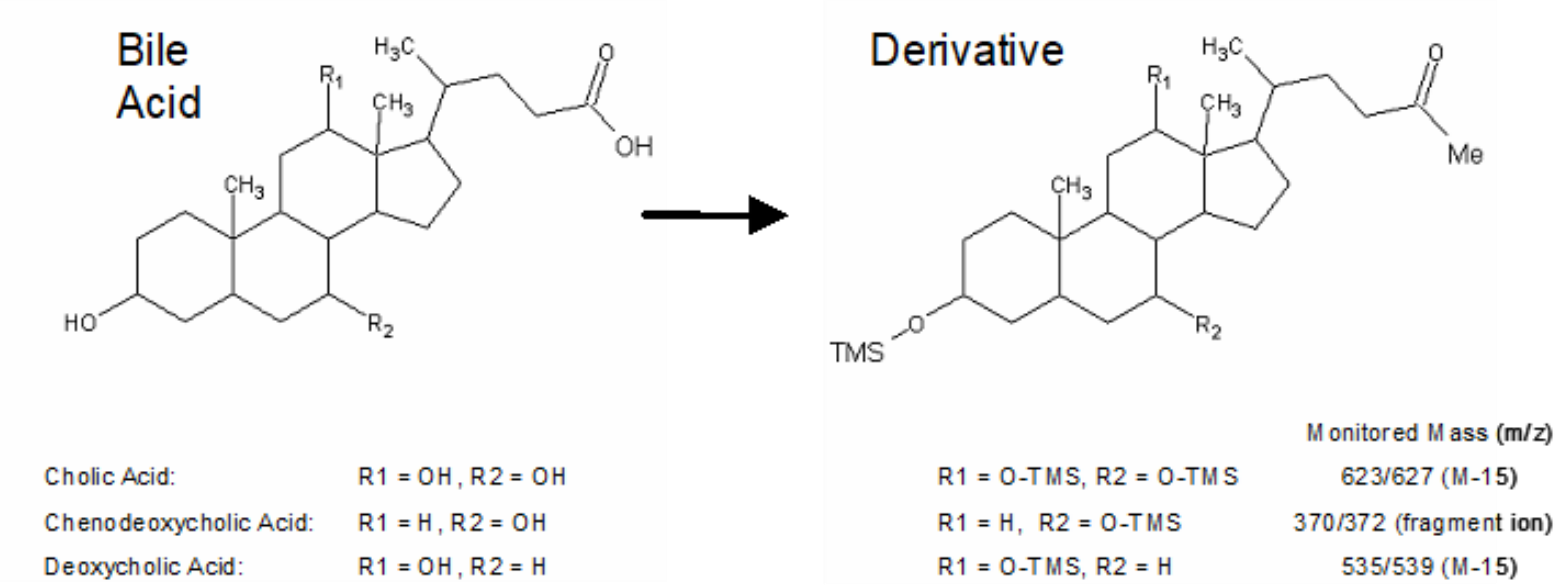
Methyltrimethylsilyl Ether Derivatives (Me TMS)



			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)

Cholic acid: *m/z* 623 / 627 (m-15)

Chenodeoxycholic acid: *m/z* 370 / 372 (fragment ion)

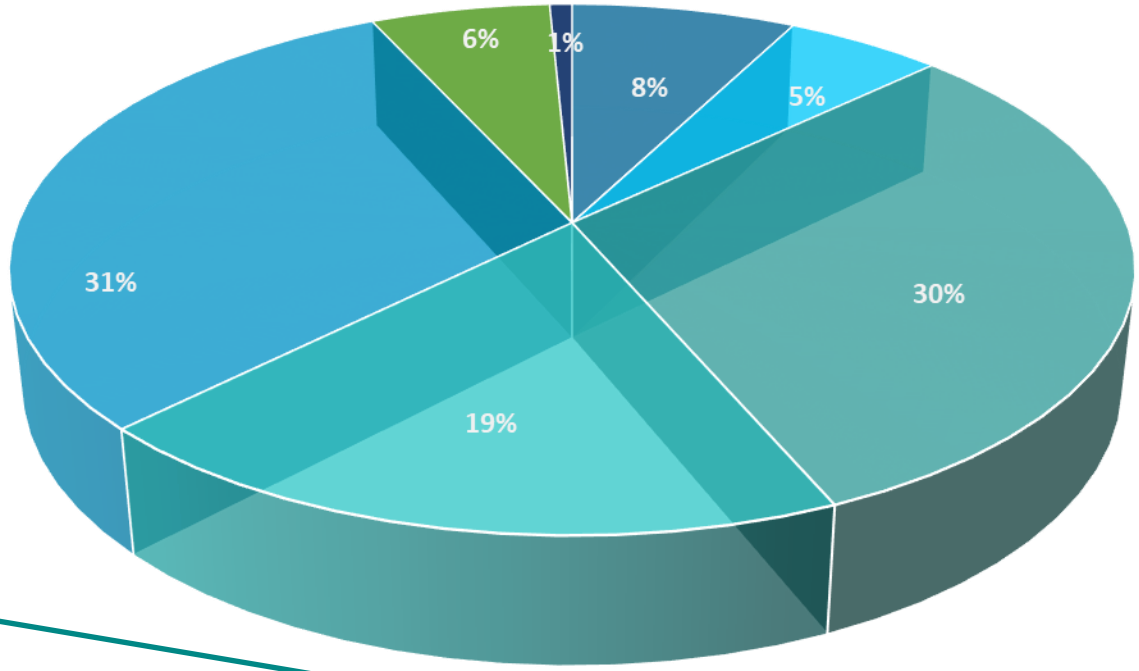
Deoxycholic acid: *m/z* 535 / 539 (m-15)

Method 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%)	

EQA Data

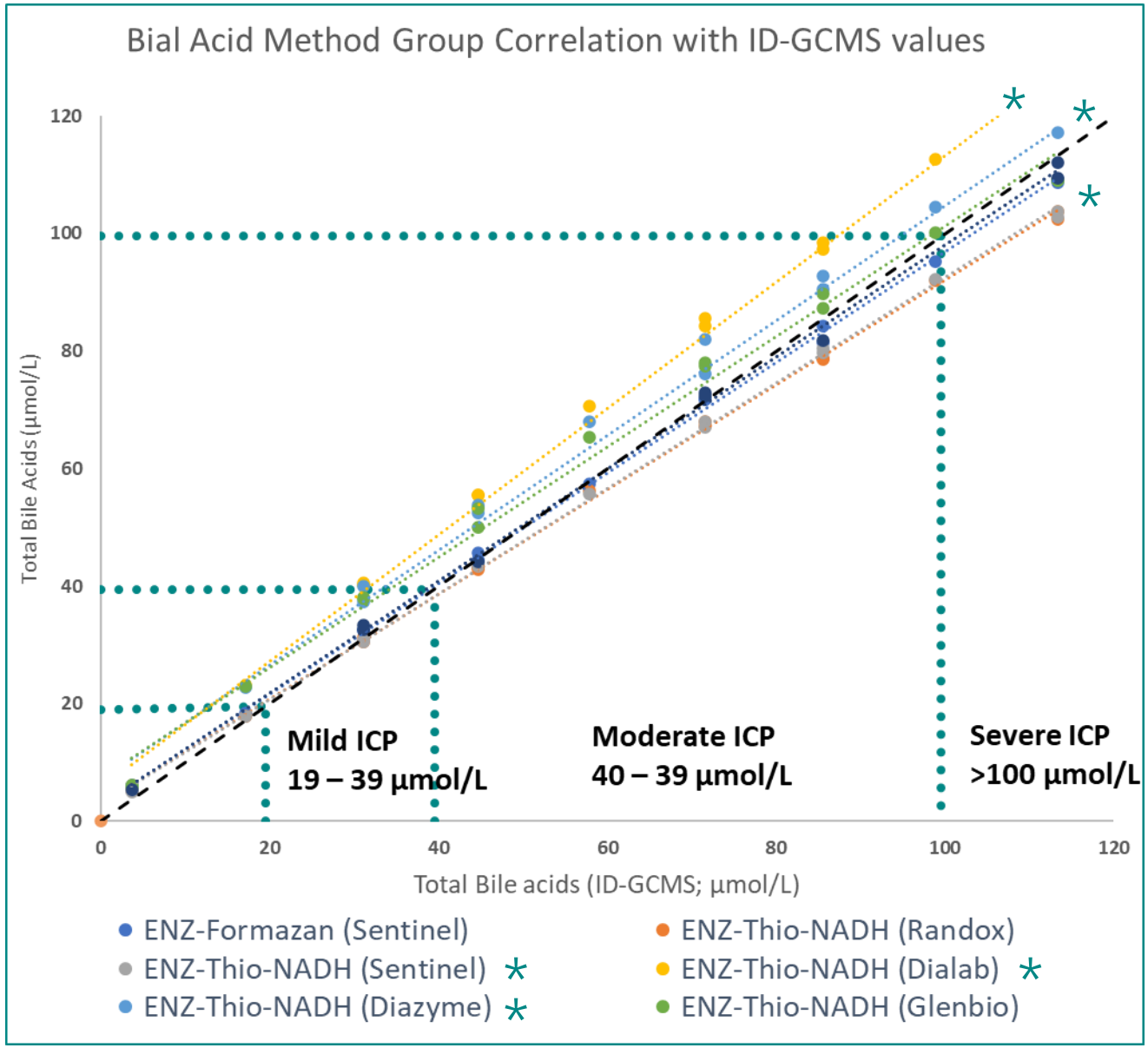
Major Total Bile acid Groups



Major groups

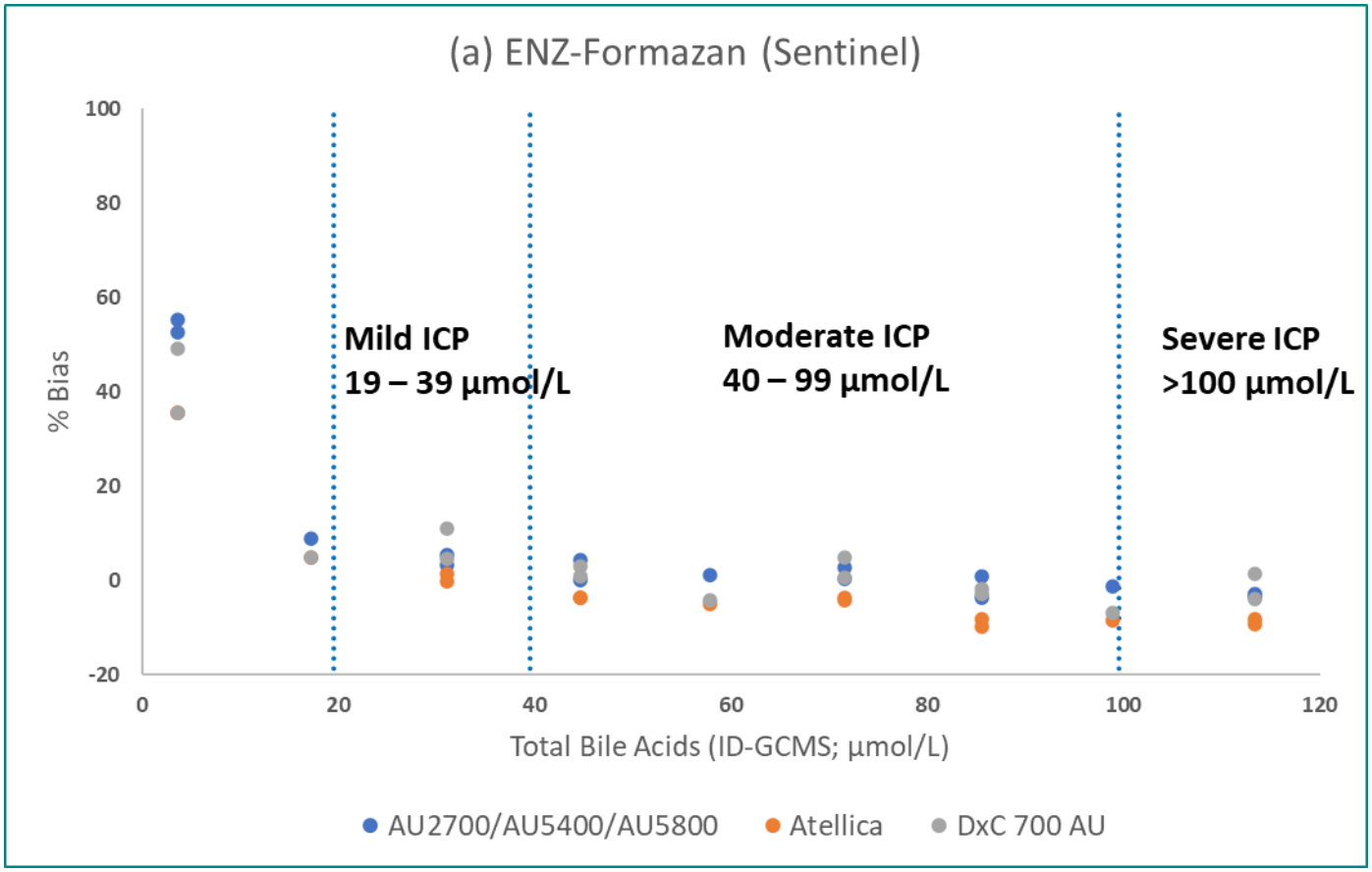
- ENZ-Formazan (Sentinel)
- ENZ-Thio-NADH (Randox)
- ENZ-Thio-NADH (Sentinel)
- ENZ-Thio-NADH (Dialab)
- ENZ-Thio-NADH (Diazyme)
- ENZ-Thio-NADH (Glenbio)
- ENZ-Thio-NADH (Beckman)

Correlation with ID-GCMS

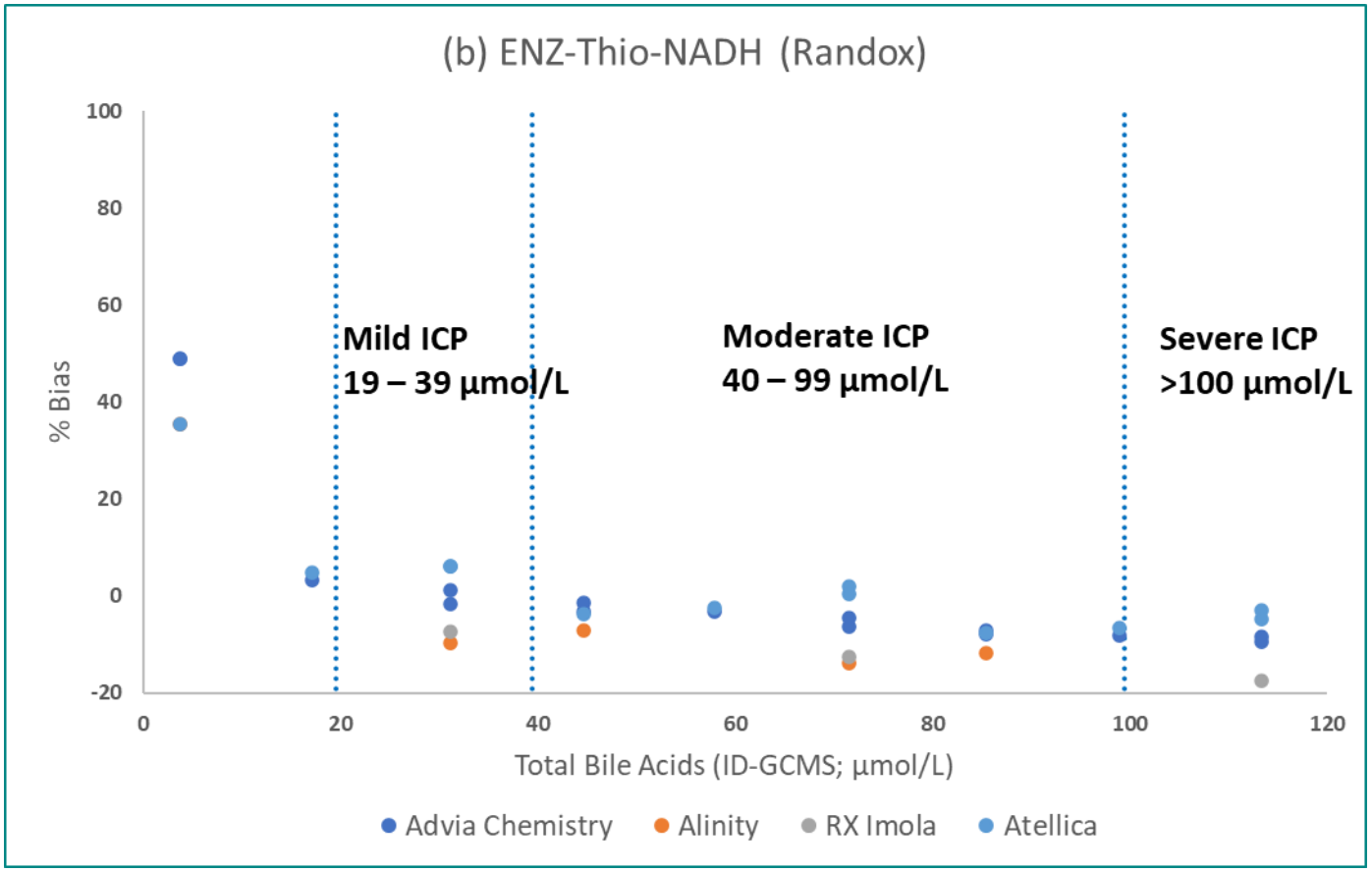


Comparing all of the current methods, proportional errors between 2.5 and -17% and constant errors between 3-5 µmol/L were observed

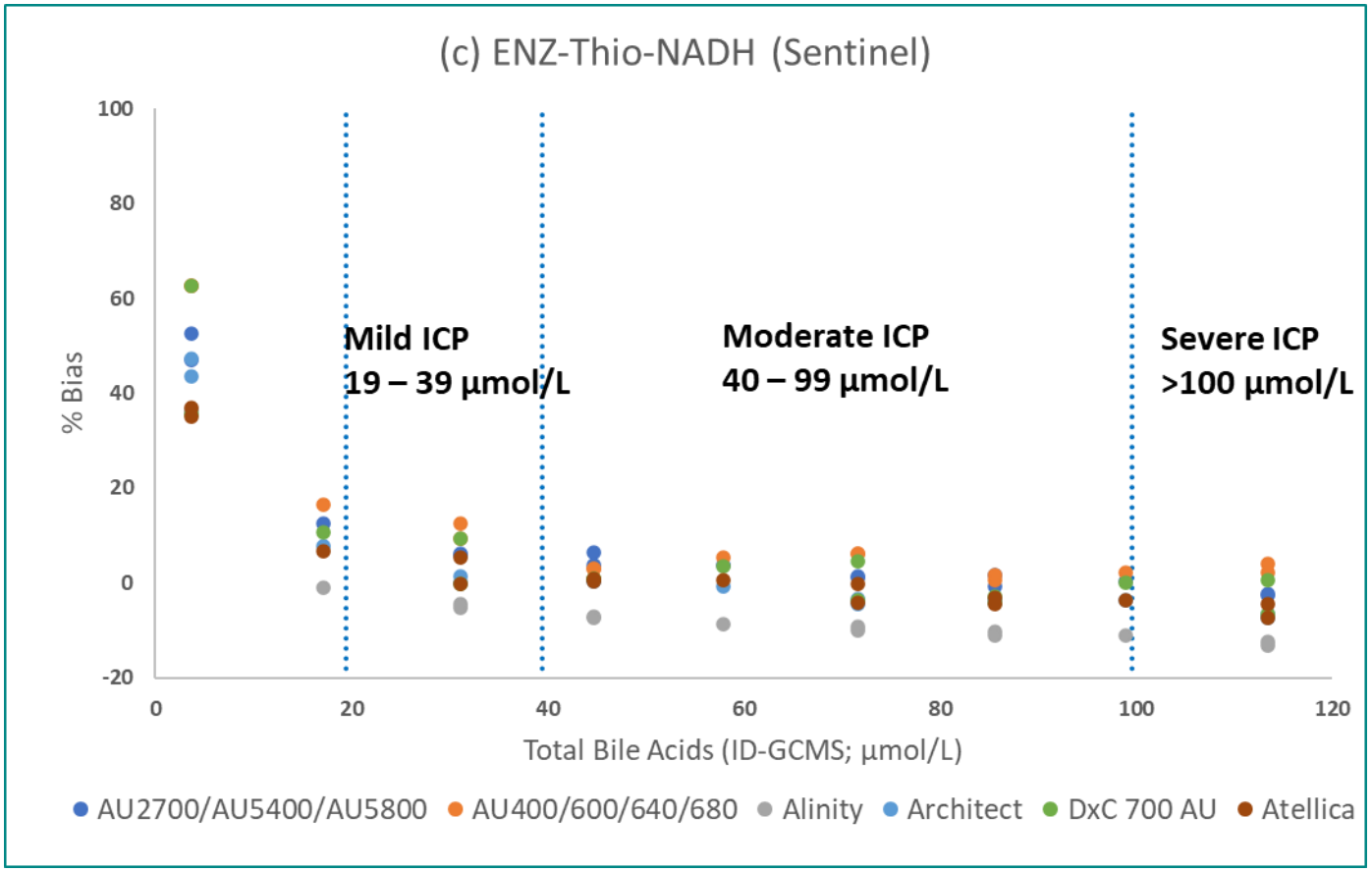
Enz- Formazan (Sentinel)



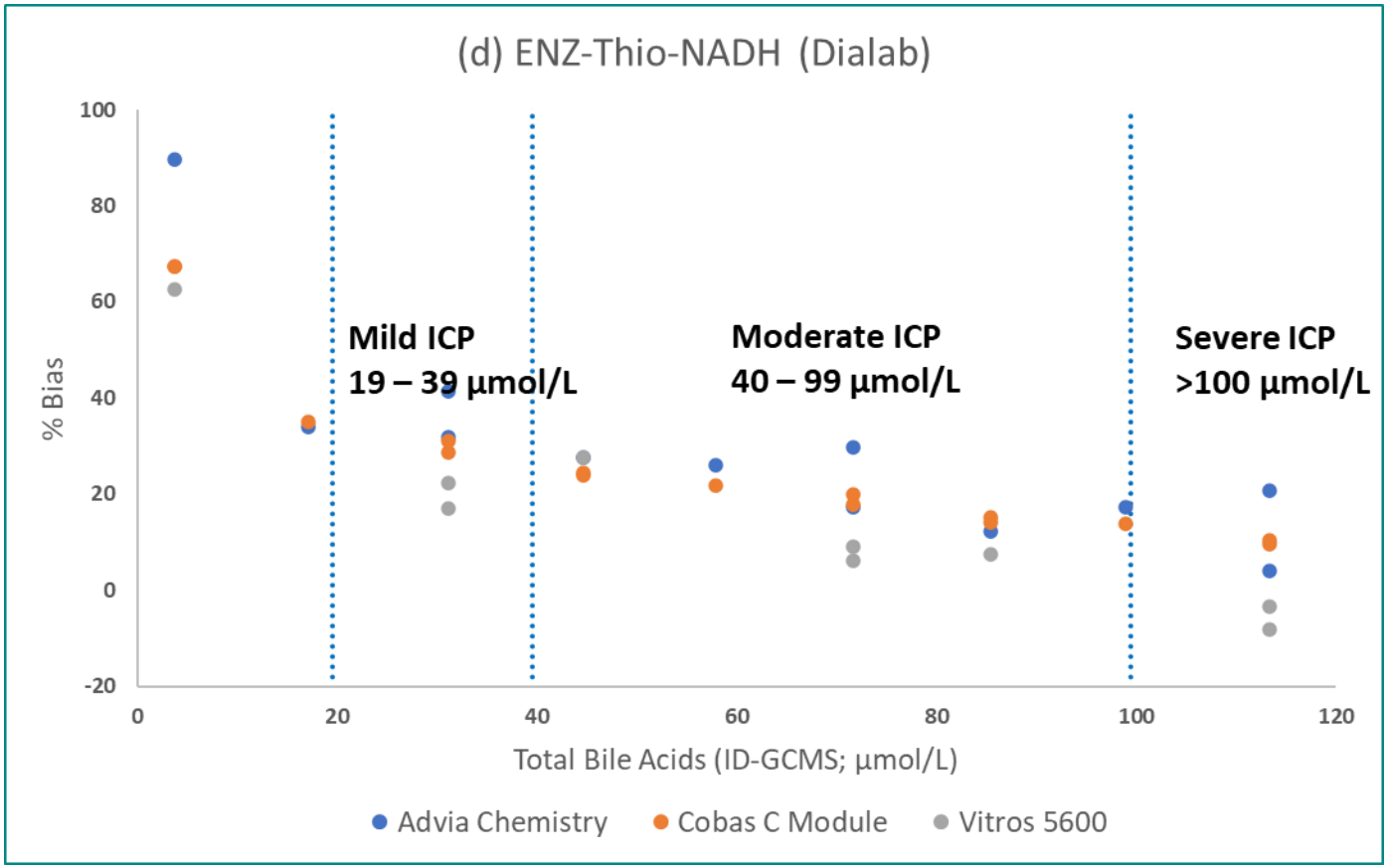
Enz-Thio-NADH (Randox)



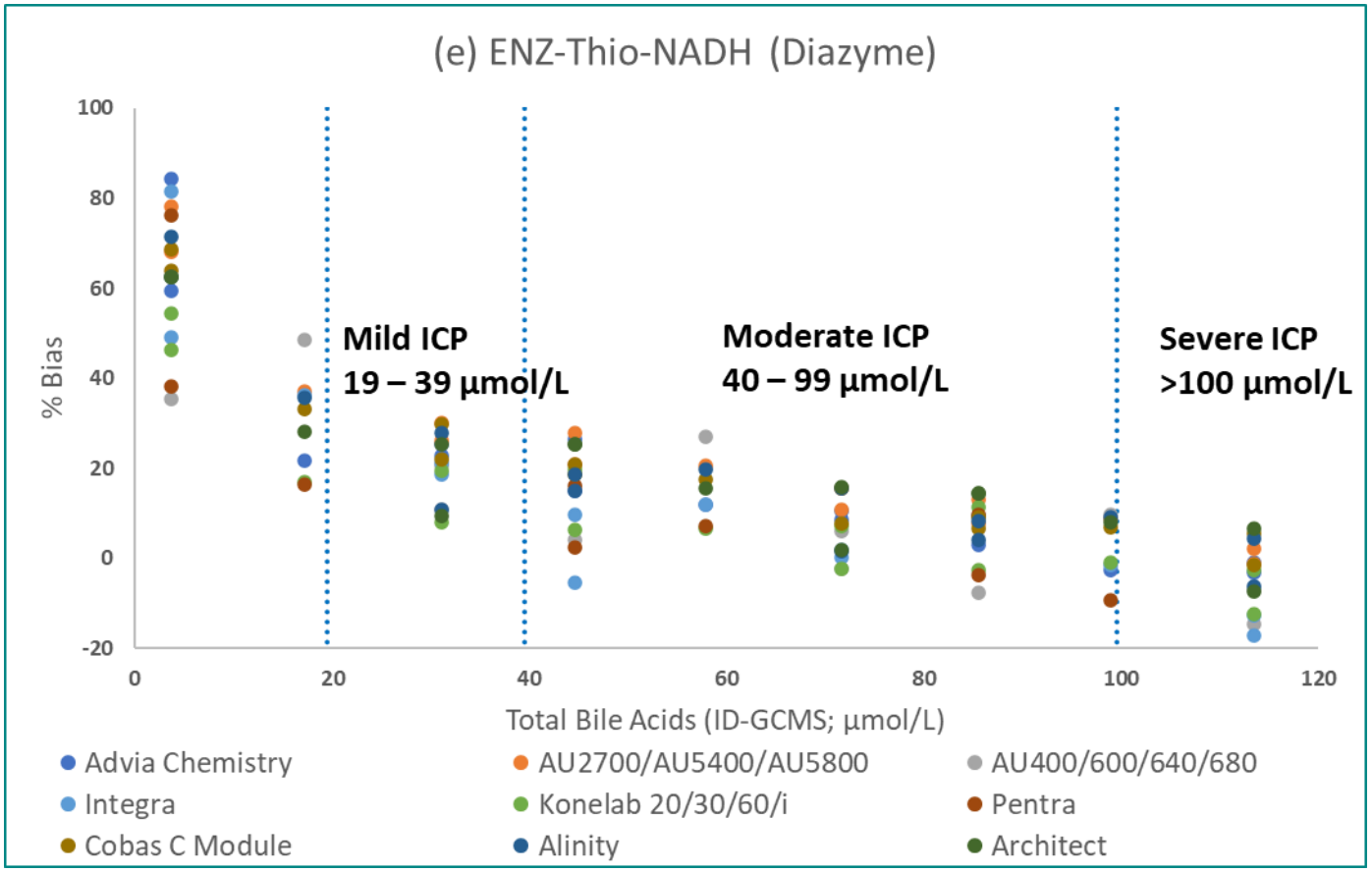
Enz-Thio-NADH (Sentinel) *



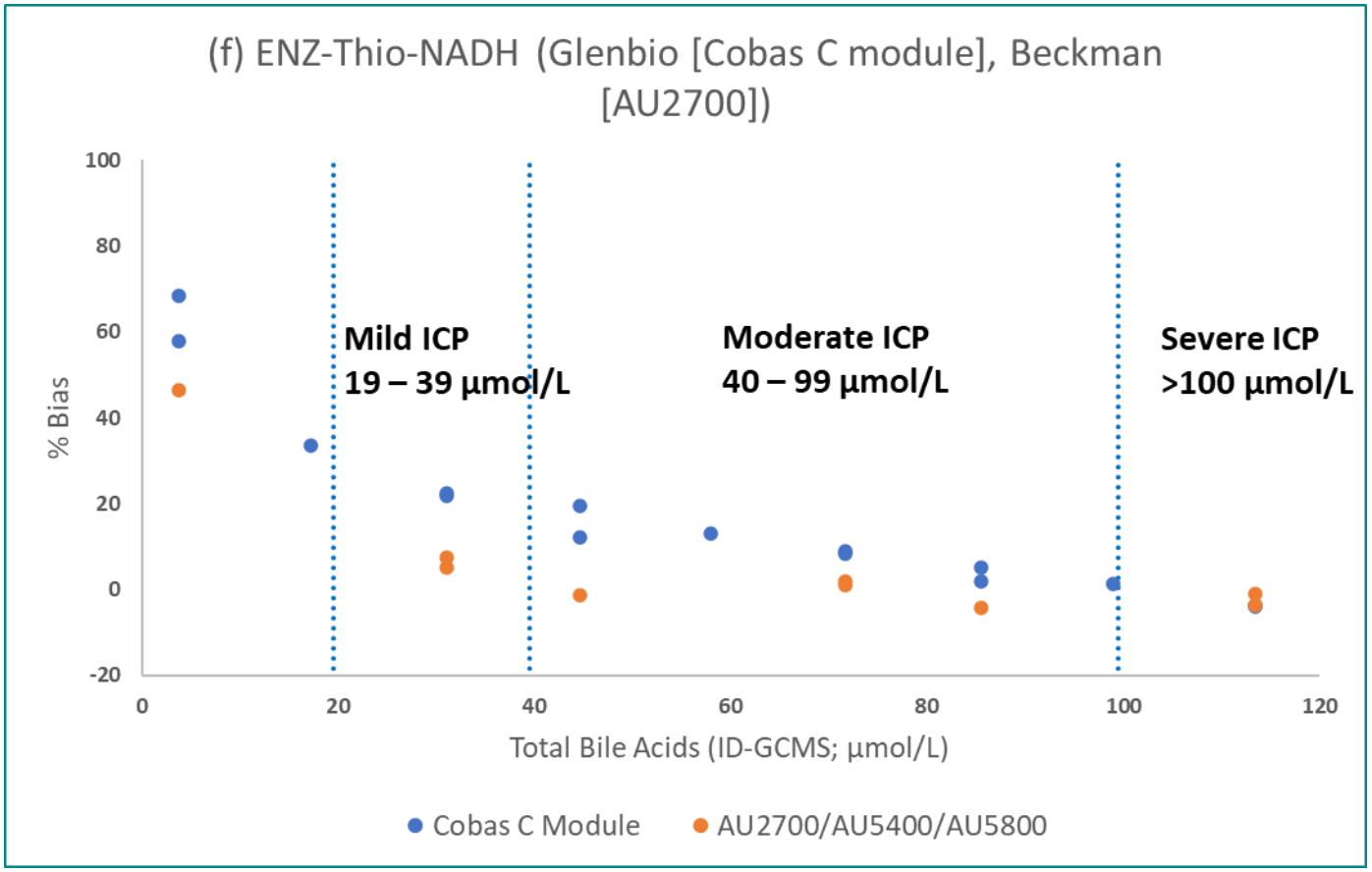
Enz-Thio-NADH (Dialab) *



ENZ-Thio-NADH (Diazyme) *



ENZ-Thio-NADH (Glenbio [Cobas C Module], Beckman [AU2700])



Summary and 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 / SOMANZ guidelines, is based on TBA values.
- There is therefore a potential for misclassification of risk dependent on the TBA method or instrument platform used

Bile Acids Study

Normal pools: linear series, TBA approx. 5 -100 µmol/L. Cholic Acid:Deoxycholic Acid 3.4:1 ratio (approx. ratio seen in ICP).

Study pools:

Pools containing single Bile Acids:
GCA - glycocholic acid
GCDCA - glycochenodeoxycholic acid
TCA - taurocholic acid
TCDCA - taurochenodeoxycholic acid
UDCA - Ursodeoxycholic acid

Series of pools with mixed Bile Acids with and without addition of Ursodeoxycholic acid

Aims
To assess method specificity to individual Bile Acids
To assess affect of presence of Ursodeoxycholic acid on analytical performance

Thank you