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Bile Acids SchemeGuide

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Contents

1. Scheme Details and Repetoire.

List of Analytes and Frequency of Distribution

Frequency : Monthly Number of samples: 3

Analyte	Approx. Range Covered	
Total Bile acids	5 - 100	µmol/l

This guide illustrates the design of the Scheme from sample preparation to dispatch of participants' reports. It includes data on interlaboratory variation, method bias and stability over the 12 month batch.

The bile acid scheme provides ID-GCMS target values for the individual bile acids, chenodeoxycholic acid, cholic acid and deoxycholic acid. In the absence of a true reference method the sum of the individual bile acids provides the current 'gold standard' for Total Bile Acids.

2. Source Material and Serum Integrity

The material is human serum, tested negative for HIV, Hepatitis B and C at donor level, spiked with a mixture of bile acids. WEQAS samples are prepared to reflect the bile acids found in obstetric cholestasis, with Cholic acid used as the predominant bile acid. Additional bile acids in the form of Chenodeoxycholic acid and Deoxycholic acid are also included. The overall ratio is usually 3.4: 1 for Cholic acid and the other bile acids respectively.

Although every effort is made to ensure that the material is free from any known infectious agent, the samples should be handled as for clinical specimens.

2.1 Sterility

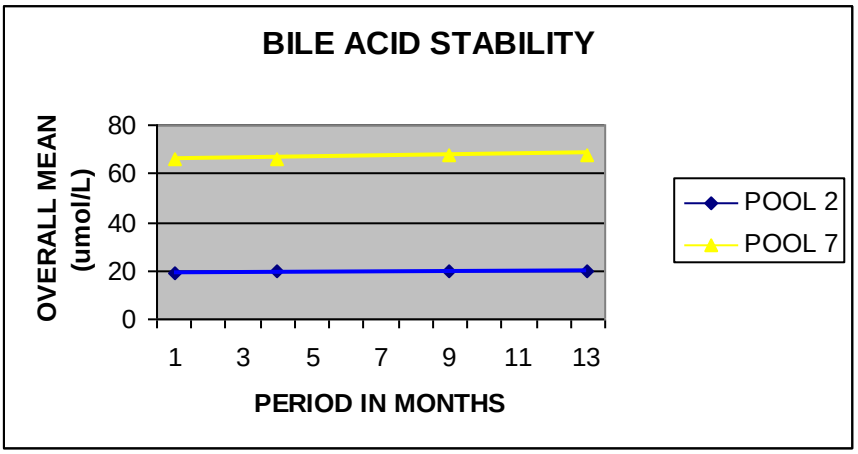
The pools are filtered aseptically down to a 0.2µm, and gentamicin added to maintain sterility. **Preservatives such as sodium azide are not added as these are known to inhibit certain immuno enzymatic methods**. Great care is taken to ensure that aseptic techniques are used throughout all procedures to maintain sterility. The serum is dispensed aseptically into 0.5ml aliquots and stored at -20°C until dispatched. The samples are dispatched by first class mail as frozen samples packaged in containers conforming to Post Office guidelines.

2.2 Sample Stability

Short term stability.
Stability experiments were carried out over a period of 14 days at room temperature, fridge(4°) and at -20°C. The total bile acid concentration over this period showed no deterioration at any of the specified temperatures.

Long term stability at -20°C

The 2 pools relate to processed serum samples stored at -20°C and dispatched over a period of several months. Pool 2 and Pool 7 (Batch 101205) were each dispatched 3 times over a 13 month period.



3 ID-GCMS Target assessment

The ID-GCMS method analyses the three major bile acids present: Cholic acid, Deoxycholic acid and Chenodeoxycholic acid as separate specific entities. Gravimetric analysis is used throughout, allowing uncertainty measurements to be estimated according to GUM, with traceability by use of gravimetrically prepared QC material. Each bile acid is chemically derivatised to form the methyl trimethylsilyl ether derivative prior to analysis (figure 2). Standardisation was achieved by use of the purest form of each bile acid available (table 1). No certified material is currently available for these bile acids. Analytical control was assessed using gravimetrically prepared serum as internal QC material (table 2). For the purposes of EQA evaluation, the results for each of the three bile acid targets are combined to produce a total bile acid target value. For the purpose of this recovery experiment, where single bile acids were spiked, the target value represents each individual bile acid. This target value would also include any endogenous bile acid present in the base serum.

3.1 Bile acid Recovery to ID- GCMS

The recovery of the routine bile acid methods were compared with the "weighed in " value and GCMS data. Recovery was calculated for the pools spiked with the individual bile acids: cholic acid, deoxycholic acid and chenodeoxycholic acid and a mixture of the three bile acids.

Table 1 – Recovery data 2005

Distribution Code	Distribution Date	Pool Code	Overall Mean (umol/l)	Base (umol/l)	Spiked value (umol/l)	Spiked with	% recovery from GCMS data
BA1	15/10/04	260804/61A	42.4	3.9	30	CDCA+ DCA	112
BA1	15/10/04	260804/61B	6.1	3.9	3	CDCA+ DCA	101
BA 2	03/11/04	111004/1	15.7	14.1	0		100
BA 2	03/11/04	111004/2	56.7	14.1	50	DCA	78
BA 3	20/12/04	101204/1	9.94	8.7	0		100
BA 3	20/12/04	201204/2	56.9	8.7	45	CA	75
BA 4	17/01/05	130105/1	87.2	5	100	CA	68
BA 4	17/01/05	130105/2	39.4	5	25	CA	107
BA 5	14/02/05	260804/61A	50.4	3.9	30	CDCA+ DCA	112
BA 5	14/02/05	111004/1	16.1	14.1	0		100
BA 6	14/03/05	260105/552/1	8.4	2.7	5	CDCA+ DCA	101
BA 6	14/03/05	260105/552/4	62.1	2.7	60	DCA+CA	80
BA 7	18/04/05	260105/559/3	50.1	3.3	50	CDCA	87
BA 7	18/04/05	260105/559/1	5.5	3.3	5	CDCA	47
BA 8	16/05/05	260105/559/4	3.6	3.3	0		88
BA 8	16/05/05	260105/560/2	5.7	3.2	3	DCA	90

3.2 Recovery Experiment 2008.

The recovery study was repeated in November 2008. The recovery experiment was carried out on three pools spiked with approximately 100µmol/l of one of each of the major bile acids - cholic acid, chenodeoxycholic acid and deoxycholic acid.

The following Table shows a summary of the data. The predominant group is represented by the Enz-Thio-NADH method (86% of scheme participants), with the Enz-Formazan group representing 6% of scheme participants and the Sentinel Enz-Formazan group 8% of scheme participants.

Table 2 – Recovery data 2008 - Bile Acid Recovery Experiment: comparison with GCMS method.

POOL ID	CHOLIC ACID µmol/L				CHENODEOXYCHOLIC µmol/L				DEOXYCHOLIC µmol/L			
	ID-GCMS Target				ID-GCMS Target				ID-GCMS Target			
POOL A (sample 4)	96.59											
POOL B (sample 5)					91.22							
POOL C (sample 6)									89.69			
Returned results	mean	SD	n	% recovery	mean	SD	n	% recovery	mean	SD	n	% recovery
overall	82.97	5.28	73	85.90	60.29	7.25	74	66.09	97.42	9.62	77	108.61
Enz-Thio-NADH	83.12	4.44	66	86.05	58.62	5.30	65	64.26	94.91	6.54	64	105.82
Enz-Formazan	76.88	10.73	5	79.59	65.78	8.45	5	72.11	114.26	22.11	5	127.39
Enz-Formazan (Sentinel)	116.97	3.99	6	121.10	102.70	2.45	6	112.58	112.83	3.29	6	125.79

Poor recoveries were observed for chenodeoxycholic and cholic acid for all methods. However, as similar specificities were observed for all the bile acid compounds for the Sentinel method this could potentially be addressed through re-standardisation. The variability in specificity of the other methods however suggests that harmonisation of these methods may not be achieved easily.

The study highlights the importance of using reference methods to assign target values (refer to section 3 on statistical analysis), rather than consensus mean and presents strong evidence on the variability in specificities of the methods for the different bile acids.

The full report for this experiment is available on request.

3.3 Recovery experiment 2012

Recovery experiments were repeated in November 2012 (distribution BA93), with the inclusion of Ursodeoxycholic acid.

Ursodeoxycholic acid is a dihydroxycholic bile acid. This physiologically represents about 3% of total bile acid concentration. It is synthesised from 7-ketolithocholic acid which is a product of bacterial oxidation of Chenodeoxycholic. Chemically Ursodeoxycholic acid differs from Chenodeoxycholic only in the equatorial oxidation of the 7β hydroxyl group. This makes Ursodeoxycholic acid more hydrophilic than the other bile acids. This high polarity correlates with the low potential to form micelles, and is the reason for its extremely low toxicity. Pharmacologically it is administered for the treatment of cholestatic disease (biliary cirrhosis, obstetric cholestasis) and gallstones. Its therapeutic effect in hepatic and cholestatic disease is thought to be due to a relative exchange of lipophilic toxic bile acid (cholic acid) for the hydrophilic cytoprotective, non toxic Ursodeoxycholic acid.

Four extra samples were therefore distributed to all participants of the bile acid scheme, each containing only one of the individual bile acids.

A summary of the method data is provided in Table 3.

Table 3 – Recovery data 2012. Bile Acid Recovery Experiment: comparison with GCMS Method

POOL ID	CHOLIC ACID µmol/L				DEOXYCHOLIC µmol/L			
POOL A (sample 4)	ID-GCMS Target 103.18				ID-GCMS Target			
POOL B (sample 5)					108.78			
POOL C (sample 6)								
POOL D (sample 7)								
Returned results	mean	SD	n	% recovery	mean	SD	n	% recovery
			11					
overall	101.18	7.54	1	98.06	137.80	15.87	110	126.68
Enz-Thio-NADH	99.89	6.59	95	96.81	141.27	15.64	94	129.87
Enz-Formazan	89.5	1.50	5	86.74	137.00	15.00	2	125.94
Enz-Formazan (Sentinel)	112.41	4.90	15	108.95	119.42	5.08	15	109.78
POOL ID	**URSODEOXYCHOLIC µmol/L**				**CHENODEOXYCHOLIC µmol/L**			
POOL A (sample 4)	Spiked Target				ID-GCMS Target			
POOL B (sample 5)								
POOL C (sample 6)					77.14			
POOL D (sample 7)	100							
Returned results	mean	SD	n	% recovery	mean	SD	n	% recovery
			10					
overall	57.81	8.44	7	57.81	56.05	7.30	107	72.66
Enz-Thio-NADH	56.00	4.44	98	56.00	54.25	4.61	95	70.32
Enz-Formazan	51.50	0.5	2	51.50	51.00	2.00	2	66.11
Enz-Formazan (Sentinel)	90.47	3.33	15	90.47	77.05	2.88	12	99.88

Table 3 shows a wide variation in the performance of the commercially available methods for the analysis of total bile acids. For Cholic acid, there is improvement from the previous study, with good agreement with the ID-GCMS method for the Enz-Thio-NADH group with a negative bias of -3%. For Enz-Formazan a negative bias of -13% was, and the Sentinel group had a positive bias of +9%. For Deoxycholic acid the Enz-Thio-NADH method had a positive bias of +30%. The Enz-Formazan and ENZ-Formazan (Sentinel) had a positive bias of +26% and +10% respectively. For Chenodeoxycholic acid, these methods had a negative bias of -30% and -34% respectively, with a recovery for the Sentinel kit of 99.9%. The data for Ursodeoxycholic acid mirrors that of Chenodeoxycholic acid.

As in prevuious studies, similar specificities were observed for all the bile acid compounds for the Sentinel method with improved concordance with the GCMS method.

The study highlights the importance of using reference methods to assign target values rather than consensus mean and presents strong evidence on the variability in specificities of the methods for the different bile acids.

The full report for this experiment may be obtained on request.

4. Statistical Analysis

Please refer to the accompanying Participants Manual for full details on statistical analysis and interpretation of results.

Typical Report

Scheme: Bile Acids. Distribution Code: BA96.
Final. Report Issued: 12/10/12

Total Bile Acids (umol/l)		1	2	3	Analyte SDI
Reported Result		19.0	88.0	55.0	
Method Corrected Result		19.00	88.00	55.00	
ENZ-Thio-NADH	Mean	19.12	87.93	55.69	
	SD	1.39	4.56	3.03	
	Number	104	99	101	
	Uncert.	0.136	0.459	0.302	
Modular	Mean	18.53	85.59	54.35	
	SD	1.13	2.49	2.36	
	Number	17	17	18	
	Uncert.	0.274	0.604	0.557	
Overall	Mean	19.05	88.53	55.91	
	SD	1.33	4.50	3.03	
	Number	122	116	119	
	Uncert.	0.121	0.418	0.277	
Reference Values					
Ref. Value Uncertainty					
Non-scoring Reference Values ID-GCMS		13.29	86.40	49.89	
WeQas SD		1.82	7.51	4.36	
SDI		-0.06	0.01	-0.16	0.08

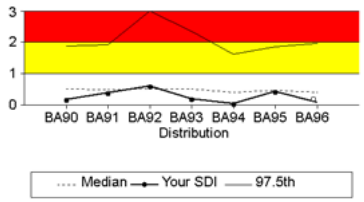
Please note: Linear regression uses CF corrected data.

Total Error

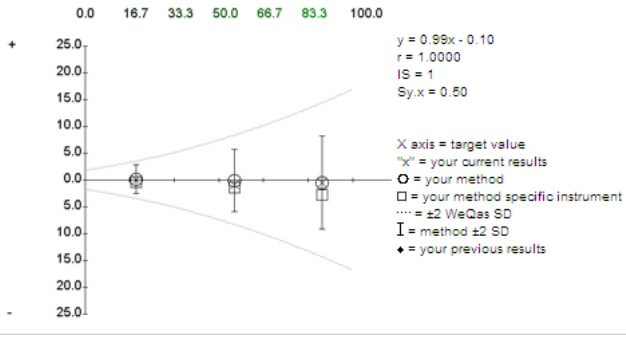
SDI is a measurement of your total error and will include both inaccuracy and imprecision.

This Distribution BA96
Your average analyte SDI for the 3 samples is 0.08

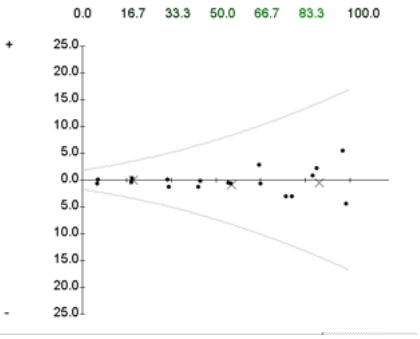
Previous SDI



This Distribution *BA96*



Previous Distributions



GCMS target value information is shown on the front of the report. See example below:

Comments:

Distribution BA96

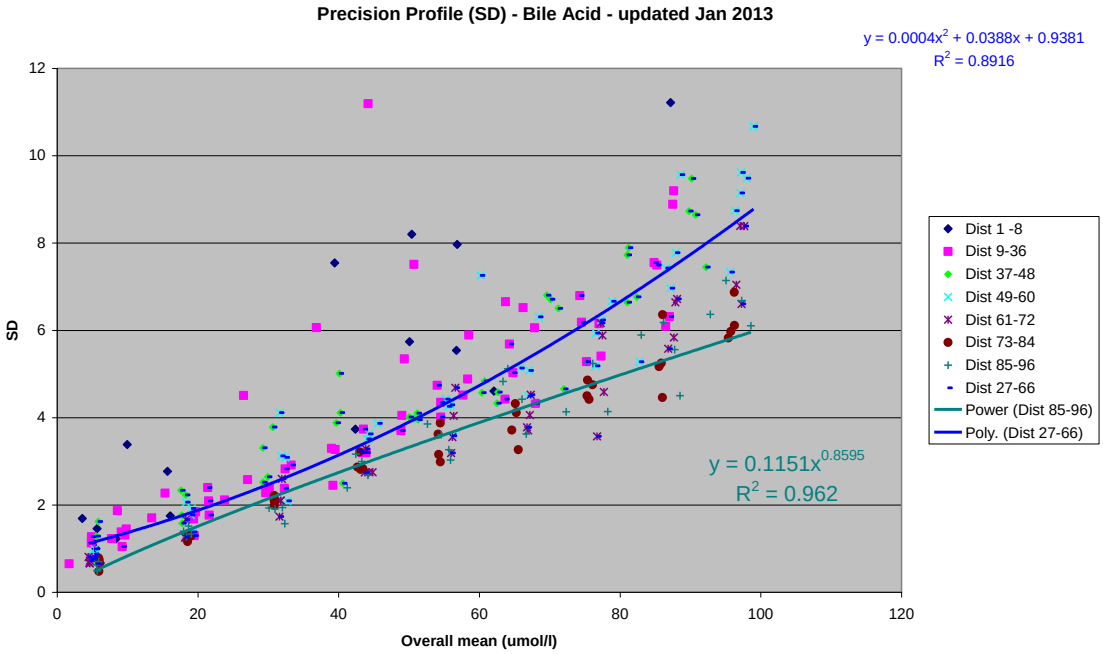
		Sample No 1	Sample No 2	Sample No 3
	Total bile Acid	13.287	86.397	49.888
GCMS Targeted Values	Chenodeoxycholic Acid	0.254	0.254	0.254
	Cholic Acid	10.361	68.805	39.402
	Deoxycholic Acid	2.672	17.338	10.232

4.1 Performance Criteria

Standard deviation limits used in the report are based on precision profiles calculated over several batches and are fixed for a given level of analyte. They are reviewed yearly and reflect the state of the art of the methods used.

Precision profiles

A plot of the overall Standard Deviation against concentration is given over the distributions. The data reflects the interlaboratory variation and the bias between the methods currently in use.



4.2 Method Performance

Analytical performance of methods and instruments are available to participants for *all Schemes* on request. An overall summary of this data is issued with each distribution report.

A summmary sheet example is shown overleaf:

Bile Acid Summary Sheet BA96

Distribution:	BA96
Distribution Date:	26-Sep-12
Analyte:	**Total Bile Acids (µmol/L)**

Method	Instrument		1	2	3
		Overall Mean	19.07	88.58	55.96
		Overall SD	1.32	4.48	3.00
		Est. Uncertainty of Consensus	0.120	0.418	0.276
		Overall Number	121	115	118
		Reference Value NS ID-GCMS	13.29	86.40	49.89
ENZ-Thio-NADH		Method Mean	19.14	87.99	55.74
		Method SD	1.37	4.55	3.01
		Est. Uncertainty of Consensus	0.135	0.460	0.301
		Number	103	98	100
	Advia 1200/1650/1800/2400	Instrument Mean	19.14	85.83	54.57
		Instrument SD	1.81	6.09	3.84
		Number	16	16	16
	AU2700/AU5400/AU5800	Instrument Mean	18.85	87.77	55.87
		Instrument SD	0.89	4.74	2.60
		Number	6	6	6
	AU400/600/640/680	Instrument Mean	19.56	92.10	58.24
		Instrument SD	0.56	3.63	2.69
		Number	9	9	9
	Modular	Instrument Mean	18.65	85.81	54.56
		Instrument SD	1.05	2.40	2.26
		Number	16	16	17
	Konelab 20/30/60/i	Instrument Mean	17.27	79.93	50.13
		Instrument SD	1.79	5.98	4.92
		Number	3	3	3
	Architect	Instrument Mean	19.16	91.00	57.50
		Instrument SD	1.10	2.08	1.80
		Number	7	6	6
	DX	Instrument Mean	19.76	89.01	57.41
		Instrument SD	1.17	2.60	1.77
		Number	8	7	7
	Vitros 5,1 FS	Instrument Mean	19.80	90.33	57.89
		Instrument SD	0.64	2.78	1.78
		Number	7	7	7
	Cobas C Module	Instrument Mean	19.23	89.40	56.77
		Instrument SD	0.63	4.34	2.08
		Number	21	23	22
ENZ-FORMAZAN	Modular	Method Mean	17.00	82.00	50.50
		Method SD	1.0	1.0	1.5
		Est. Uncertainty of Consensus	0.707	0.707	1.061
		Number	2	2	2
ENZ-Formazan (Sentinel)		Method Mean	18.80	92.29	57.99
		Method SD	0.50	2.37	1.21
		Est. Uncertainty of Consensus	0.129	0.593	0.303
		Number	15	16	16
	Architect	Instrument Mean	18.86	91.74	57.92
		Instrument SD	0.46	1.93	1.26
		Number	14	13	14
	DX	Instrument Mean	19.0	93.5	58.5
		Instrument SD	1.0	2.5	0.5
		Number	2	2	2