Solid Matrix EQA Scheme (SOM@S)

Annual Report: 2015-16

7th March 2017

EQA@S

External Quality
Assessment at Surrey

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Introduction

Running since 2012-13 the solid matrix EQA scheme provides EQA specimens for laboratories that undertake the analysis of solid samples for copper and iron. The scheme is coordinated by External Quality Assessment at Surrey which runs the scheme and is part of UKNEQAS and based at the Surrey Research Park.

Clinically, in patients suspected of having Wilson's Disease or haemochromatosis, a liver biopsy sample may be taken for the determination of copper or iron to aid diagnosis (Wilson's and haemochromatosis respectively). To ensure accurate testing methods and facilitate suitable QA/QC in these complex determinations this scheme sends out small mass (10 - 20 mg) powdered samples of animal organs or other suitable organic solid materials, which are analysed by the participants for their copper and iron content. The analytical procedure used requires the organic materials sent to be converted to a liquid prior to analysis. This step can be carried out in a number of ways using various combinations of reagents and heating methods. Ultimately this complex step can lead to significant errors being introduced due to contamination or under-recovery of the analyte from the matrix. The scheme is designed to assess this step of the process with the aim of improving the participant's performance.

Distribution Process

Twenty participants were registered in 2015-16 of which two were not returning results. A single distribution contains 3 tissue samples (Table 1), a covering letter and an answer sheet. Packages are sent out quarterly and labs are given 4 to 6 weeks to return the results and also the methodology they used (Table 3) in preparing the samples. Reports are then compiled and returned to the participant.

Materials Distributed in 2015-16

		Certificate concentration (µg/g)		2015-2016			
Reference code	Tissue	Iron	Copper	1st distribution	2nd distribution	3rd distribution	4th distribution
ERM-BB186	Pig Kidney	255	36.5	A1 A2 A3			
ERM-BB184	Bovine Muscle	75	2.31		B1 B2 B3		D1 D2 D3
ERM-BB422	Fish Muscle	9.4	1.67			C1 C2 C3	
		Date of distribution		26/05/2015	19/08/2015	30/10/2015	09/12/2015
		Date of return		31/07/2015	31/10/2015	31/12/2015	29/02/2016

Table 1: Overview of distributed materials and dispatch dates.

Each specimen was individually weighed into a 1.8ml ThermoFisher™ cryogenic micro-centrifuge tube. The samples contained approximately 15mg of dried tissue sample. Two out of the three samples in each distribution contained a spike of either copper or iron. Where spiked the individual mass of dried tissue sample and mass of the spike, were noted to calculate the expected concentration per tube.

Sample	Spiked element	Spike volume (µl)	Standard concentration (mg/L)
A2	Iron	20	1000
A3	Copper	20	1000
B1	Iron	30	1000
B2	Copper	10	1000
C2	Iron	30	1000
C3	Copper	10	1000

Table 2: Sample spike volumes and concentrations

Methodology Overview

		Mean recovery			Standard deviation of mean recovery			Range of mean recovery		
Method of digestion (full details given)	**Analyser**	**Fe**	**Cu**	**Mean**	**Fe**	**Cu**	**Mean**	**Fe**	**Cu**	**Mean**
HNO_3 (A) TMAH (B,C,D)	ICP-MS	105.0	123.4	114.2	15.7	21.5	18.6	55.2	21.5	38.3
HNO_3 Microwave	ICP-MS	98.6	90.9	94.8	17.0	18.2	17.6	74.5	18.2	46.3
HNO_3 80°c Heating block		103.1	117.0	110.0	18.1	35.5	26.8	68.6	35.5	52.0
90°c Hotblock for 6 hours in HNO_3		76.9	104.9	90.9	38.5	42.6	40.5	103.3	42.6	72.9
HNO_3 Hot plate	ICP-MS	105.4	111.2	108.3	17.5	30.6	24.1	54.9	30.6	42.8
HNO_3 Hot plate	ICP-MS	92.7	112.8	102.7	16.5	30.4	23.5	57.2	30.4	43.8
HNO_3 Teflon digestion bomb 110°c 18 hours	ICP-MS	111.8	95.9	103.9	22.1	53.9	38.0	84.9	53.9	69.4
$HNO_3 + H_2O_2$ Microwave		106.6	97.9	102.2	13.3	84.2	48.8	40.0	84.2	62.1
65% ultra pure HNO_3 MARS CEM	ICP-MS	N/A	113.0	113.0	N/A	58.7	58.7	N/A	58.7	58.7
CEM Microwave	ICP-MS	102.3	128.4	115.4	15.7	32.3	24.0	64.6	32.3	48.5
HNO_3 MARS 5 Microwave	ICP-MS	93.2	135.7	114.4	8.0	85.6	46.8	21.5	85.6	53.6
HNO_3 Oven 50°c 48 hours	ICP-MS	104.3	119.7	112.0	38.4	50.3	44.3	155.2	50.3	102.8
HNO_3 Oven 80°c 24 hours	ICP-OES	189.2	205.2	197.2	180.5	116.6	148.5	567.0	116.6	341.8
HNO_3 Heating system/method 80°c	EAA/ETAAS	93.1	53.1	73.1	65.7	35.2	50.5	135.3	35.2	85.2
65% HNO_3 8 hours No heating		16.2	19.8	18.0	1.7	3.8	2.8	3.3	3.8	3.6

Labs that performed well within a 25% threshold of recovery and standard deviation and within a range of 75
Labs that performed with borderline results
Labs that performed well outside of the thresholds

Table 3: Individual methods used by participants compared with their mean recovery, standard deviation of recovery and range of recovery over all distributions.

Three participants (17% of the laboratories returning results) failed to return information on digestion or analysis methods, or both.

Results overview

	Iron				Copper			
	Target value (µg/g)	Mean value (µg/g)	SD	CV	Target value (µg/g)	Mean value (µg/g)	SD	CV
A1	255.0	225.1	20.2	8.5	36.5	33.4	3.3	9.3
A2	1588.3	1525.8	249.6	13.1	36.5	33.2	1.7	5.0
A3	255.0	225.9	27.1	9.1	1369.8	1464.4	345.5	22.3
B1	2075.0	2318.6	306.5	13.2	2.3	3.4	3.1	90.1
B2	75.0	75.1	6.1	7.2	669.0	958.5	173.3	18.1
B3	75.0	72.5	6.2	7.7	2.3	2.2	1.1	50.0
C1	9.4	8.6	4.0	46.6	1.7	1.8	0.7	40.3
C2	2009.4	2140.6	283.1	30.1	1.7	1.7	0.5	31.1
C3	9.4	9.2	3.9	30.1	668.3	745.0	264.8	35.5
D1	75.0	75.0	9.1	12.2	2.3	2.1	1.0	46.2
D2	75.0	74.3	5.0	6.1	669.0	891.6	144.0	16.2
D3	1408.3	1729.7	333.9	14.9	2.3	2.5	1.3	53.0

Table 4: Overview of results. The values shown in green for A2, B1, C2, D3 (iron) and A3, B2, C3, D2 (copper) are the mean spike values for the sample group therefore the variation in vial spike values are not taken into account. Individual values are used in the reports to give a more accurate bias calculation.

	Iron					Copper				
	Mean recovery	High value	Low value	SD	CV	Mean recovery	High value	Low value	SD	CV
A1	88.3	105.8	23.1	20.2	22.9	91.6	121.6	32.9	18.4	20.1
A2	94.9	126.6	48.3	20.8	22.0	91.0	130.1	46.6	16.4	18.0
A3	88.6	121.6	20.8	22.0	24.9	118.3	173.8	94.6	21.2	17.9
B1	117.0	216.8	77.9	33.3	28.5	149.2	510.8	0.0	136.9	91.8
B2	102.2	130.7	88.4	11.1	10.8	158.6	309.6	106.1	49.4	31.1
B3	96.6	110.8	83.1	8.3	8.6	121.4	419.9	0.0	94.3	77.6
C1	122.7	477.7	6.1	105.0	85.6	127.5	305.4	47.9	62.1	48.7
C2	106.6	130.5	72.2	14.7	13.8	131.4	317.4	59.9	70.8	53.9
C3	150.7	652.1	81.9	145.8	96.7	111.0	158.3	1.4	44.1	39.7
D1	102.5	134.7	85.8	14.9	14.5	105.0	287.4	0.0	65.0	61.9
D2	102.2	130.7	88.4	11.1	10.8	134.5	177.3	92.9	22.3	16.6
D3	104.3	136.0	90.7	14.7	14.1	106.0	216.5	0.0	56.1	53.0

Table 5: Overview of recovery's across the distributions. This data takes into account the variation of individual vial spike values and is a better indication of overall performance.

Copper (target value 2.31µg/g)			Iron (target value 75µg/g)		
Results	**B3**	**D1**	**Results**	**B3**	**D1**
Mean	2.38	2.29	Mean	72.46	76.84
SD	0.96	0.80	SD	6.20	11.18
Range	3.76	2.83	Range	20.80	36.65
High	4.50	3.80	High	83.10	101.00
Low	0.74	0.97	Low	62.30	64.35
Recovery	**B3**	**D1**	**Recovery**	**B3**	**D1**
Mean	102.97	99.04	Mean	96.62	102.46
SD	41.57	34.65	SD	8.26	14.91
Range	162.77	122.42	Range	27.73	48.87
High	194.81	164.50	High	110.80	134.67
Low	32.03	42.08	Low	83.07	85.80

Table 6: Comparison of results and recovery between first (B3) and second (D1) dispatches of ERM-BB184 Bovine Muscle

	Material	Target value (µg/g)		Average bias		Number of labs	% improved	Average bias improvement
				B3	D1			
Over recovery on B3	ERM-BB184 Bovine muscle	Cu	2.31	36.00	7.36	6	33	-28.64
		Fe	75	6.76	1.57	3	33	-5.19
Under recovery on B3	ERM-BB184 Bovine muscle	Cu	2.31	-26.58	-12.63	5	60	13.95
		Fe	75	-5.45	-2.75	10	40	2.70

Table 7: Percentage of labs with improved result bias after first (B3) and second (D1) dispatch of ERM-BB184 Bovine Muscle. Participants are divided into those that under or over recovered on the first dispatch.

	Distribution	Material	Certificate values (µg/g)		Average bias		Number of labs	% of labs with improvement
					Unspiked	Spiked		
Under recovery on unspiked sample	A	ERM-BB186	Fe	161	-15.8	-5.1	11	55
			Cu	5.98	-12.6	5.6	12	25
	B	ERM-BB184	Fe	75	-7.4	17.4	11	55
			Cu	2.31	-41.9	47.7	7	57
	C	ERM-422	Fe	9.4	-36.4	-17.6	8	75
			Cu	1.67	-26.4	-2.7	8	50
	D	ERM-BB184	Fe	75	-9.4	30.1	7	43
			Cu	2.31	-30.5	21.5	9	22
Over recovery on unspiked sample	A	ERM-BB186	Fe	161	3.2	3.9	3	0
			Cu	5.98	8.6	19.6	3	33
	B	ERM-BB184	Fe	75	7.8	15.6	4	25
			Cu	2.31	61.6	62.3	9	44
	C	ERM-422	Fe	9.4	68.5	6.4	8	63
			Cu	1.67	60.4	13.4	9	56
	D	ERM-BB184	Fe	75	14.3	16.0	7	43
			Cu	2.31	58.3	31.7	6	50

Table 8: Percentage of labs with an improved results bias for a spiked sample compared to an unspiked sample sent in the same distribution. Results highlighted green show an improvement when spiked and samples highlighted blue show no improvement.

	Iron			Copper			Total		
Distribution	**Number of results**	**Results within CRM limits**	**%**	**Number of results**	**Results within CRM limits**	**%**	**Number of results**	**Results within CRM limits**	**%**
A	28	9	32	30	8	27	58	17	29
B	30	11	37	32	3	9	62	14	23
C	32	15	47	34	10	29	66	25	38
D	28	11	39	30	3	10	58	14	24
Total	118	46	39	126	24	19	244	70	29

Table 9: Unspiked results across all distributions with number and percentage of results that fell within set CRM limits

Distribution reports

TEQ@S

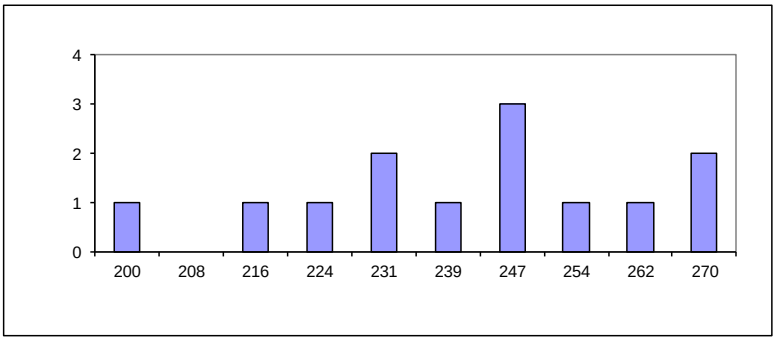
Trace Element Quality at Surrey
UK NEQAS for Trace Elements
Solid Matrix Scheme

Distribution: Jun 2015 | Report date: Aug 2015

Specimen	Description	**IRON**	Comments
A1	Pig Kidney	255 µg/g	59 µg/g (A1) omitted
A2	Pig Kidney + Fe spike	1526 µg/g (Mean Spike Value)	776 µg/g (A2) omitted
A3	Pig Kidney + Cu spike	255 µg/g	53 µg/g (A3) omitted

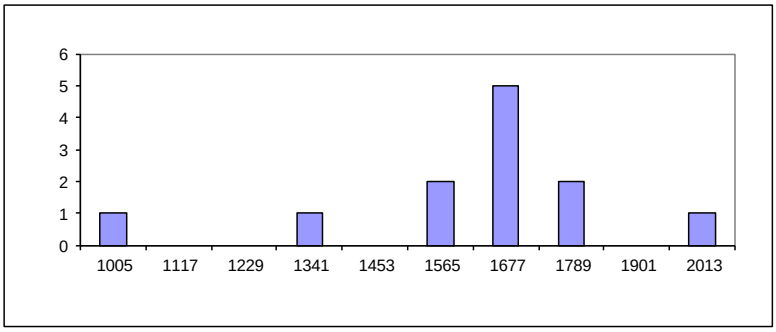
Specimen A1

Number	ALTM	SD	CV(%)
13	238	20.2	8.5



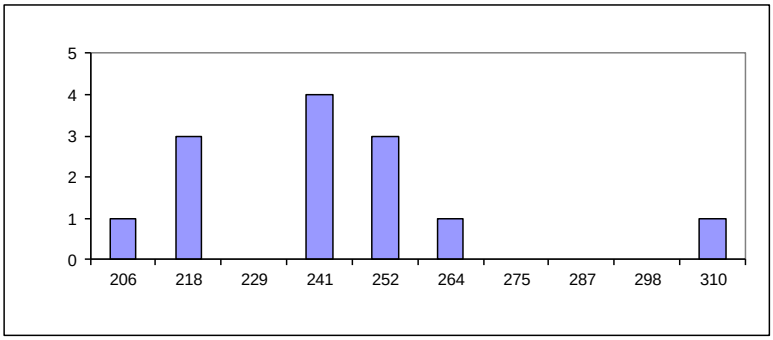
Specimen A2

Number	ALTM	SD	CV(%)
12	1588	249.6	13.1



Specimen A3

Number	ALTM	SD	CV(%)
13	239	27.1	9.1



UK NEQAS

TEQ@S

Trace Element Quality at Surrey
UK NEQAS for Trace Elements
Solid Matrix Scheme

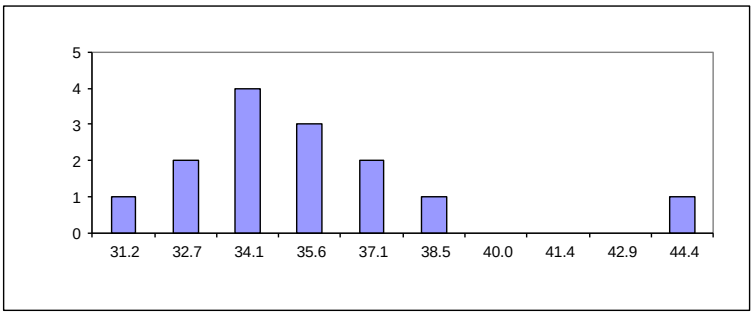
Distribution: Jun 2015 | Report date: Aug 2015

Specimen	Description	**COPPER**
A1	Pig Kidney	36.5 µg/g
A2	Pig Kidney + Fe spike	36.5 µg/g
A3	Pig Kidney + Cu spike	0.0 µg/g (Mean Spike Value)

Comments
12.0 µg/g (A1) omitted
17.0 and 47.5 µg/g (A2) omitted
360.0 µg/g (A3) omitted

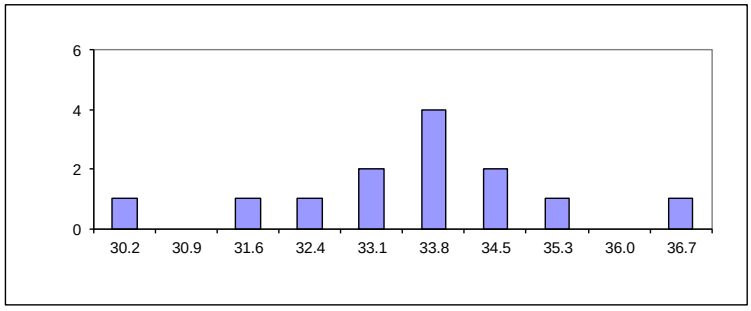
Specimen A1

Number	ALTM	SD	CV(%)
14	35.0	3.3	9.3



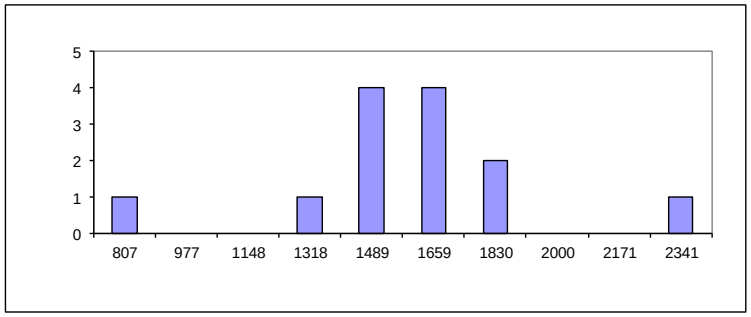
Specimen A2

Number	ALTM	SD	CV(%)
13	33.4	1.7	5.0



Specimen A3

Number	ALTM	SD	CV(%)
13	1549.3	345.49	22.3



TEQ@S

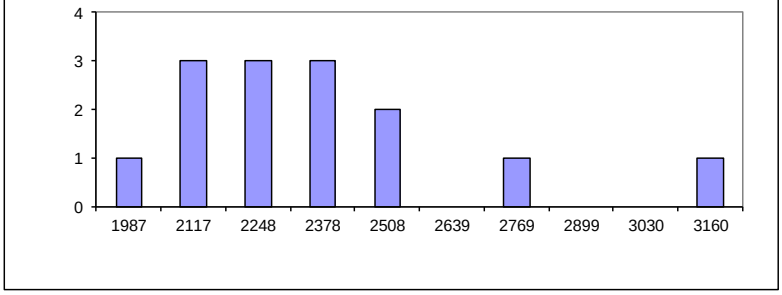
Trace Element Quality at Surrey
UK NEQAS for Trace Elements
Solid Matrix Scheme

Distribution: Sep 2015 Report date: Nov 2015

Specimen	Description	**IRON**	Comments
B1	Bovine Muscle + Fe Spike	2461 µg/g (Mean Spike Value)	
B2	Bovine Muscle + Cu Spike	75 µg/g	4457 µg/g (B1) omitted
B3	Bovine Muscle	75 µg/g	98 µg/g (B2) omitted

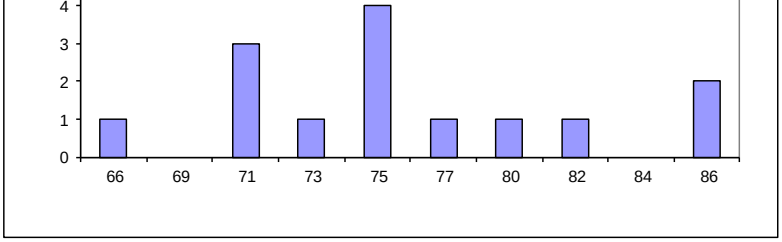
Specimen B1

Number	ALTM	SD	CV(%)
14	2319	306.5	13.2



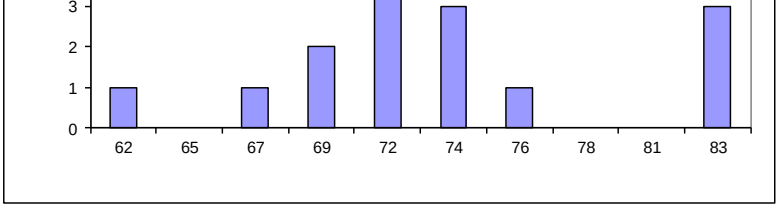
Specimen B2

Number	ALTM	SD	CV(%)
14	75	6.1	7.2



Specimen B3

Number	ALTM	SD	CV(%)
15	72	6.2	7.7



TEQ@S

Trace Element Quality at Surrey
UK NEQAS for Trace Elements
Solid Matrix Scheme

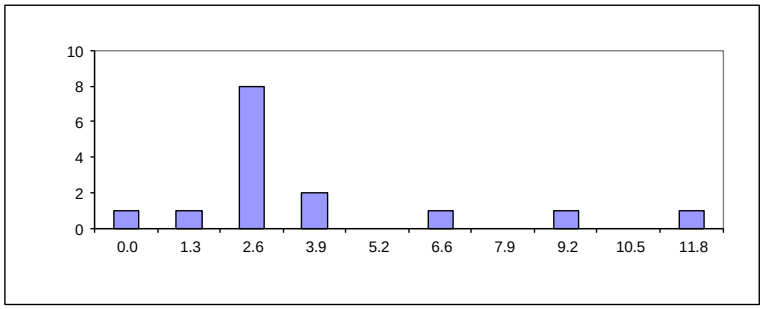
Distribution: Sep 2015 Report date: Nov 2015

Specimen	Description	COPPER
B1	Bovine Muscle + Fe Spike	2.3 µg/g
B2	Bovine Muscle + Cu Spike	1023.1 µg/g (Mean Spike Value)
B3	Bovine Muscle	2.3 µg/g

Comments
39.2 µg/g (B1) omitted
1992.0 µg/g (B2) omitted
9.7 µg/g (B3) omitted

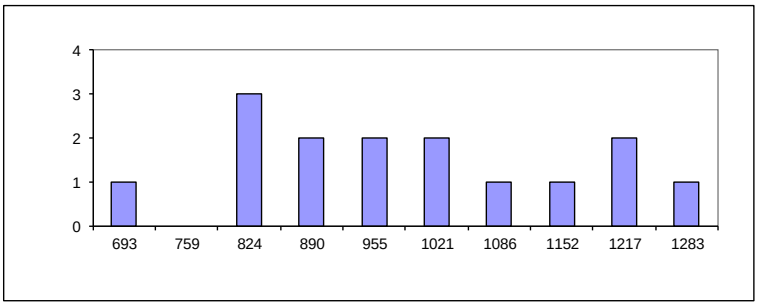
Specimen B1

Number	ALTM	SD	CV(%)
15	3.4	3.1	90.1



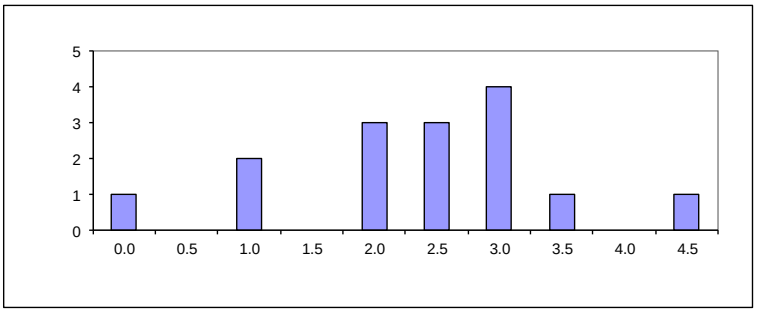
Specimen B2

Number	ALTM	SD	CV(%)
15	958.5	173.3	18.1



Specimen B3

Number	ALTM	SD	CV(%)
15	2.2	1.11	50.0



TEQ@S

Trace Element Quality at Surrey
UK NEQAS for Trace Elements
Solid Matrix Scheme

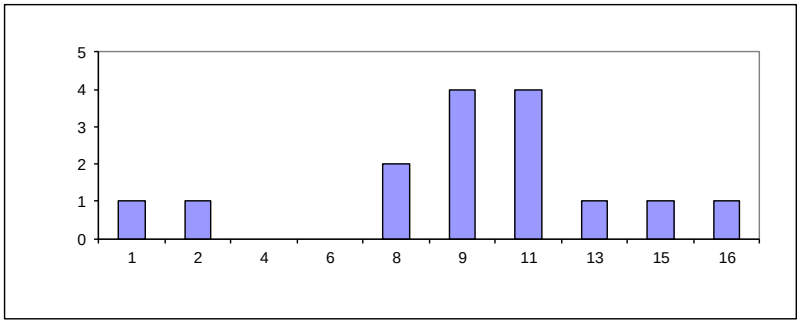
Distribution: Nov 2015 | Report date: Jan 2016

Specimen	Description	IRON
C1	Fish Muscle	9.4 µg/g
C2	Fish Muscle + Fe Spike	1896.1 µg/g (Mean Spike Value)
C3	Fish Muscle + Cu Spike	9.4 µg/g

Comments
44.9 µg/g (C1) omitted
367.7 and 2.1 µg/g (C2) omitted

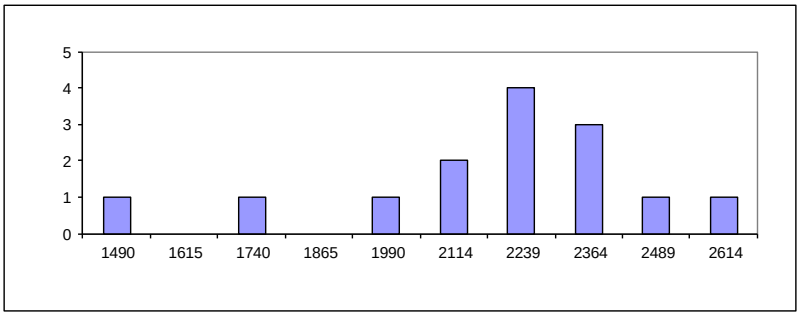
Specimen C1

Number	ALTM	SD	CV(%)
15	9	4.0	46.6



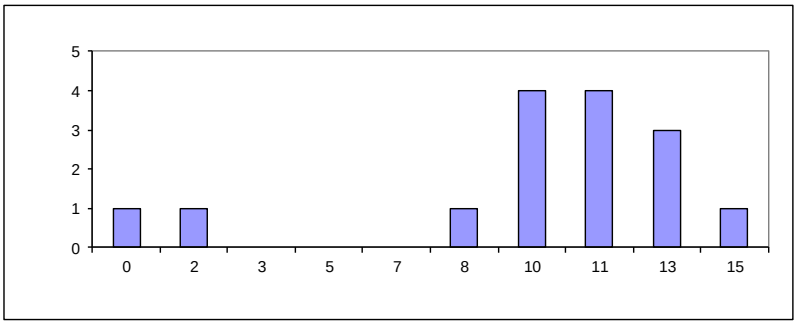
Specimen C2

Number	ALTM	SD	CV(%)
14	2141	283.1	11.4



Specimen C3

Number	ALTM	SD	CV(%)
15	9	3.9	30.1



TEQ@S

Trace Element Quality at Surrey
UK NEQAS for Trace Elements
Solid Matrix Scheme

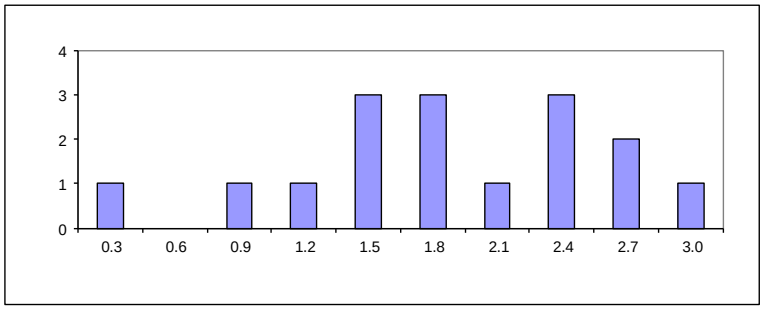
Distribution: Nov 2015 Report date: Jan 2016

Specimen	Description	COPPER
C1	Fish Muscle	1.7 µg/g
C2	Fish Muscle + Fe Spike	1.7 µg/g
C3	Fish Muscle + Cu Spike	701.7 µg/g (Mean Spike Value)

Comments
5.1 µg/g (C1) omitted
5.3 and 4.64 µg/g (C2) omitted
9.3 µg/g (C3) omitted

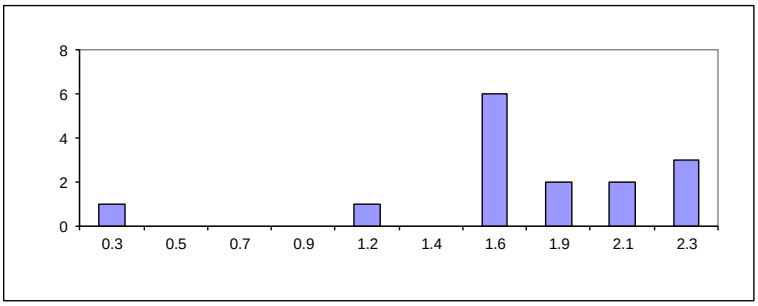
Specimen C1

Number	ALTM	SD	CV(%)
16	1.8	0.7	40.3



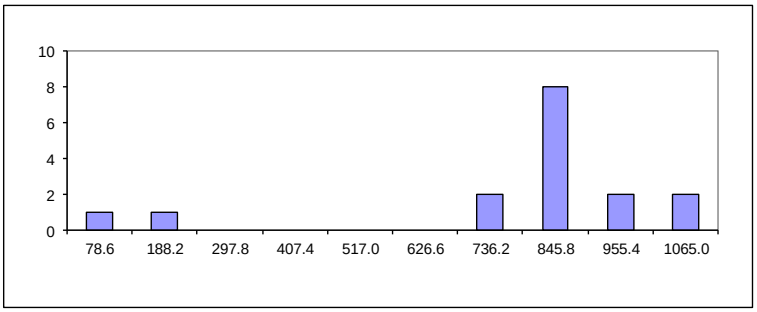
Specimen C2

Number	ALTM	SD	CV(%)
15	1.7	0.5	31.1



Specimen C3

Number	ALTM	SD	CV(%)
16	745.0	264.78	35.5



TEQ@S

Trace Element Quality at Surrey
UK NEQAS for Trace Elements
Solid Matrix Scheme

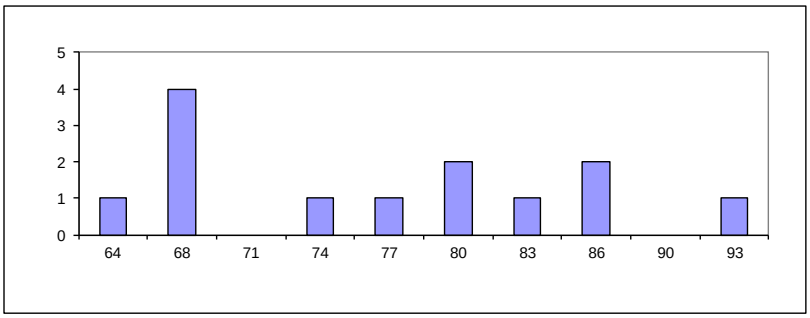
Distribution: Jan 2016 | Report date: Mar 2016

Specimen	Description	**IRON**
D1	Bovine Muscle	75 µg/g
D2	Bovine Muscle + Cu Spike	75 µg/g
D3	Bovine Muscle + Fe Spike	1730 µg/g (Mean Spike Value)

Comments
101.0 µg/g (D1) Omitted
101.5 and 102.0 µg/g (D2) Omitted

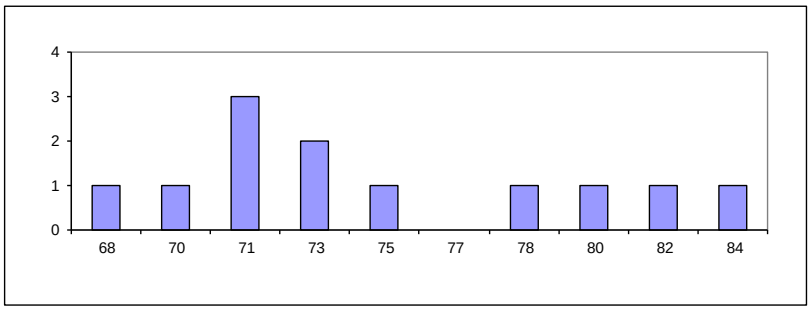
Specimen D1

Number	ALTM	SD	CV(%)
13	75	9.1	12.2



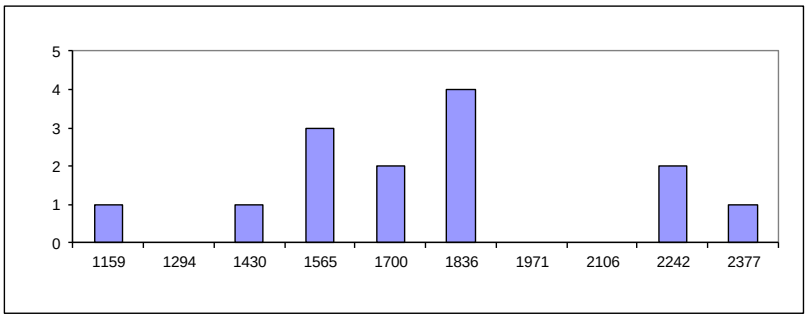
Specimen D2

Number	ALTM	SD	CV(%)
12	74	5.0	6.1



Specimen D3

Number	ALTM	SD	CV(%)
14	1730	333.9	14.9



TEQ@S

Trace Element Quality at Surrey
UK NEQAS for Trace Elements
Solid Matrix Scheme

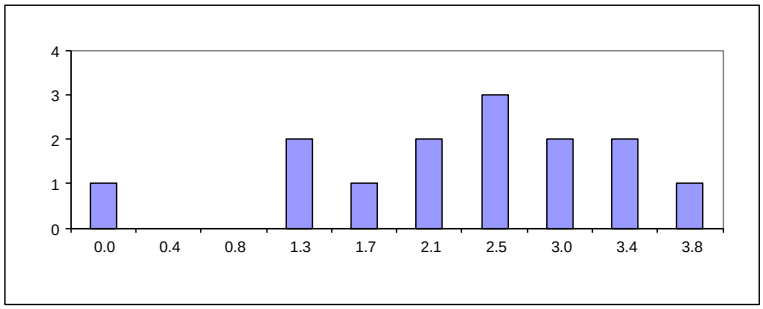
Distribution: Jan 2016 | Report date: Mar 2016

Specimen	Description	COPPER
D1	Bovine Muscle	2.3 µg/g
D2	Bovine Muscle + Cu Spike	832.3 µg/g (Mean Spike Value)
D3	Bovine Muscle + Fe Spike	2.3 µg/g

Comments
6.6 µg/g (D1) Omitted
1.5 µg/g (D2) Omitted

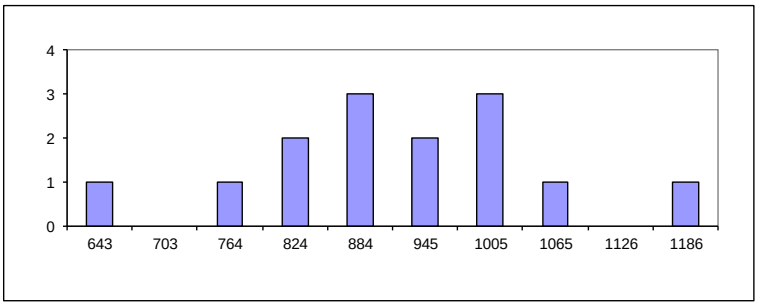
Specimen D1

Number	ALTM	SD	CV(%)
14	2.1	1.0	46.2



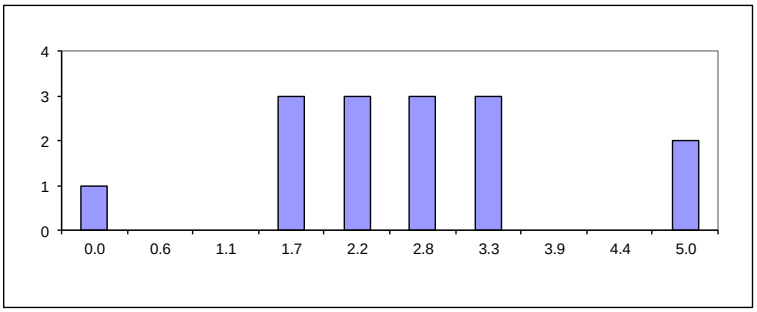
Specimen D2

Number	ALTM	SD	CV(%)
14	891.6	144.0	16.2



Specimen D3

Number	ALTM	SD	CV(%)
15	2.4	1.30	53.0



Discussion

The participant results for this scheme year showed that 70 of the 244 results returned for unspiked samples were within acceptable limits as set by the certified values from suppliers of the materials used. In percentage terms this means 29% of the results were within the CRM limits compared to 23% last year. Deviations from the certified value occurred both above and below the target across all distributions and analytes.

Acceptable results were not evenly spread across the 4 distributions. Table 9 shows that the third distribution had the highest percentage of samples within the CRM limit; where 38% of results returned were within the limits set. This compares to 29% for the first distribution, 23% for the second and 24% for the fourth. Iron had a higher percentage of recovery within acceptable limits (39%) compared to copper at 19%.

Table 6 details how labs improved their performance in cases where the same material has been distributed twice in the scheme year.

Results were also split into those that had returned results with a positive bias (over-recovery is most likely due to contamination) and those with a negative bias (under-recovery is most likely due to loss of materials). Table 7 shows that those that under-recovered in their first distribution improved more than those that over-recovered: 60% of labs that under-recovered copper and 40% of labs which under-recovered iron improved; compared to 33% for both copper and iron for the labs which over-recovered. Failure to improve on over-recovery could be due to continuing contamination issues; here the use of multiple digestion blanks may help remove the bias. Where labs have consistently under recovered over both sample distributions we would recommend a reassessment of their digestion conditions, with a view to better mineralisation of the samples.

Table 8 shows the improvements participants made with the same sample material when it was spiked to increase the concentrations of copper and iron. Overall, 57% of the labs with over-recovery on the unspiked samples and 44% with under-recovery went on to improve their bias on the spiked samples.

Recovery results were also not evenly spread across the 4 distributions. The first distribution showed under recovery with the mean at 95.5%. An over recovery mean of 123% was seen with the second distribution with improvement of 118% for the third distribution and 113% for the fourth. Overall copper had the highest over recovery at 118%, compared to iron at 106%.

This year the lab that produced the most accurate results (table 3) used TMAH digestion (tetramethylammonium hydroxide – a strong alkali) in 3 out of the 4 distributions. Although largely accurate, there was imprecision in results with those using a heating block or microwave to aid digestion. Using acid for digestion without heating has given the least accurate results with a mean recovery of less than 20%. Most labs used ICP-MS for analysis and there was little difference between the accuracy of the copper and iron returns, with an overall error of 6% for iron and 16% for copper which is a slight improvement on last year where both had an average error of around 20%.

Of the materials distributed, the Pig Kidney (Distribution A) gave the most accurate returns for both copper and iron. The mean recovery of copper for distribution A was 100% with the lowest mean standard deviation (SD) of 18.6 and a mean coefficient of variation (CV) of 18.7**.** By comparison the next best performance was Bovine Muscle (Distribution D) which gave a mean recovery of 115% for copper, with a mean SD of 47.8 and a mean CV of 43.8.

Conclusions

There has been a slight increase year on year in the accuracy of the returned results, with 70 out of 244 (29%) results being within acceptable bounds this year, compared with 60 out of 264 (23%) for 2014 and 58 out of 304 (19%) from 2013.

The scheme has helped to highlight areas of the analytical procedure used for the preparation of liver biopsy samples that require improvement to achieve the best possible accuracy. In general the best accuracy was achieved by TMAH digestion; further data would be needed to determine if this is preferable over the more common HNO_3. The lab with the most under recovery did not heat the sample and therefor it is advised to use a method of heating during digestion. In analysis, ICP-MS appears to give the best results. Information on methods used by labs is invaluable to us as it helps to give advice and guide people towards the best method for digestion and analysis and identifying potential issues which prevent precise and accurate

recovery rates. This will be the focus of the 2016-2017 scheme with participants requested to provide full details of the method used and pre-digested samples being included in the dispatches to give a clearer indication of method bias against analysis bias with a view to determining best practice.

The organizers would like to thank the participants for their involvement in the scheme and request that they draw its existence to the attention of other laboratories who may wish to take part.

If participants have any comments on this report or the scheme in general then these should be directed to Dr Chris Harrington (Scheme Manager) at the TEQAS office (rsc-tr.guildford-eqa@nhs.net).
