

LGC Experience in Certification of Reference Materials by Interlaboratory Comparisons



4005

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LGC Limited

- **Outline**
 - **LGC Reference Materials**
 - **Accreditation Status**
 - **Interlaboratory studies**
 - **When used**
 - **Organisation**
 - **Data treatment**
 - **Value assignment**
 - **Issues**

LGC Certified Reference Materials

- ***Matrix materials***

- ***Food***
- ***Environment***
- ***Clinical***
- ***Industry***
- ***Forensic***

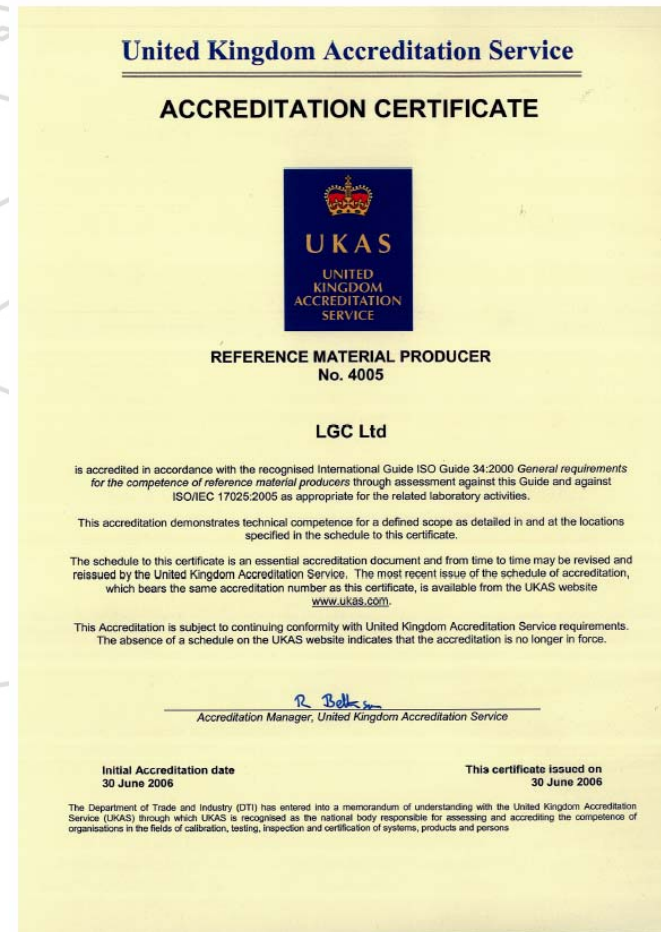
- ***Single substance
organic materials***

- ***Purity***
- ***Melting point***
- ***Enthalpy of fusion***
- ***Elemental content***

~150 materials

Relevant Guidelines

- **ISO/IEC 17025**
 - **General Requirements for the Competence of Calibration and Testing Laboratories**
- **ISO Guide 34**
 - **General Requirements for the Competence of Reference Material Producers**
- **ISO Guide to the Expression of Uncertainty in Measurement (GUM)**



Reference Material Production

- ***Sourcing***
 - ***Preparation / purification***
 - ***Sub-sampling***
 - ***Homogeneity***
 - ***Characterisation***
 - ***Stability***
 - ***Certification***
 - ***Post certification activities***
- 

RM Production Planning

Produce a written project plan for production of each reference material

- **Production Steps**
- **Material specification**
- **Sourcing, preparation and sub-division**
- **Homogeneity assessment**
- **Stability assessment**
- **Characterisation of the assigned value(s)**
- **Calculation of the assigned value and its uncertainty**
- **Documentation and storage of the material**
- **Sales and storage and distribution**
 - **LGC Standards**

Project Planning Form	
WORKING TITLE	
(Reference:)	
PPF-Type Certification	Status: DRAFT under discussion
Project information	
Integrated scientific area:	
Action number:	
(C)RM-project responsible:	
Resources needed:	Internal Delegate: External Collaborator: 0 FTE days; €k
Action Leader:	
Service providers:	
Processing:	
Dispatch:	
Stability Monitoring:	
Quality Management:	
(C)RM identifier	(C)RM-Matrix:
CRM: <input type="checkbox"/>	Target parameters:
Proficiency testing: <input type="checkbox"/>	
Feasibility study (FWP V&V): <input type="checkbox"/>	
Others: <input type="checkbox"/>	
EUR-report already available: <input type="checkbox"/> EUR	

Material Certification

- **Certification Panel**
 - *In house group*
 - *Project team members*
 - *Independent experts (e.g. statistics)*
 - *Quality Team representative*
- **European Reference Materials Co-operation**
 - *Technical review by experts from German (BAM) and European (IRMM) metrology and reference material organisations*
 - *ERM Panel approval*

Activities of Certification Panel

- **Review:**
 - *the intended use/original specification of the material*
 - *the expertise of the RM producer / subcontractors*
 - *the approaches used to characterise the property values*
 - *the approaches used to assess homogeneity and stability*
 - *supplementary information from the characterisation studies*
 - *statistical processing of raw analytical data and results*
- **Examine**
 - *records on the sourcing and preparation of the RM*
- **Approve**
 - *documentation for the reference material*
- **Authorise**
 - *the release of the RM for use*
- **All essential details of the review and examination of data are recorded**

Certificate (ISO Guide 31)



CERTIFICATE OF ANALYSIS

ERM[®] - AC020a

<i>trans</i> -5,6-Dihydro-4-methoxy-6-(2-phenylethenyl)-2H-pyran-2-one (Kavain)		
Parameter	Certified value ¹ (mass %)	Uncertainty ² (mass %)
Purity	99.8	0.2

1) The certified value is traceable to the analytical methods and standards used in the characterisation study and described in the certificate.

2) The quoted uncertainty is the half-width of the expanded uncertainty calculated using a coverage factor (k) of 2.45, which gives a level of confidence of approximately 95 %.

This certificate is valid for 12 months from the date of shipment provided the sample is stored under the recommended conditions.

The minimum amount of sample to be used is 2 mg.

NOTE

European Reference Material ERM[®] - AC020a was produced and certified under the responsibility of LGC according to the principles laid down in the Technical Guidelines of the European Reference Materials' co-operation agreement between BNM-LGC-IRM. Information on these guidelines is available on the Internet (<http://www.erm.com.org>).

Accepted as an ERM[®], Teddington, August 2006.

Signed: _____

Dr John Marriott, UK Government Chemist
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Queens Road
Teddington
Middlesex
TW11 6LY, UK



4026

All following pages are an integral part of the certificate.

ERM[®] - AC020a
Page 1 of 4

DESCRIPTION OF THE SAMPLE

A batch of D,L-kavain obtained from a commercial supplier of chemical reagents was mixed and dispensed as 10 mg units into screw-capped amber glass vials.

The material was considered to be homogeneous on the basis of High Performance Liquid Chromatography with UV detection (HPLC-UV) measurements on 10 randomly selected 2 mg portions, which showed no significant variation in purity value.

The identity of the material was confirmed by 250 MHz ¹H-NMR spectroscopy and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS/MS) utilising accurate mass measurement for determination of elemental formulae in combination with product ion MS.

The material contains a mixture of D and L kavain; the proportion of these isomers in the material is not necessarily the same as that found in nature.

INTENDED USE

The primary use of this reference material is for the calibration of methods for the determination of kavain in herbal products, foodstuffs and other relevant matrices.

ANALYTICAL METHOD USED FOR CERTIFICATION

High Performance Liquid Chromatography with UV Detection (HPLC-UV)

The purity of the material was determined by HPLC-UV using a reversed phase column (150 × 2 mm, Phenomenex Luna C18 (2), 5 μm), with an isocratic mobile phase consisting of 55 % water and 45 % acetonitrile and a flow rate of 0.2 mL/min. Solutions of kavain in acetonitrile (0.01 mass %) were prepared from 9 units of the material. The purity of the material was quantified by peak area normalisation using UV detection at λ=246 nm.

Differential Scanning Calorimetry

A Polymer Labs STA 625 differential scanning calorimeter was used, with a sample size of approximately 2 mg in aluminium pans and a heating rate of 1.3 °C/min. The instrument was calibrated using a high purity indium certified reference material (LGC2901). A total of 6 determinations were carried out.

Gas Chromatography Flame Ionisation Detection (GC-FID)

The purity of the material was determined by GC-FID using a DB-1 column (60 m × 0.25 mm id, d = 0.25 μm) with a constant flow of helium as the carrier gas (1 mL/min) and column temperature programme of: 30 °C for 1 minute, ramp @ 10 °C to 40 °C, ramp @ 5 °C to 200 °C for 1 minute, ramp @ 1 °C to 270 °C for 20 minutes with the Flame Ionisation Detector (FID) at a temperature of 280 °C. Solutions of kavain in dichloromethane (0.1 mass %) were prepared from 10 portions of the material. Each solution was analysed by cold on-column injection of 1 μL. The purity of the material was quantified by peak area normalisation.

The data from these three techniques is shown in Table 1.

In addition to the above, moisture and inorganic material was also determined:

Moisture

Moisture was determined by coulometric Karl Fischer titration and the value subtracted from the purity determined by HPLC-UV and GC-FID.

Inorganic Content

The percentage of inorganic material was assessed by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES), and confirmed by ash determination at 550 °C. The value was subtracted from the purity value determined by HPLC-UV and GC-FID. The percentage inorganic content was not subtracted from the DSC purity value as this technique allows for the presence of inorganics in the sample.

Homogeneity Testing

- **To identify variations in analyte concentrations between the units**
- **Determine an uncertainty for the homogeneity**
- **Key requirements**
 - **method of high precision**
 - **repeatability conditions**
 - **same method, same reagents, same equipment, same analyst over shortest possible time-scale**
 - **sample size - should reflect amount taken for day to day analysis**

Homogeneity Testing

- **All materials are tested for homogeneity**
- **All analytes in a given matrix are tested**
- **Usually 10 to 30 units analysed in duplicate**
 - **3 times the cubed root of n .**
- **Units analysed in random order, not 'as bottled'**
- **Statistical analysis (ANOVA)**
 - **used to obtain s_{method} and s_{bb}**
- **The larger of s_{method} and s_{bb} is used to estimate μ .**

Stability Testing

- **Approaches**

- ***New stability study***
 - ***Use published and readily available information***
 - ***Use of data from related materials***
 - ***materials, packaging and storage***
 - ***Accelerated testing***
 - ***Isochronous testing***
- 

Isochronous Testing

Accelerated Stability Testing Programme

Storage Time (months)	Storage Temperature				
	37 °C	20 °C	4 °C	-20 °C	-70 °C
1	✓	✓	✓	✓	✓
2	✓	✓	✓	✓	✓
3	✓	✓	✓	✓	✓

Characterisation Approaches

- ***Single Primary (Definitive) Method***
 - ***cost effective if methodology and equipment is readily available***
- ***Accurate Gravimetric Preparation***
 - ***valid and effective where analyte(s) can be added to the matrix homogeneously***
- ***Two or More Independent Methods***
 - ***preferable to have validation information available for methods (precision and accuracy)***
- ***Inter-laboratory Study***

Inter-laboratory Studies

- ***Qualified participants***
- ***QC material supplied with candidate material***
- ***Detailed protocol provided to participants***
- ***Safety data sheets prepared and supplied***
- ***Robust statistical data processing***

- ***Number and availability of participants***

Inter-laboratory Studies - Protocol

- ***Timescale***

- ***“The analyses and reporting of results should be carried out within 4 weeks of receipt of the samples or by March 20th 2007 – whichever is soonest.”***

- ***Candidate Material***

- ***“Five replicate analyses should be performed on this material, to ensure that your laboratory median is a good estimate of the true value of the analytes being measured.”***
- ***“Laboratories should report the results of ALL the replicate analyses that they carry out and should NOT discard any data.”***
- ***“Results should be quoted to 2 decimal places and NOT truncated.”***

Inter-laboratory Studies - Protocol

• QC Material

- “Three replicate analyses should be performed on the QC material for each analyte.”***
- “Laboratories should report the results of ALL the replicate analyses that they carry out and should NOT discard any data.”***
- “Results should be quoted to 2 decimal places and NOT truncated.”***

• Analytical Method to be used

- “Laboratories are free to use a method of their own choice.”***
- “It should be a method they are familiar with and for which they have appropriate method validation data.”***
- “Such data should include information on the precision and trueness of the method.”***

Inter-laboratory Studies - Protocol

- **Quantitative Determination of the Analyte**
 - *“Where the analyte is determined by an instrumental technique (e.g. Mass Spectrometry), the instrument should be calibrated using a reliable standard of the analyte in question.”*
 - *“It is important that you report the source and purity of your calibrant to help with the assessment of your data.”*
- **Reporting of Results**
 - *“All results should be reported in the Excel spreadsheet provided.”*
 - *“Background information, including method details, should be entered in the Word document provided.”*
 - *“The documents should be returned by email if possible.”*
 - *“Both sets of forms should be returned by March 20th to the organiser.”*

Data Processing

- **Robust Statistics**

- **Robust statistical approaches are automated mathematical systems which detect and down weight outliers**
- **Appropriate steps must be taken to ensure the suitability of the data set to be processed**

LGC robust statistical processing stages:

- ***Establish a QC limit and reject labs which do not meet requirements***
- ***Examine raw data for technical issues***
 - ***E.g. inappropriate methods, incorrect reporting***
- ***Ensure underlying normality of data set***
- ***Robust analysis by more than one method***
- ***Assign certified values and uncertainties using a robust estimator***

Establishing a QC limit:

- ***Use a QC material***
- ***Set limits of acceptability***
 - ***Analyte and level***
 - ***Type of matrix of QC***
 - ***Similarity of QC to candidate material***
 - ***10 % to 30 % of known value***
- ***Plot all participant data graphically***
- ***Remove all laboratories whose mean QC value falls outside the limit***

Investigating technical issues:

- ***Data reported in correct units?***
- ***Appropriate method capable of required accuracy and precision?***
- ***Is reported data wrong by an integer factor?***
- ***Was the material stored properly before analysis?***

Normality of data:

- ***Almost all statistical processes in common usage require data to be normally distributed***
- ***Tests available to assess normality***
 - ***Kolomogorov-Smirnov-Lilliefors***
 - ***Shapiro-Wilks***
 - ***Excel normal probability plot***
- ***Outlying data may interfere with normality tests***

Robust processing

- **Median/MADe**

- *Median of each laboratory data set is calculated and the median of medians is then taken as the robust mean of the total dataset*
- *MAD is the median of the absolute differences*
- *MADe = MAD / 0.6745*
 - *Equivalent to standard deviation*

- **H15 estimator (Hubers estimator)**

- *Uses winsorisation to reduce the effect of outliers*
- *Winsorisation modifies outlying results to reduce their effect on the mean of the data*

Robust processing

- ***Median/MADe & H15 estimators can be calculated automatically using an Excel plug in prepared by the Analytical Methods Committee of the Royal Society of Chemistry***

www.rsc.org/Membership/Networking/InterestGroups/Analytical/AMC/Software

Certified Values and Uncertainty

- **Certified values**
 - *Median of the data set*
- **Uncertainty**
 - *Characterisation uncertainty is calculated from the MADe value*

$$u_{char} = s / \sqrt{n}$$

$$u_{char} = \left[MADe \cdot \sqrt{\frac{\pi}{2}} \right] / \sqrt{n}$$

Uncertainty

- **A CRM has one or more property values each with an uncertainty:**
- **Uncertainty has 3 components:**
 - **characterisation**
 - **homogeneity**
 - **stability**

$$u_{CRM} = \sqrt{u_{char}^2 + u_{hom}^2 + u_{lts}^2}$$

$$U_{CRM} = k \times u_{CRM}$$

Normal & Robust Statistics

• Normal Statistics

- All labs
- Outliers removed
- All accredited labs
- All accredited labs with outliers removed
- All non accredited labs
- Non accredited labs with outliers removed
- Data corrected for QC, all labs
- Data corrected for QC, with outliers removed

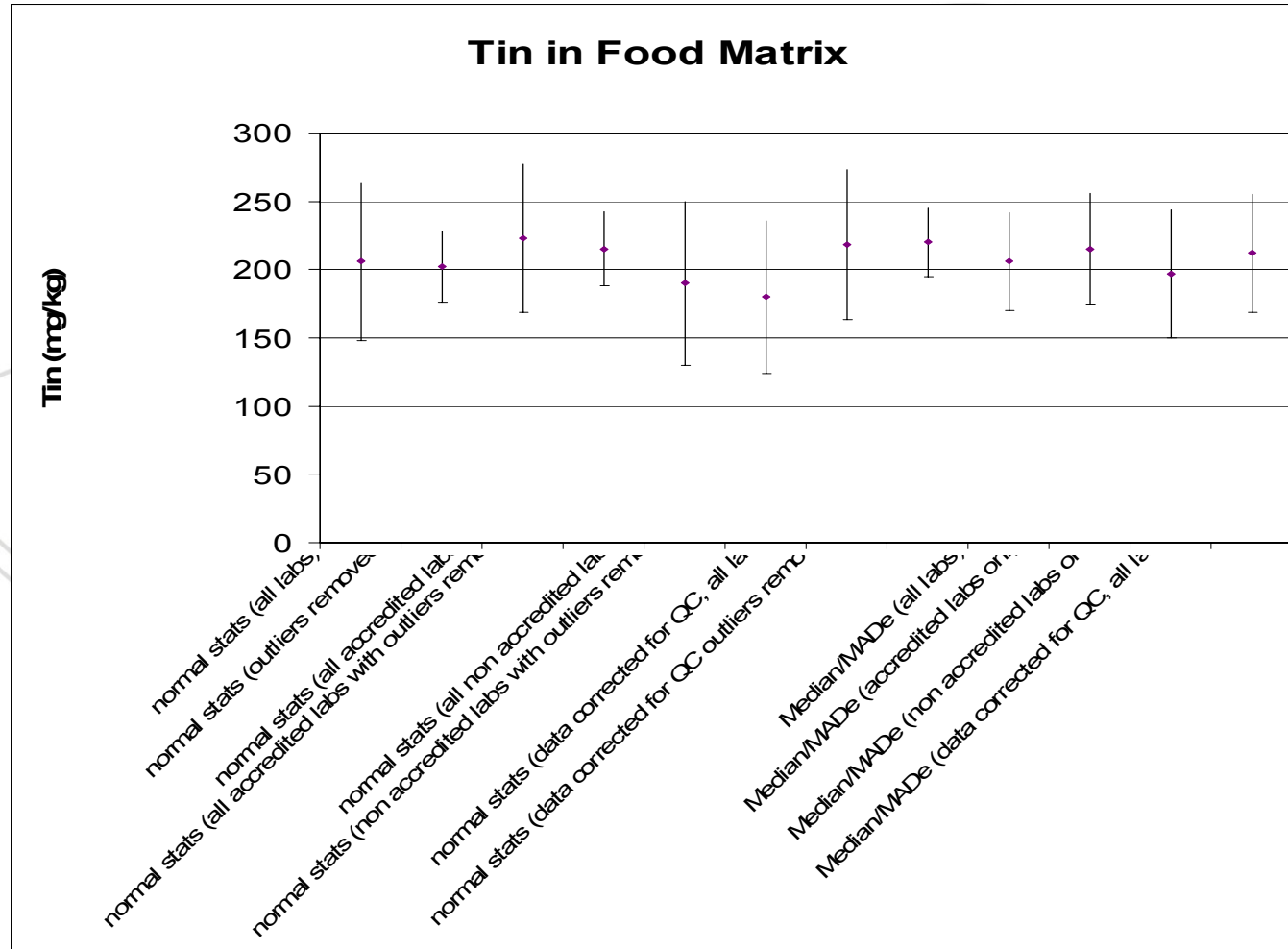
• Robust Median/MADe

- All labs
- Accredited labs only
- Non accredited labs only
- Data corrected for QC, all labs

• Other robust methods applied

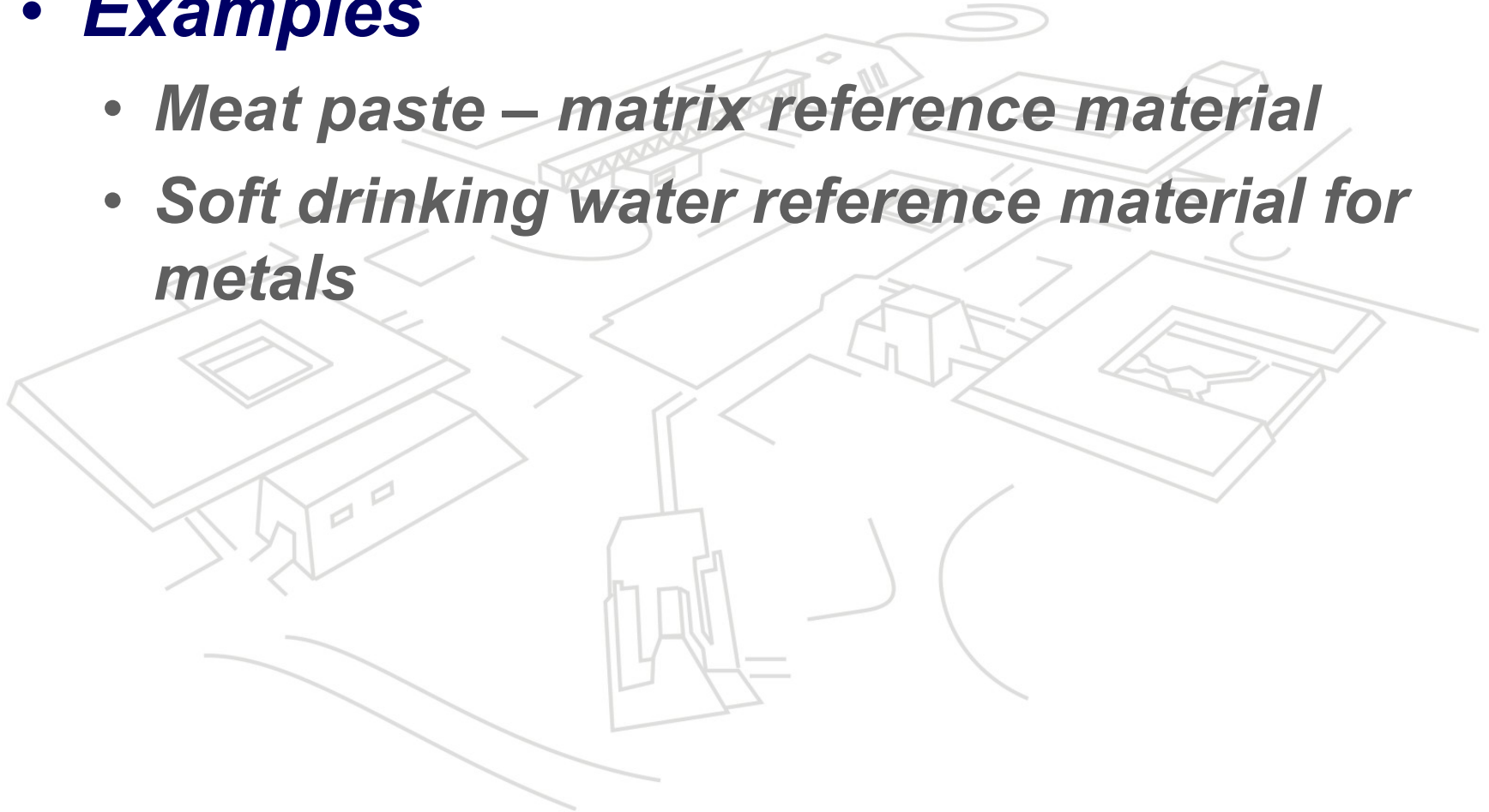
- H15 estimator
- Mixture model median

Effect of different processing approaches



- **Examples**

- **Meat paste – matrix reference material**
- **Soft drinking water reference material for metals**



CASE Study

Meat paste – matrix reference material

Legislation

- **Statutory Instrument 2003 No. 2075**
 - *The Meat Products (England) Regulations 2003*
 - *refers to EU Directive/13/EC for definitions of meat*
- **Statutory Instrument 1999 No. 1136**
 - *The Miscellaneous Food Additives (Amendment) Regulations 1999*
 - *Residual amount for cured/canned meat products:*
 - *E249 Potassium nitrite 50 mg/kg (expressed as NaNO₂)*
 - *E250 Sodium nitrite 100 mg/kg (expressed as NaNO₂)*
 - *E251 Sodium nitrate 250 mg/kg (expressed as NaNO₃) **
 - *E252 Potassium nitrate 50 mg/kg (expressed as NaNO₃) **

Homogeneity

- **10 sachets analysed in duplicate**
- **Moisture – oven 102 °C**
- **Nitrogen – Kjeldahl**
- **Fat – Weibull-Stoldt**
- **Ash - 525 °C**
- **Salt – silver nitrate titration on ashed sample**
- **Hydroxyproline – Modified BS4401 Part 11 1995**
- **Nitrate - HPLC**

Inter-laboratory Characterisation

- ***Interlaboratory study***
 - ***19 laboratories agreed to take part***
 - ***19 laboratories reported results (not for all analytes)***
 - ***13 were UK based***
- 

QC materials

- **Matrix Meat Reference Material**

- **Assigned values include:**

- **Moisture 68.8 +/- 0.1 g/100g**
- **Nitrogen 1.63 +/- 0.05 g/100g**
- **Fat 14.3 +/- 0.4 g/100g**
- **Ash 2.65 +/- 0.09 g/100g**
- **Sodium Chloride 2.19 +/- 0.09 g/100g**
- **Hydroxyproline 0.133 +/- 0.012 g/100g**
- **No value for nitrate**

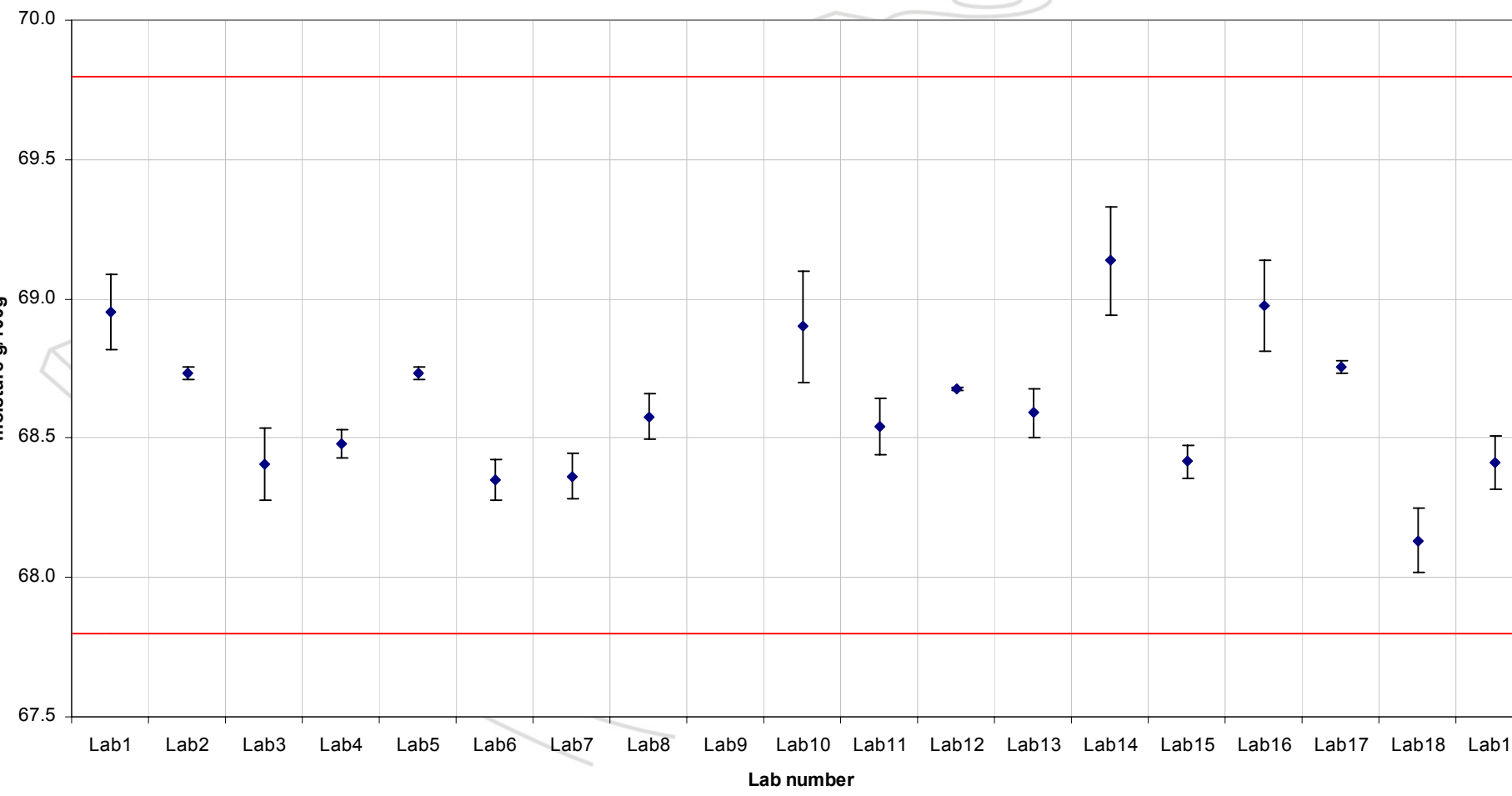
- **For nitrate only**

- **Candidate material 'spiked' with sodium nitrate**

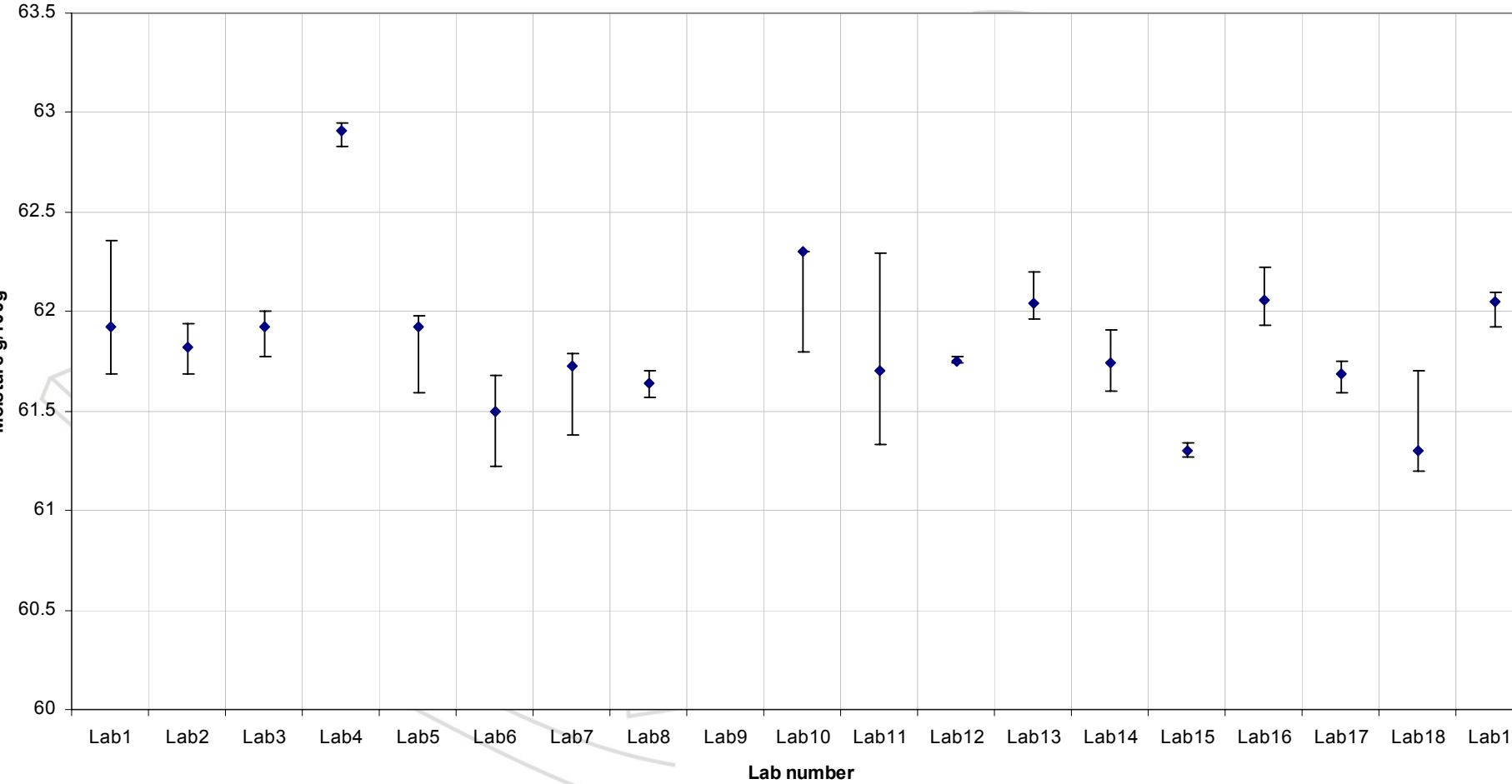
QC limits

	Certified mean	Uncertainty	Repeatability	QC limit for study
Moisture g/100g	68.8	0.1	0.5	1.0
Protein g/100g	1.63	0.05	0.1	0.2
Starch g/100g	14.3	0.4	0.5	1.0
Salt g/100g	2.65	0.09	0.1	0.2
Chloride calculated from certified NaCl content g/100g	1.33	0.05		0.2
Hydroxyproline g/100g	0.133	0.012		0.03
Nitrate as NaNO ₃ mg/kg	No certified value as prepared in –house. Expected value 326 mg/kg, so limits set as 20% expected value.			

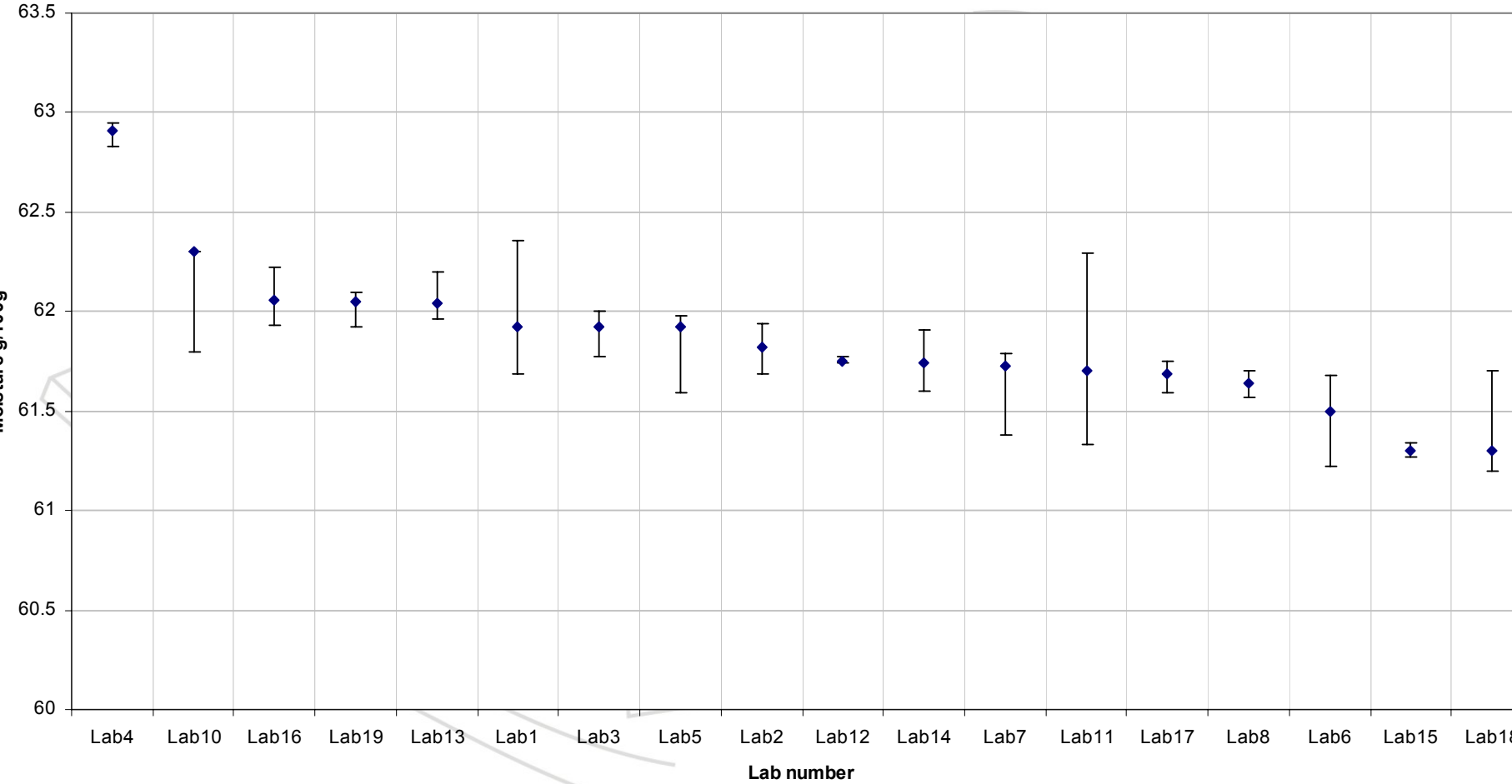
Moisture (QC material)



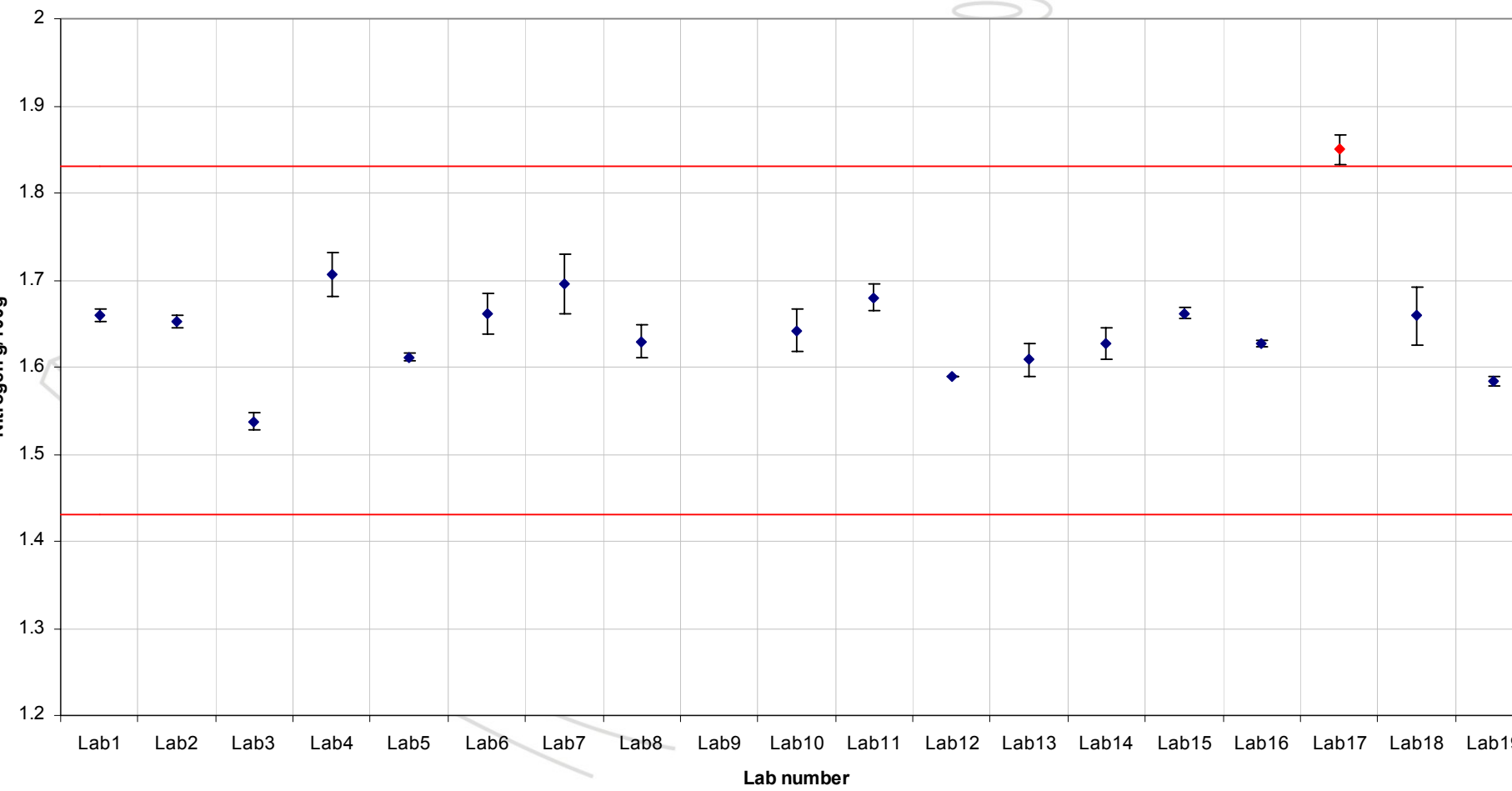
Moisture (candidate material)



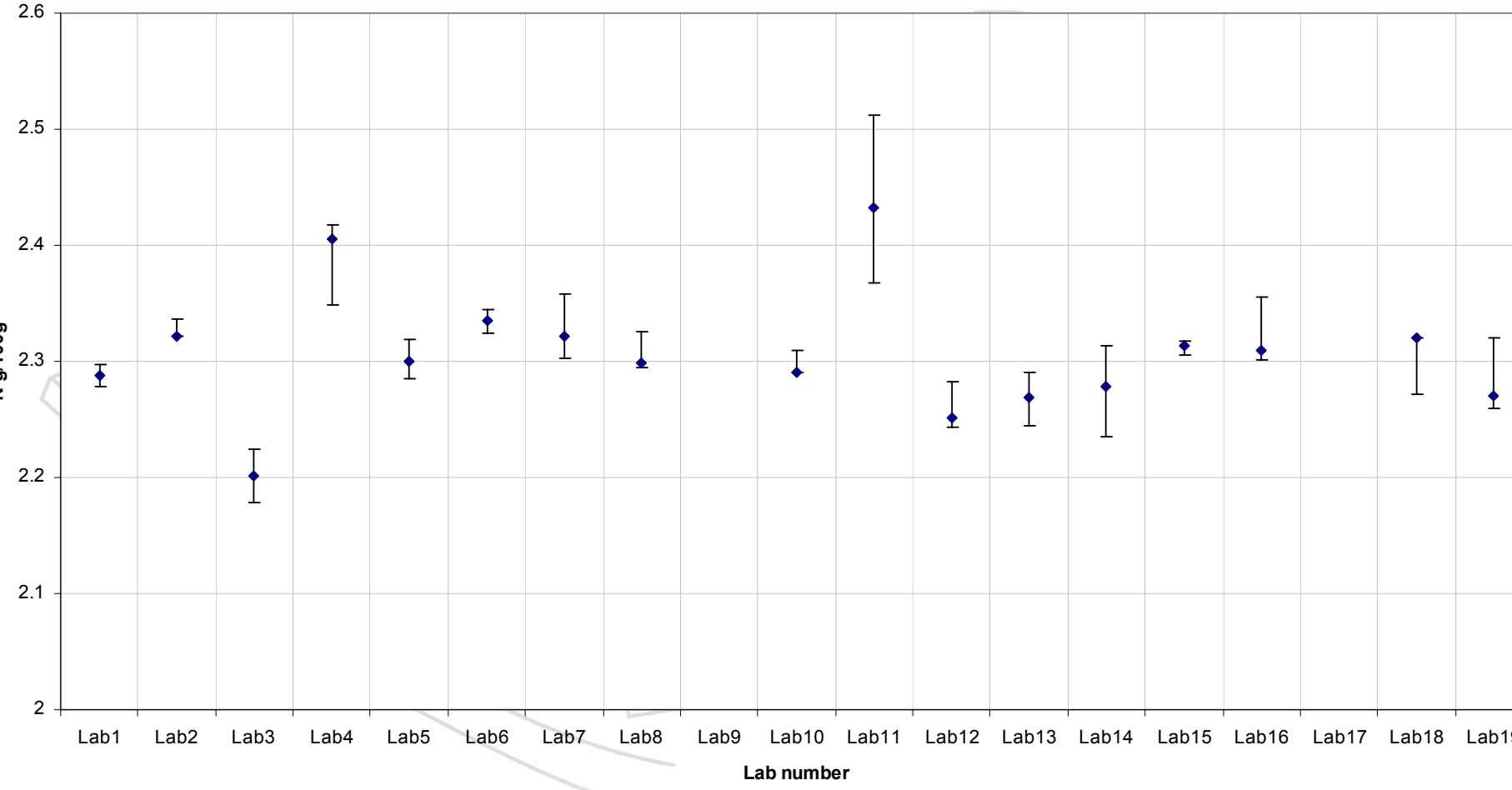
Moisture (candidate material in result order)



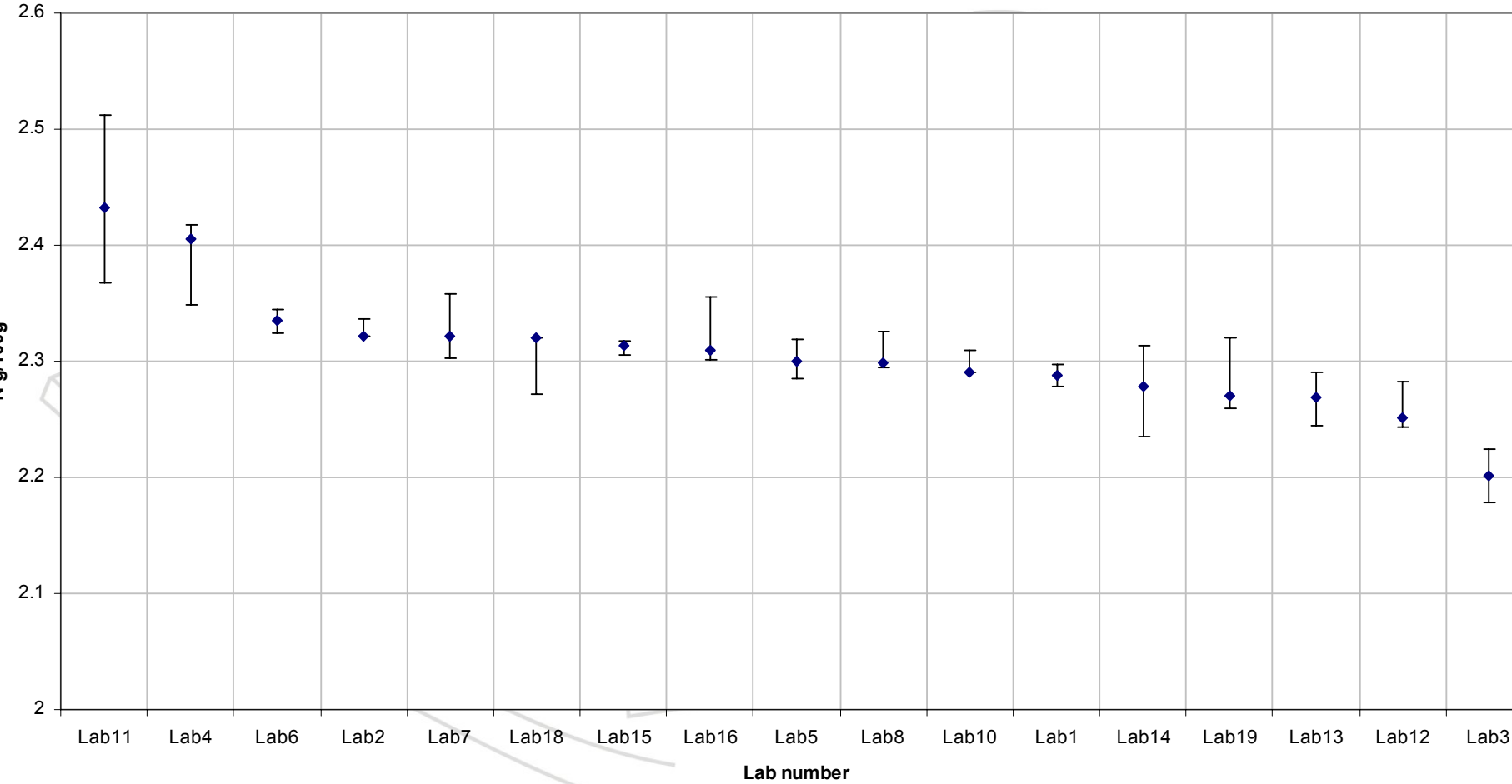
Nitrogen (QC material)



Nitrogen (candidate material)



Nitrogen (candidate material in result order)



QC rejections

	Number of labs reporting results	Number of labs failing QC test
Moisture	18	0
Nitrogen	18	1
Fat	18	1
Ash	18	0
Chloride	18	1
Hydroxyproline	13	0
Nitrate as NaNO_3	11	2

Proposed values

	Value	Uncertainty
Moisture g/100g	61.8	0.6
Nitrogen g/100g	2.30	0.08
Fat g/100g	15.0	1.4
Ash g/100g	3.32	0.10
Chloride g/100g	1.45	0.08
Chloride as Salt	2.39	0.14
Hydroxyproline g/100g	0.33	0.04
Nitrate (as NaNO ₃) mg/kg	286	82

CASE Study

Soft drinking water reference material for metals

Homogeneity

- **30 samples in duplicate for most elements**
 - **10 samples in duplicate for the IDMS elements (Cd, Fe and Zn for this material)**
- 

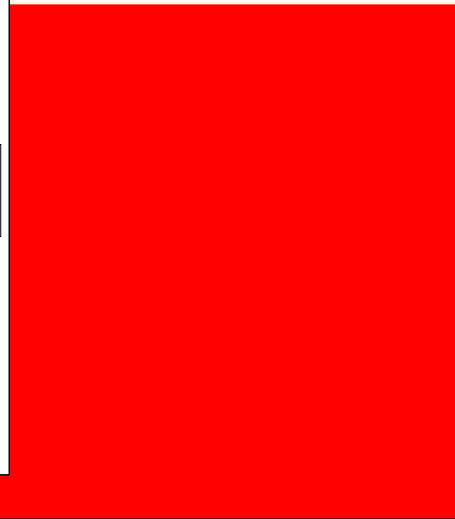
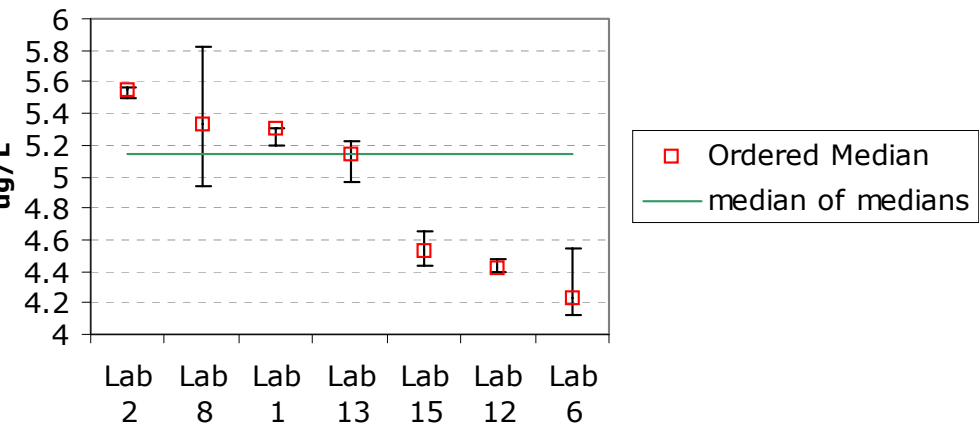
Stability

- ***Data available from the previous batch of this material shows that the material is stable for at least 60 months***
- ***No additional long-term stability measurements have been carried out for this batch of material***
- ***Previous hard water material used homogeneity data as approximation for u_{Its}***

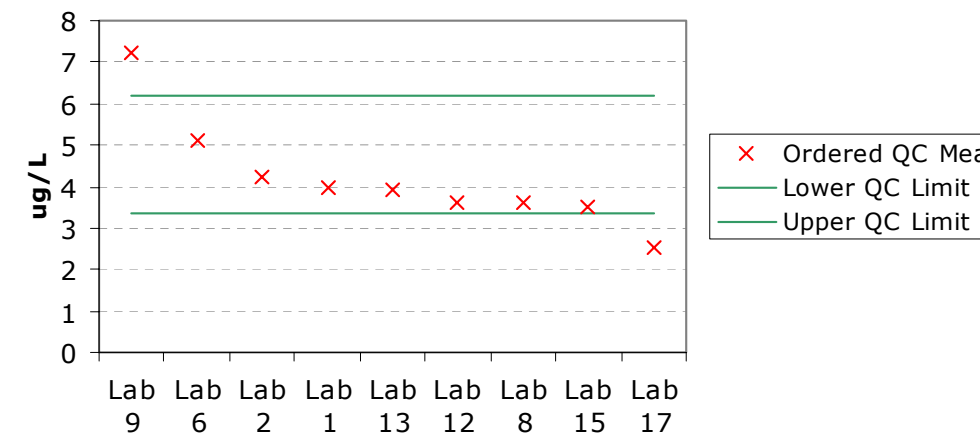
Inter-laboratory Characterisation

- ***18 laboratories agreed to take part***
 - ***17 reported data (not for all analytes)***
 - ***9 were UK based***
- 

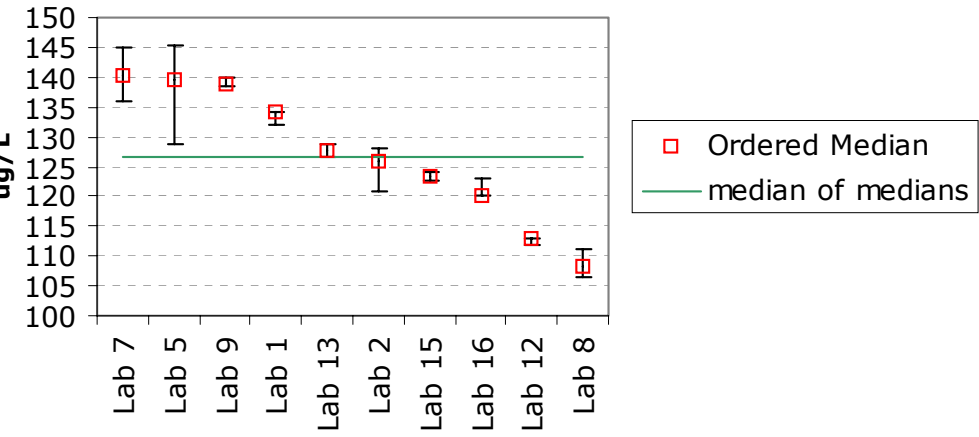
Antimony - candidate material



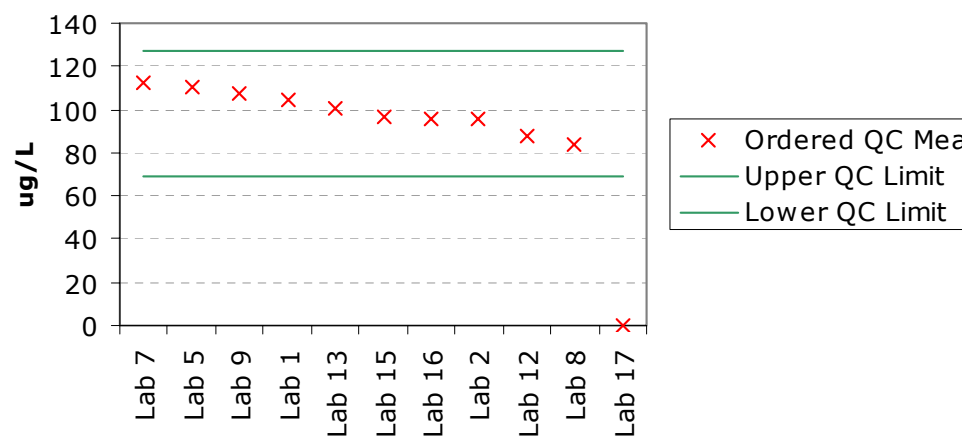
Antimony - QC data



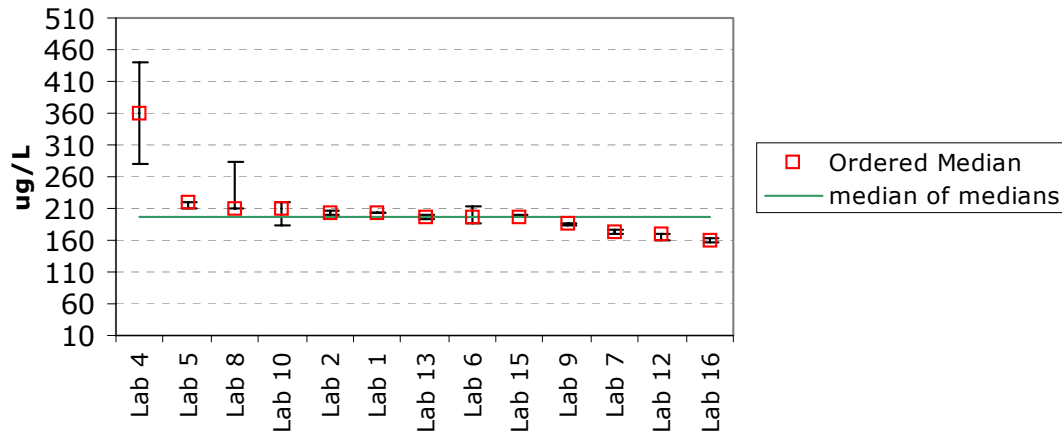
Barium - candidate material



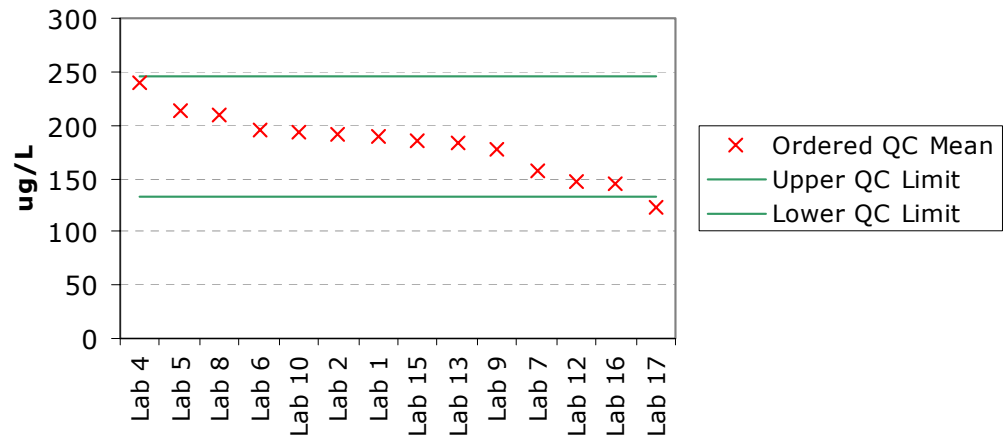
Barium - QC data



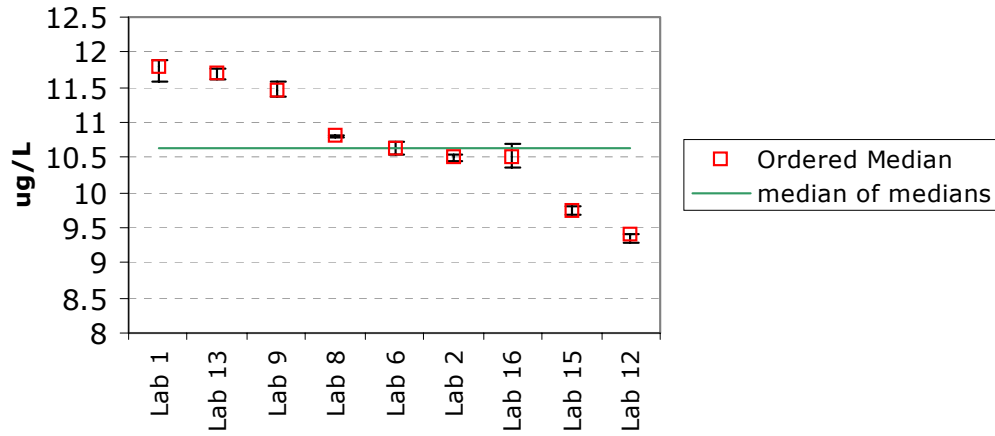
Iron - candidate material



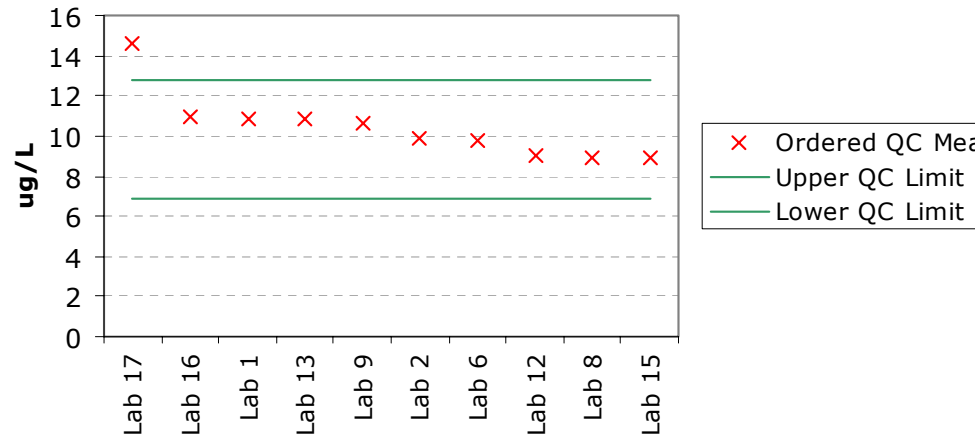
Iron - QC data



Selenium - candidate material



Selenium - QC data



Proposed Values for Certificate

	Certified value	Uncertainty	%
Aluminium	204	11	5.4%
Antimony	5.1	1	19.6%
Arsenic	10.3	1.3	13.0%
Barium	127	13	10.2%
Boron	1066	42	3.9%
Cadmium*	5.26	0.2	3.9%
Copper	2102	67	3.2%
Chromium	51	3	5.9%
Iron*	201	1.26	0.6%
Lead	26	1	3.8%
Manganese	53	4	7.5%
Nickel	21	2	9.5%
Selenium	11	2	18.2%
Zinc*	628	3.2	0.5%
Calcium	7.3	0.3	4.1%
Magnesium	1.01	0.043	4.3%
Potassium	0.37	0.04	10.8%
Sodium	5.8	0.2	3.4%

Issues and challenges

- ***Number of willing participants***
 - ***Transport of samples***
- 

Acknowledgements

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- ***Mark Pettengell***
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 - ***Chemical and Biological Metrology Knowledge Base Programme***
 - ***www.nmschembio.org.uk***