PRIMARY METHODS FOR ORGANIC CHEMICAL ANALYSIS

Willie E. May
Analytical Chemistry Division
National Institute of Standards and Technology
Gaithersburg, MD 20899

CIPM Mutual Recognition Arrangement

Objectives:

- **Establish the degree of equivalence of national measurement standards maintained by NMIs**
- **Provide for the mutual recognition of calibration and measurement certificates issued by NMIs**
- **Provide a secure technical foundation for wider agreements related to international trade, commerce and regulatory affairs**

The CIPM Mutual Recognition Arrangement (MRA) **was signed in October, 1999 by the directors of the NMIs of thirty-eight member states of the *Metre Convention,* and representatives of two international organizations.**

CIPM Mutual Recognition Arrangement

National Metrology Laboratories Must:

- **Declare measurement capabilities that underpin services delivered to customers**
- **Participate in International Key comparisons to validate claims**
- **Provide evidence of competence and Quality systems that underpins delivery of measurement services**

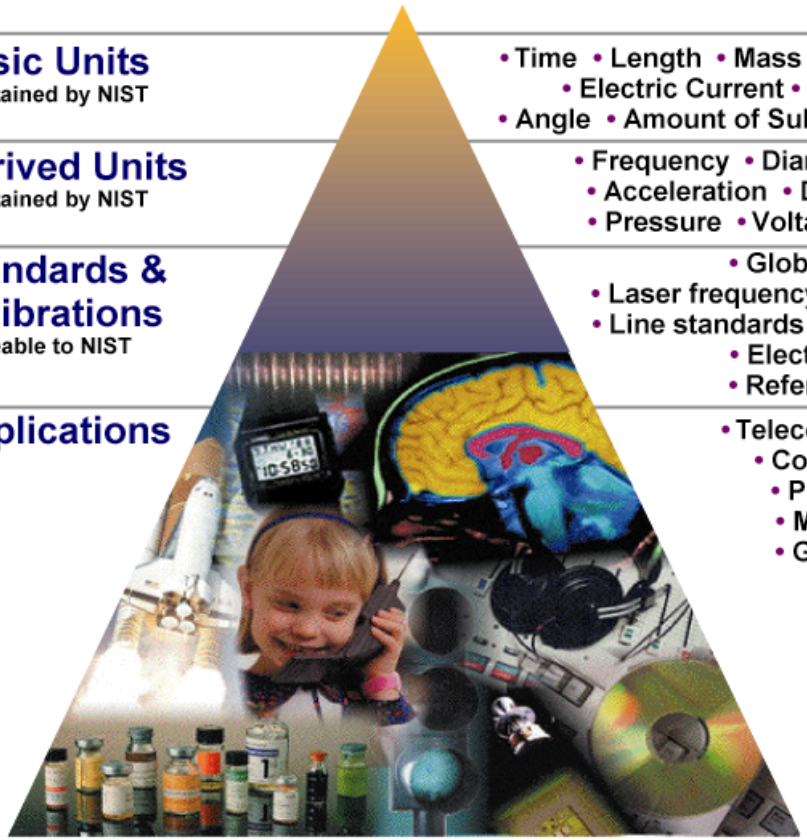
Outcome:

- **Statements of the measurement capabilities of each NMI in a database publicly available on the Web**

Impact of NIST Measurement and Standards Programs

ADVANCING TECHNOLOGY...

- Is vital for commerce and international trade
- Accounts for ~50% of U.S. economic growth
- Drives demand for new measurements and standards
- Requires that NIST maintain state-of-the art scientific facilities



LEVERAGE

Approx. $500 M/yr NIST investment (0.7% of federal R&D)

Undergirds ~$10 B/yr of private sector investment in measurements and standards

Impacts U.S. economy - More than half of $7.6 T/yr U.S. GDP in sales supported by measurement

Food Quality & Adulteration, Healthcare, Forensics, Environmental Quality, Advanced Materials, National Security, Commodities Trading .....

Challenges associated with chemical measurements of “real samples”

- tasked with accurately measuring “practically nothing” in the midst of “everything else” without the benefit of absolute or quantum-based methods
- multiplicity of methods being used
- multiplicity of analytes and matrices

"Primary Methods" for Chemical Analysis

A **primary method** of measurement was defined by the CCQM as a method having the highest metrological qualities, whose operation can be completely described and understood, for which a complete uncertainty statement can be written down in terms of SI units.

In 1998, the CCQM further agreed that:

- A **primary direct method**: measures the value of an unknown without reference to a standard of the same quantity.
- A **primary ratio method**: measures the value of a ratio of an unknown to a standard of the same quantity; its operation must be completely described by a measurement equation."

Examples	Characteristics
• Gravimetry • Coulometry • Titrimetry • Freezing Point Depression • Isotope Dilution MS	• Sound theoretical principles • Negligible systematic errors • Precise, as well as accurate

5th Meeting, CCQM, february 1999

GRAVIMETRY

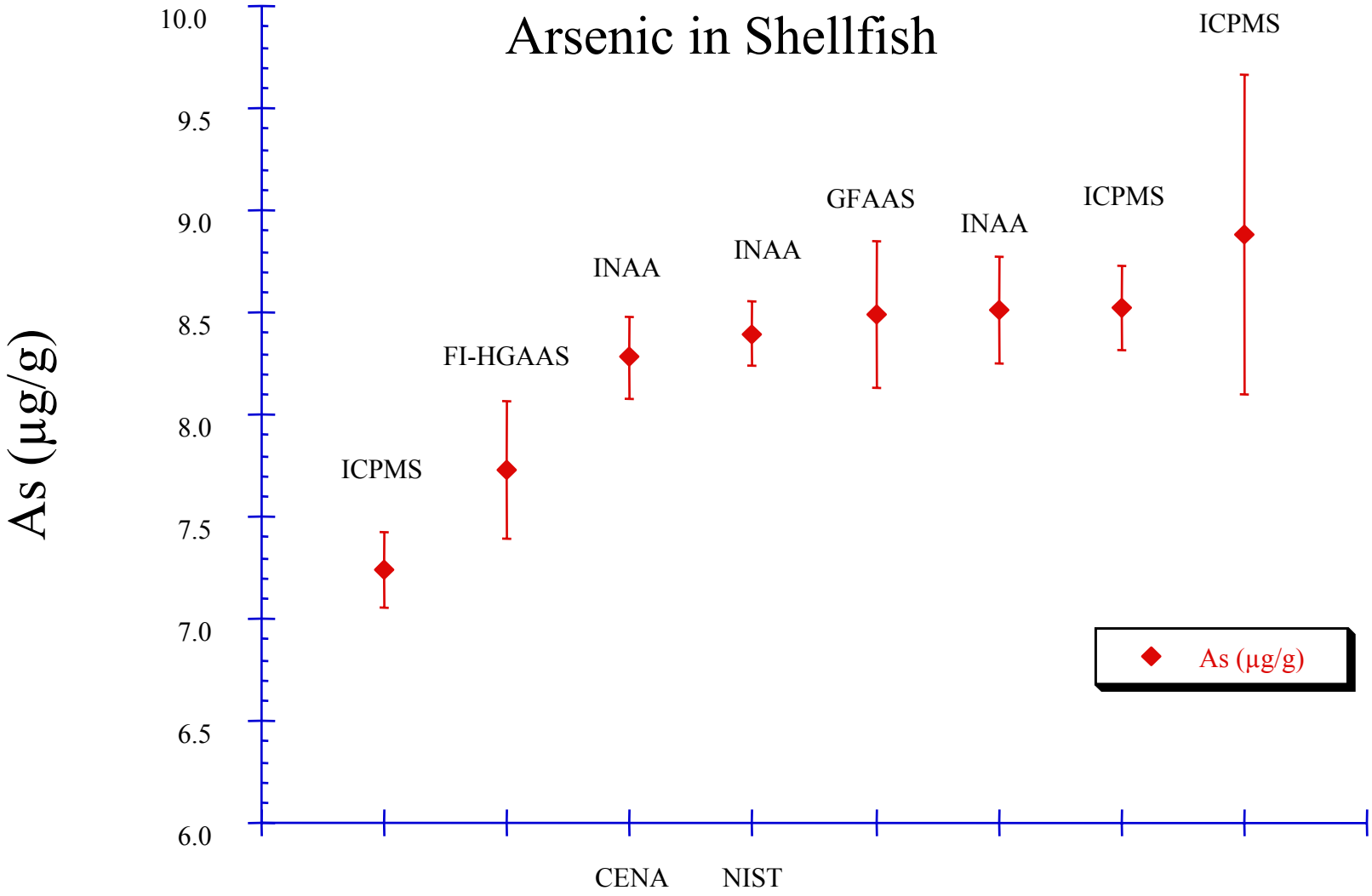
Advantages

- **Can establish SI-traceability via unit of mass**

Limitations

- **Must have enough mass for accurate weighing**
- **Must demonstrate that *analyte only* is being measured**
- **Very laborious for real world samples**

CCQM-K31



Study Period: 2002

Jan 03 Prelim. Plot

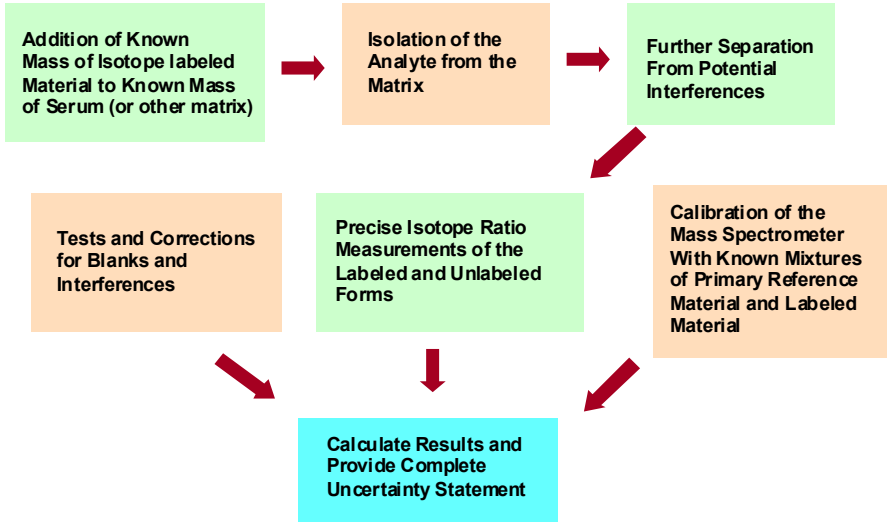
Reference Methods & SRMs for Health Status Markers in Blood/Urine

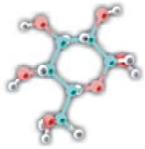
Reference Systems are Currently in Place for Many Well-Defined Markers that are:

- **Relatively small well-defined molecular or elemental species**
- **Typically, can be determined using ID/MS methodology**
- **Such as the following:**

Marker	*Disease State*
Calcium	Cancer, Blood Clotting
Chloride	Kidney Function
Cholesterol	Heart Disease
Creatinine	Kidney Function
Glucose	Diabetes
Lithium	Antipsychotic Treatment
Magnesium	Heart Disease
Potassium	Electrolyte Balance
Sodium	Electrolyte Balance
Triglycerides	Heart Disease
Urea	Kidney Function
Uric Acid	Gout
Vitamins	Nutrition Status

Isotope Dilution/Mass Spectrometry-based Definitive Methods





Glucose

Definitive Methods for Clinical Diagnostic Markers

(well-defined molecular or elemental species)

ANALYTE	*METHOD*	*CONDITION*
Calcium	ID/MS	Cancer, Blood Clotting
Chloride	ID/MS, Coulometry	Kidney Function
Cholesterol	ID/MS	Heart Disease
Creatinine	ID/MS	Kidney Function
Glucose	ID/MS	Diabetes
Lithium	ID/MS	Antipsychotic treatment
Magnesium	ID/MS	Heart Disease
Potassium	ID/MS	Electrolyte Balance
Sodium	Gravimetry, ICP/MS	Electrolyte Balance
Triglycerides	ID/MS	Heart Disease
Urea	ID/MS	Kidney Function
Uric Acid	ID/MS	Gout

NIST Standards for Chemical Measurements

Chemical standards constitute **over 2/3** of ~1,400 NIST SRM types, and **~24,000** of nearly 31,000 NIST SRM Units sold in FY02

- High Purity Neat Chemicals
- Organic Solution Standards
- Inorganic Solution Standards
- Gas Mixture Standards

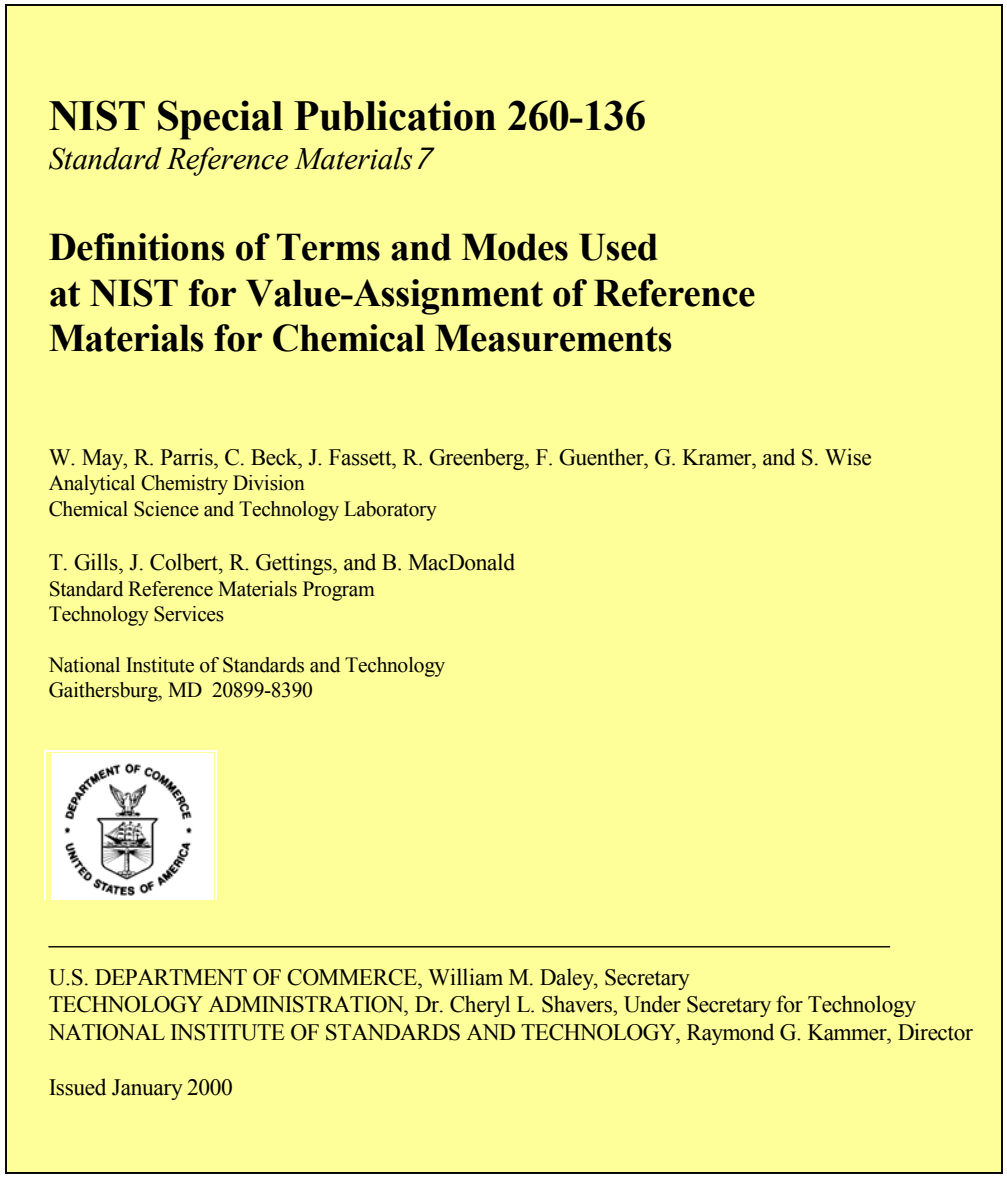
Complex Matrix Standards

- Advanced Materials
- Biological Fluids/Tissues
- Foods/Botanicals
- Geologicals
- Metals and Metal Alloys
- Petroleum/Fossil Fuels
- Sediments/Soils/Particulates
- Cements

- Molecular Spectrometry Standards
- Electrolytic Conductivity Standards
- pH / Ion Activity Standards

As a CRM Provider, NIST has:

- described seven modes currently used at NIST for value-assigning SRMs and RMs for chemical measurements
- defined data quality descriptors used at NIST for these SRMs and RMs
  - NIST Certified Value
  - NIST Reference Value
  - NIST Information Value
- linked these modes to the three data quality descriptors



NIST Special Publication 260-136
Standard Reference Materials 7

Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements

W. May, R. Parris, C. Beck, J. Fassett, R. Greenberg, F. Guenther, G. Kramer, and S. Wise
Analytical Chemistry Division
Chemical Science and Technology Laboratory

T. Gills, J. Colbert, R. Gettings, and B. MacDonald
Standard Reference Materials Program
Technology Services

National Institute of Standards and Technology
Gaithersburg, MD 20899-8390

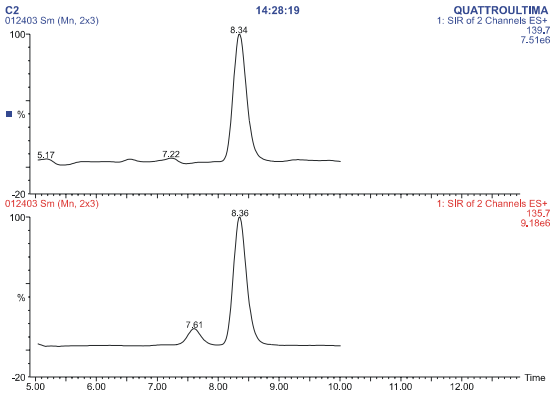
U.S. DEPARTMENT OF COMMERCE, William M. Daley, Secretary
TECHNOLOGY ADMINISTRATION, Dr. Cheryl L. Shavers, Under Secretary for Technology
NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY, Raymond G. Kammer, Director

Issued January 2000

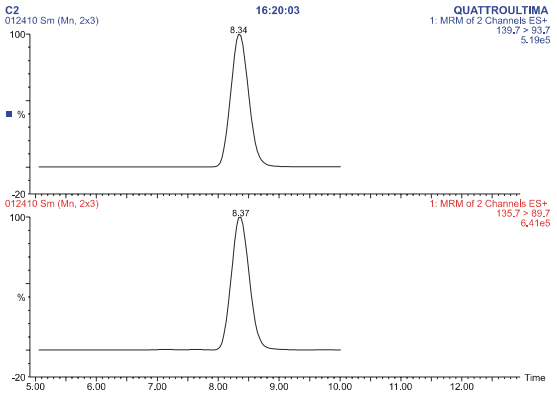
Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements

	Certified Value	Reference Value	Information Value
1. **Certification at NIST Using a Primary Method with Confirmation by Other Method(s)**	✓		
2. **Certification at NIST Using Two Independent Critically-Evaluated Methods**	✓	✓	
3. Certification/Value-Assignment **Using One Method at NIST** and Different Methods by Outside Collaborating Laboratories	✓	✓	
4. Value-Assignment Based On Measurements by Two or More Laboratories Using Different Methods in Collaboration with NIST		✓	✓
5. Value-Assignment Based on a Method-Specific Protocol		✓	✓
6. Value-Assignment Based on NIST Measurements Using a Single Method or Measurements by an Outside Collaborating Laboratory Using a Single Method		✓	✓
7. Value-Assignment Based on Selected Data from Interlaboratory Studies		✓	✓

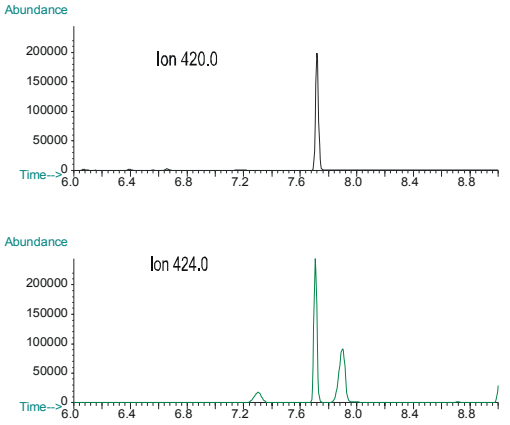
HOMOCYSTEINE



LC/MS



LC/MS/MS



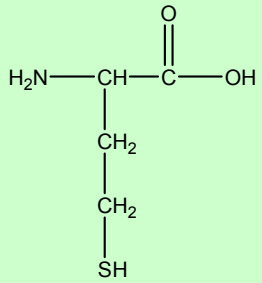
GC/MS

Three Approaches Investigated:
All resulted in successful methods

Next Steps:
Complete critical evaluation of methods

Obtain candidate SRM serum material with three levels of homocysteine and folate

Perform certification measurements using 2 of 3 methods



Results on QC Plasma Samples
micromoles/L

	GC/MS		LC/MS		LC/MS/MS	
	mean	U	mean	U	mean	U
QC1	9.6	0.3	9.6	0.4	9.4	0.4
QC2	13.3	0.5	12.9	0.7	12.4	0.6
QC3	29.8	1.0	29.3	1.5	28.9	1.3

For chemical measurements:

Various instrumental techniques have the **potential** of providing valid, comparable, acceptable measurement results; however:

- Typically the instrumental technique is only part of the measurement process
- All components of measurement process must be clearly understood and sources of bias and uncertainty identified and quantified,

Considerations for specific use:

- Specification of measurand and specificity of method
- Completeness of extraction of measurand from sample / digestion of sample
- Matrix effects
- Etc.

ISOTOPE DILUTION MASS SPECTROMETRY

Principle:

Isotopes of an element have identical chemical Properties but differ in mass

Use of Isotopes:

As internal standard for chemical analysis

Application:

Trace analysis of organic and inorganic substances in a wide range of matrices

PRINCIPLES OF INORGANIC ISOTOPE DILUTION MASS SPECTROMETRY

A known quantity of an isotope or isotopes, usually of low relative abundance, of the element of interest is added to a sample of known mass.

The sample undergoes treatment with strong acids for dissolution and destruction of all organic matter.

Measurements of isotope ratios are made using thermal ionization mass spectrometry (older technique) or inductively coupled plasma mass spectrometry (most widely used today)

Corrections are made for the natural abundances of the isotopes

Chromium (mg/kg) in CRM HISS-1 Marine Sediment (NRC Canada)

GFAAS	ID - ICPMS	INAA
10.9 ± 1.1	11.7 ± 1.3	31.4 ± 4.5

National Research Council Canada | Conseil national de recherches Canada

Footnote on HISS-1 Certificate

"†Chromium in HISS-1

It became apparent during the certification of HISS-1 that there is a significant fraction of Cr that is not easily solubilized. The certified value of **30 mg/kg** was obtained using solid sampling techniques or prolonged digestion with hydrofluoric, sulphuric and perchloric acids.

NRC · CNRC

PRINCIPLES OF ORGANIC ISOTOPE DILUTION MASS SPECTROMETRY

A known amount of an isotopically modified version of the compound of interest is added to a matrix containing the compound of interest.

The ratio between the native form and the isotopically modified form is constant throughout the sample preparation and analysis.

A mass spectrometer is used to measure the ratio between the two forms.

NIST
National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

IDMS – has POTENTIAL for being a primary technique

but there are pitfalls that one must be aware of:

- Isotope Effects
- Non-equilibration
- Chemical Interferences
- Cross Contributions
- Calibration Errors
- Instrument Instability
- Memory Effects
- Differences In Fragmentation

More than 80 CCQM Comparison Completed or in Progress

Health
- clinical diagnostic markers *(cholesterol/heart disease, diabetes/glucose, creatinine/kidney function, trace hormones)*
- electrolytes *(Na, K, Ca)*
- Pb in blood
- Anabolic steroids in urine

Food
- pesticide residues
- antibiotics in meat
- growth hormones in meat
- vitamins and minerals
- drinking water *(EPA List)*

Environment
- air *(EPA HAPs List)*
- soil/sediments
- biological tissues
- waste water *(EPA List)*

General Studies
- *pH*
- *Electrolytic conductivity*

Advanced Materials
- semiconductors
- metal alloys
- polymers and plastics

Forensics
- drugs of abuse
- explosive residues
- breathalyzer *(ethanol-in-air)*
- DNA profiling

Commodities
- emissions trading *(SO_2 in stack emissions)*
- sulfur in fossil fuels
- natural gas
- sucrose
- cement *(Ca, Si, Al, S, Ti, Na, Mg)*
- source of origin/adulteration

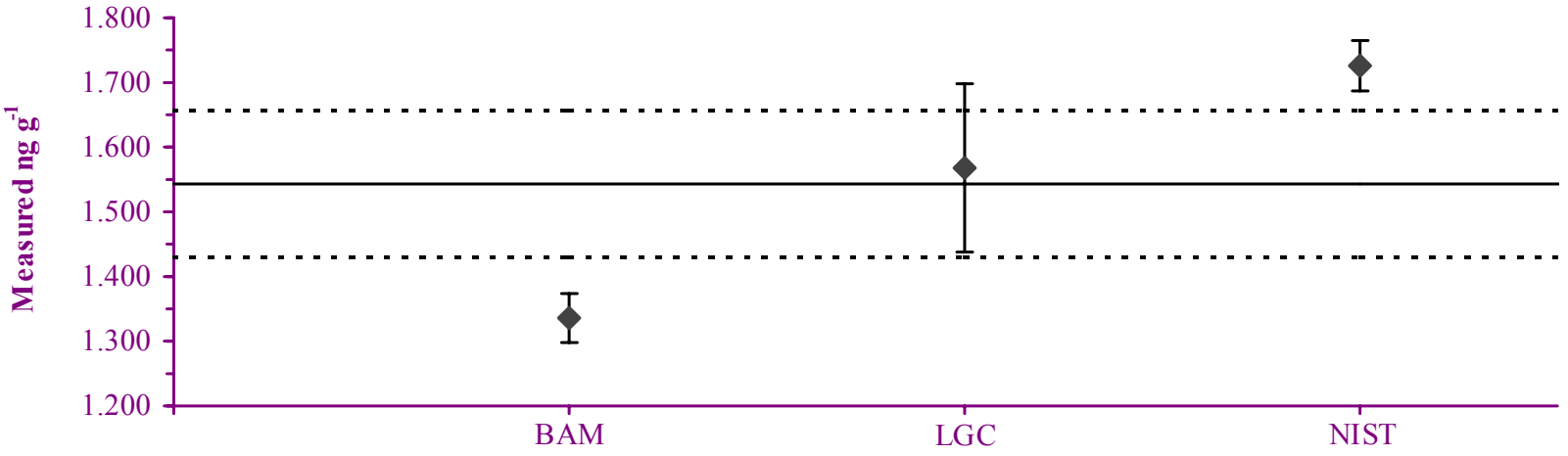
Biotechnology
- DNA Quantitation
- GMO

CCQM-P27: LSD in Human Urine

Final study report agreed to by Working Group

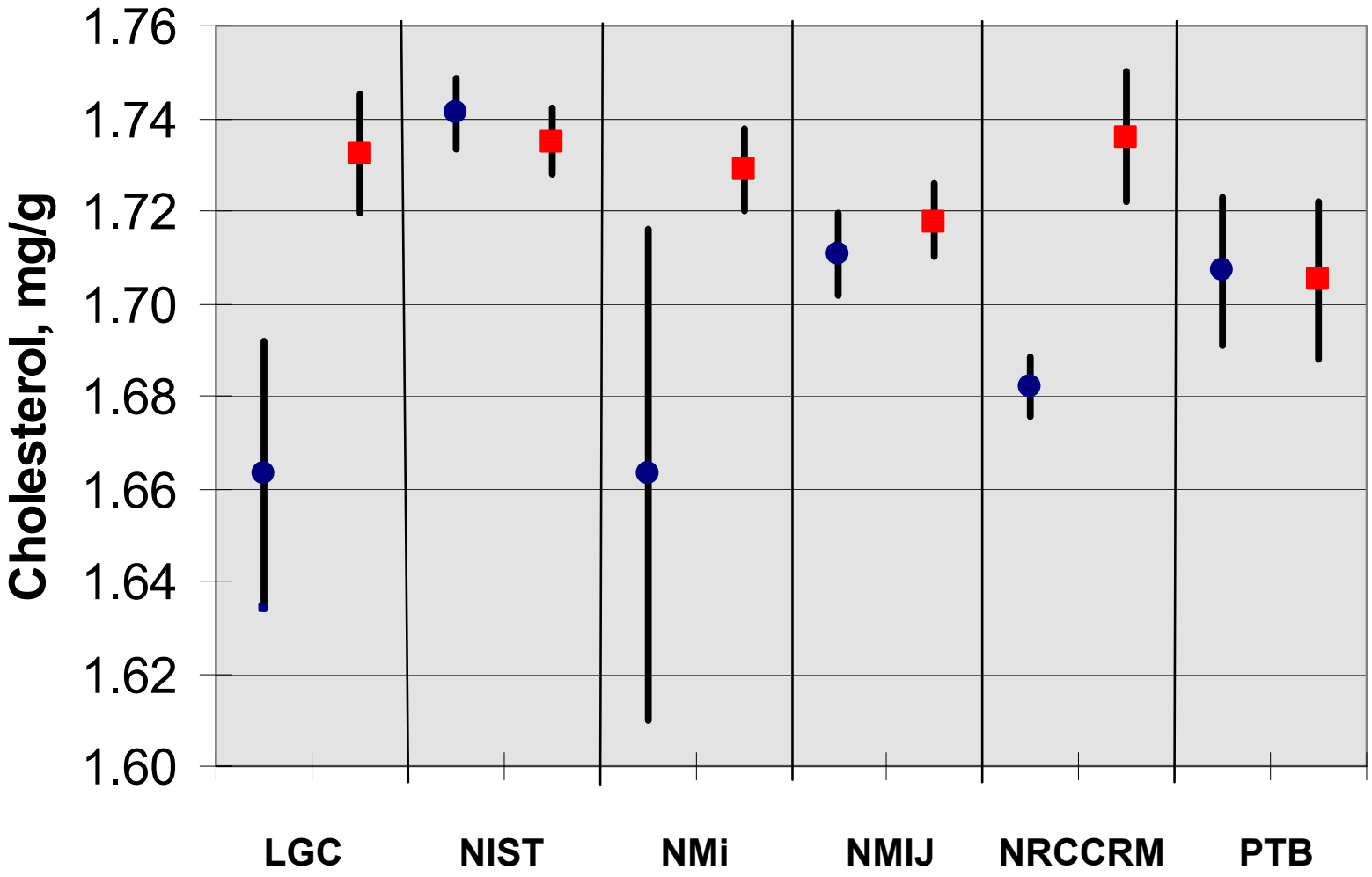
Consensus: The limited numbers of results from this study show considerable variation between participants (RSD 12.7%). It is apparent that accurate measurements of LSD at the trace level in a urine matrix are not readily achievable at the present time. It is noted that intra-laboratory results were very good but inter-laboratory results were poor. This may indicate that more attention should be paid to the accuracy and purity of calibration standards.

WG recommends new pilot study Forensic Drugs in Urine. Will delay starting until ??? Needs Coordinator Laboratory and agreement as to proposed design.



CCQM – Comparison of Results for Cholesterol in Serum in 1999 Pilot Study ● and in 2000 Key Comparison ■

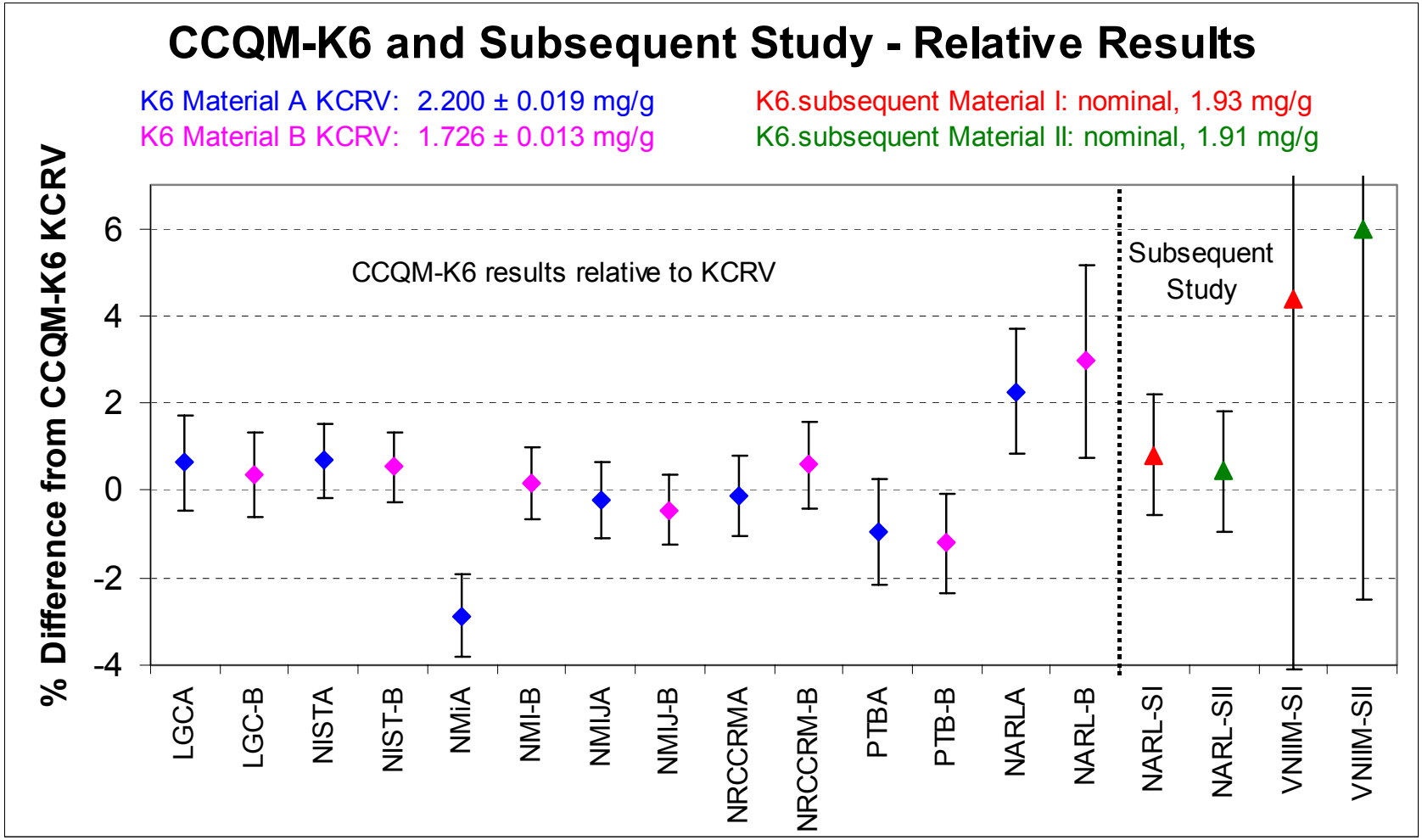
mean ± U: 1.700 ± 0.029 mg/g (Pilot: CCQM-P6 Matl A_p)
1.726 ± 0.013 mg/g (Key: CCQM-K6 Matl B)



Cholesterol in Human Serum

A subsequent comparison to CCQM-K6 to provide Appendix B data for cholesterol in human serum for NARL and VNIIM with NIST serving as the link to CCQM-K6.

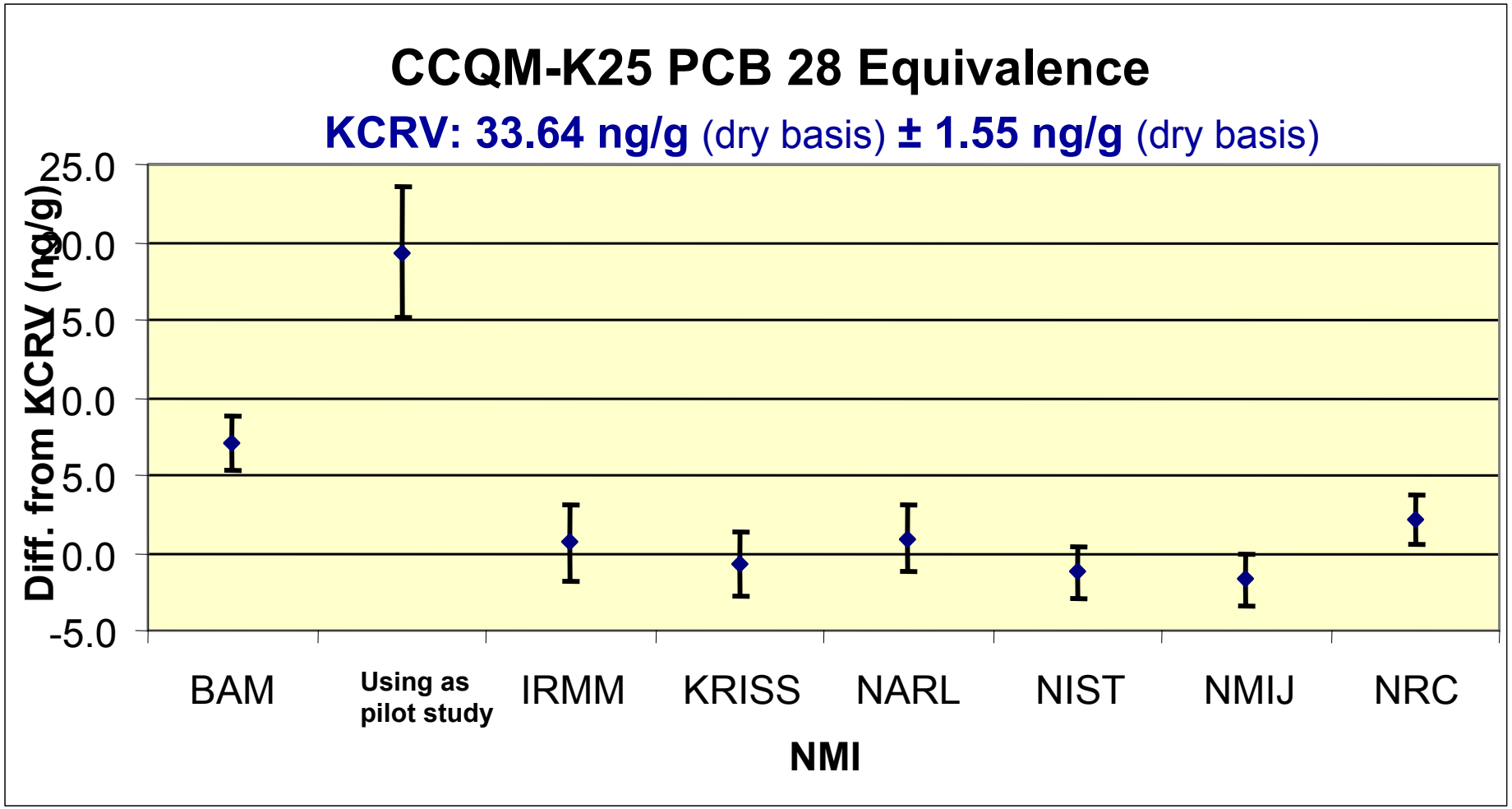
CCQM-K6-Subsequent Materials: Frozen Human Serum - IMEP 17 Materials I and II



K6 results are plotted as % differences from KCRVs

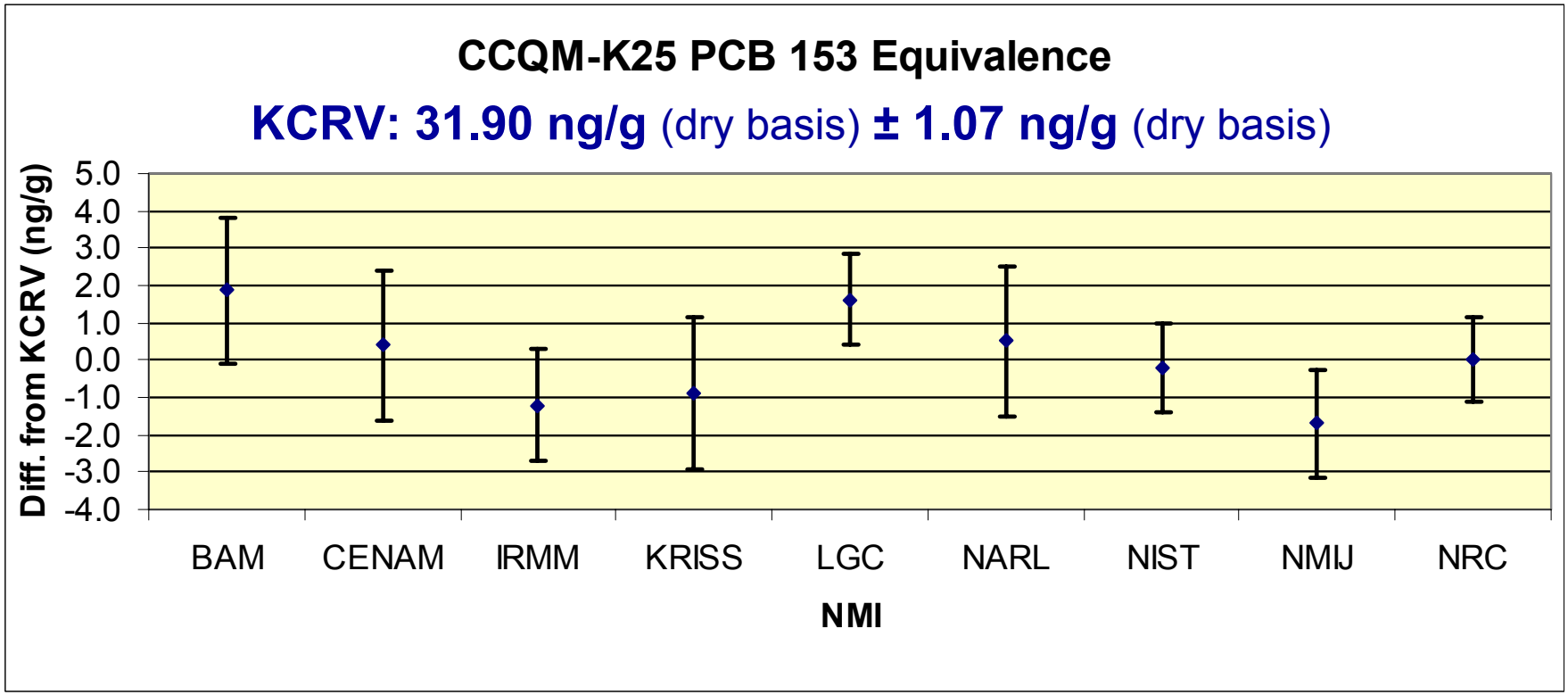
Subsequent results are plotted relative to NIST results in K6S and are offset by average (NIST-KCRV) result from K6 (NIST Ref Pt)

CCQM-K25: PCB Congeners in Sediment

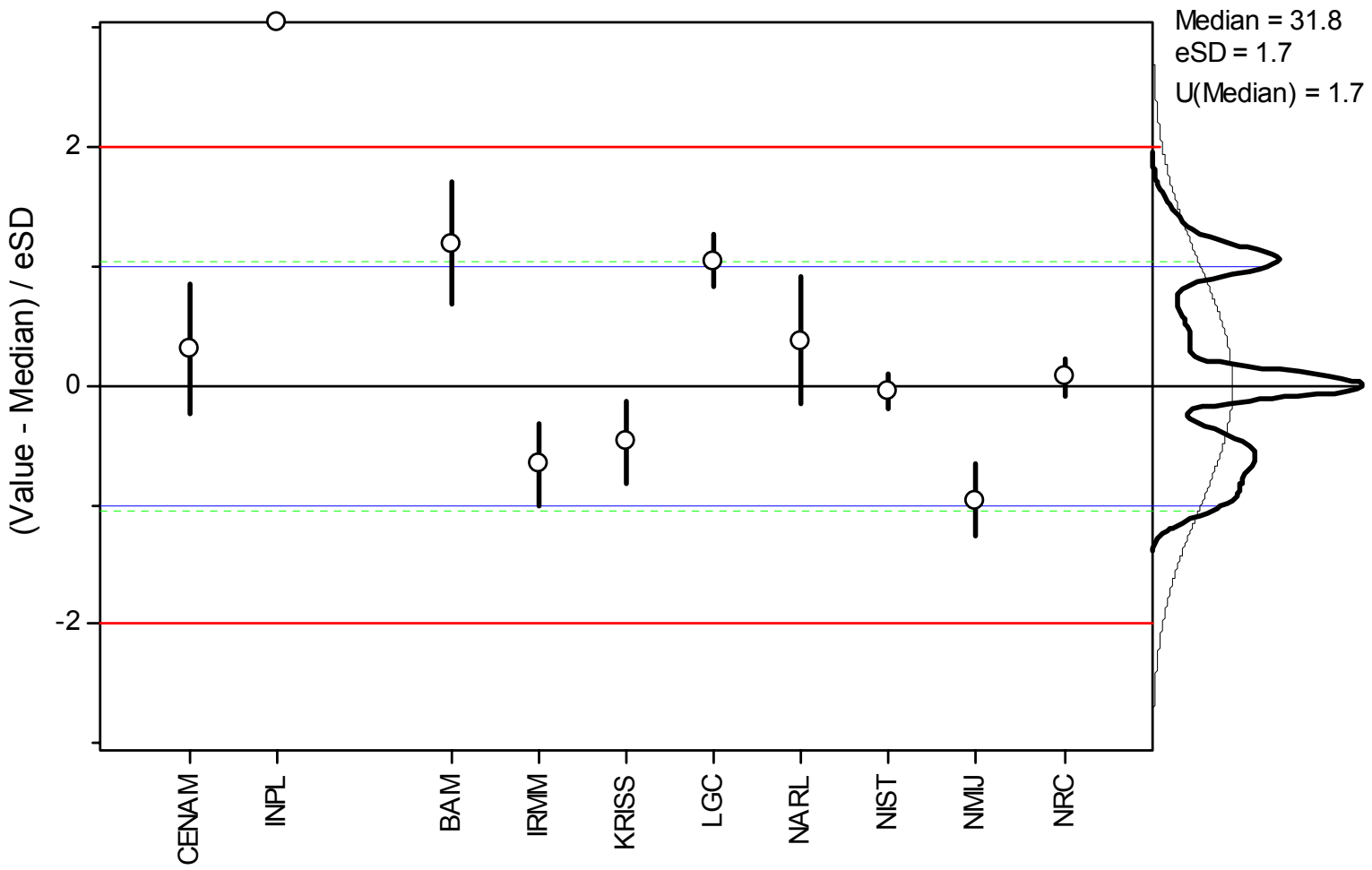


CENAM did not report data for PCB 28 due to a coelution with PCB 50; LGC did not report data for PCB 28 due to "problems."

CCQM-K25: PCB Congeners in Sediment



CCQM-K25 PCBs in Sediment: PCB 153



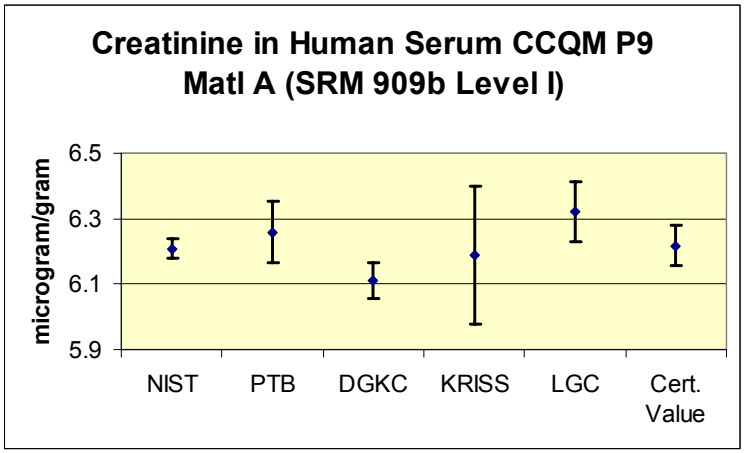
It is recommended that the KCRV for each of the five congeners be assigned as the mean, U, of the eligible results, excluding any statistical outliers. CENAM's results were not included because they did not use carbon-13 labeled PCB congeners as the internal standards/surrogates.

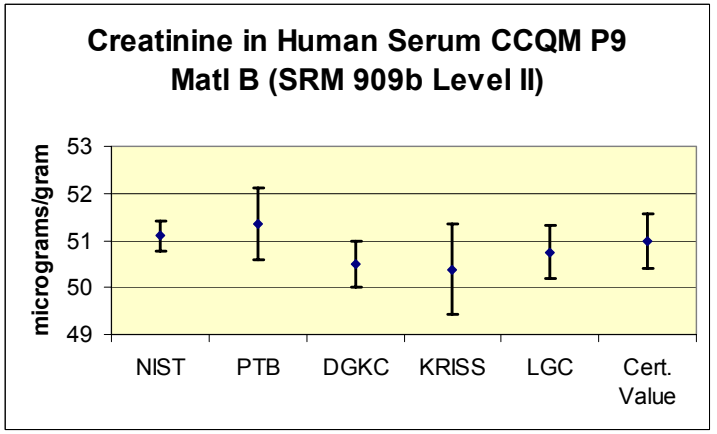
KCRV 31.90 ng/g (dry basis) + 1.07 ng/g (dry basis)

Creatinine in Serum

CCQM-P9

WG Consensus: Successful; proceed to Key Comparison



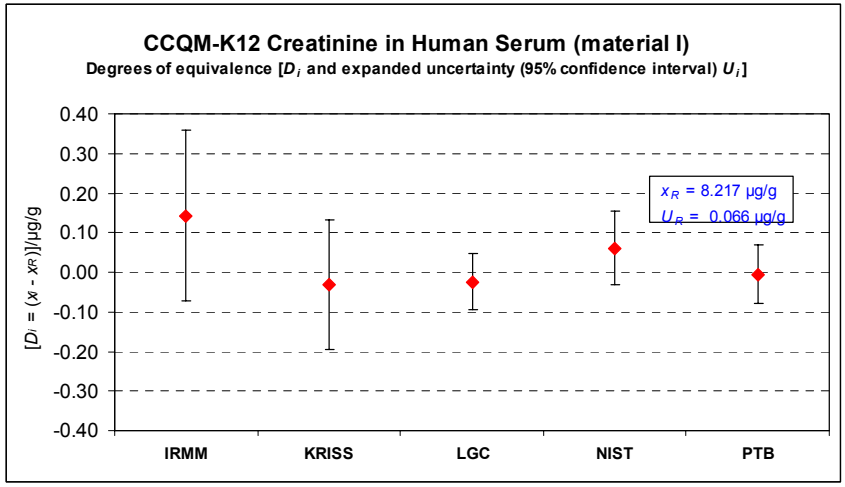


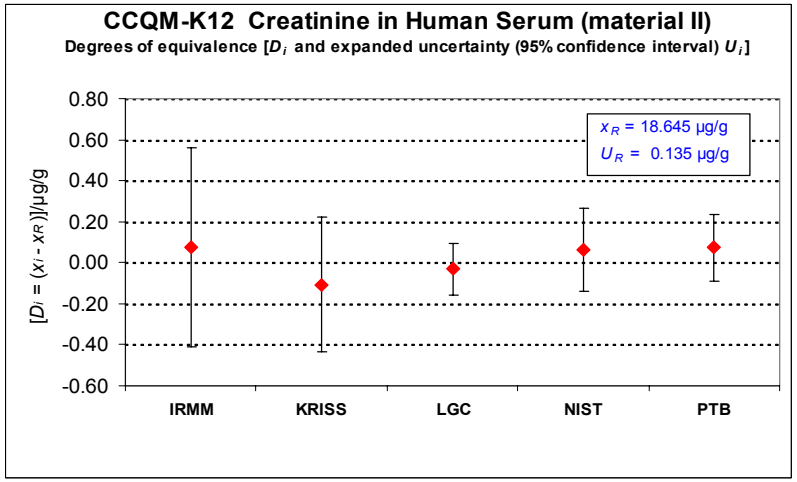
CCQM-K12

Coordinating laboratory: NIST

Study samples: IMEP-17 materials I and II

Korea requested that its results be withdrawn as they had learned that the IMEP-17 results were released prior to Korea's reporting of K12 results. As creatinine was only determined in Material I by IMEP participants, Material II was still an unknown, and Korea results for Matl I and II were consistent, WG recommended, in this specific case, that both results be kept in K12.





For each K12 material, the KCRV was assigned as the mean ± U of the eligible results.

The results from IRMM were not used for the KCRV calculations as they did not participate in CCQM-P9.

CCQM-K21: p,p'-DDT in Fish Oil – Draft B Report presented and approved by WG at Nov. 2001 mtg; now to be circulated to WG chairs for final approval.

Figure 3
Degrees of Equivalence for Sample A

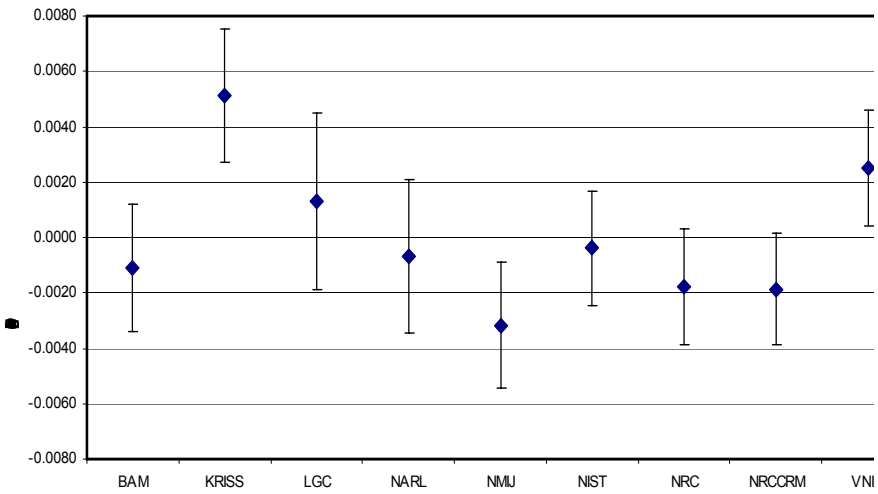
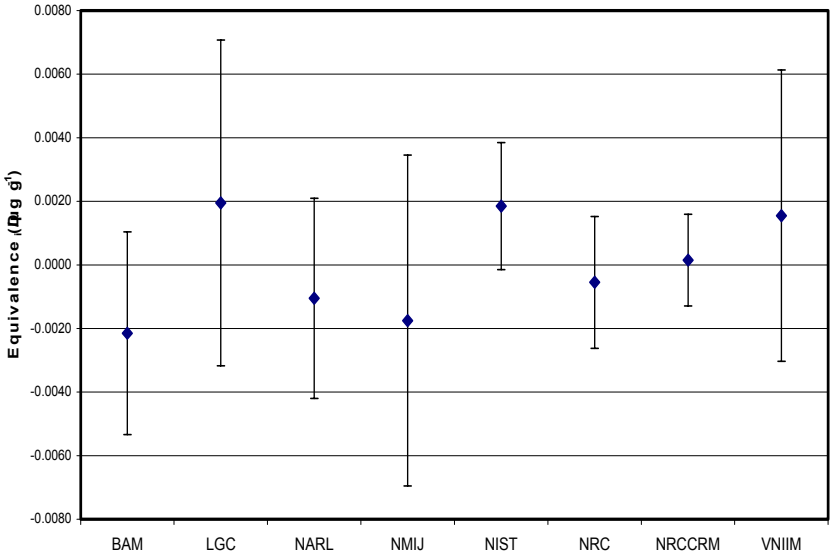


Figure 4
Degrees of Equivalence for Sample B



Key Comparison Reference Value (KCRV): T KCRV was calculated as the mean of the results with the standard deviation of the mean taken as the standard uncertainty of the KCRV. The data contain a mix of degrees of freedom, consequently the Satterthwaite approximation was used, resulting in a coverage factor for Sample A of 2.306 (8 degrees of freedom) and a coverage factor for Sample B of 2.365 (7 degrees of freedom).

Sample A KCRV: 0.0743 ± 0.0020 mg g-1 corresponding to a 95% confidence interval of 0.0723 mg g-1 to 0.0763 mg g-1.

Sample B KCRV: 0.1655 ± 0.0014 mg g-1 corresponding to a 95% confidence interval of 0.1641 mg g-1 to 0.1669 mg g-1.

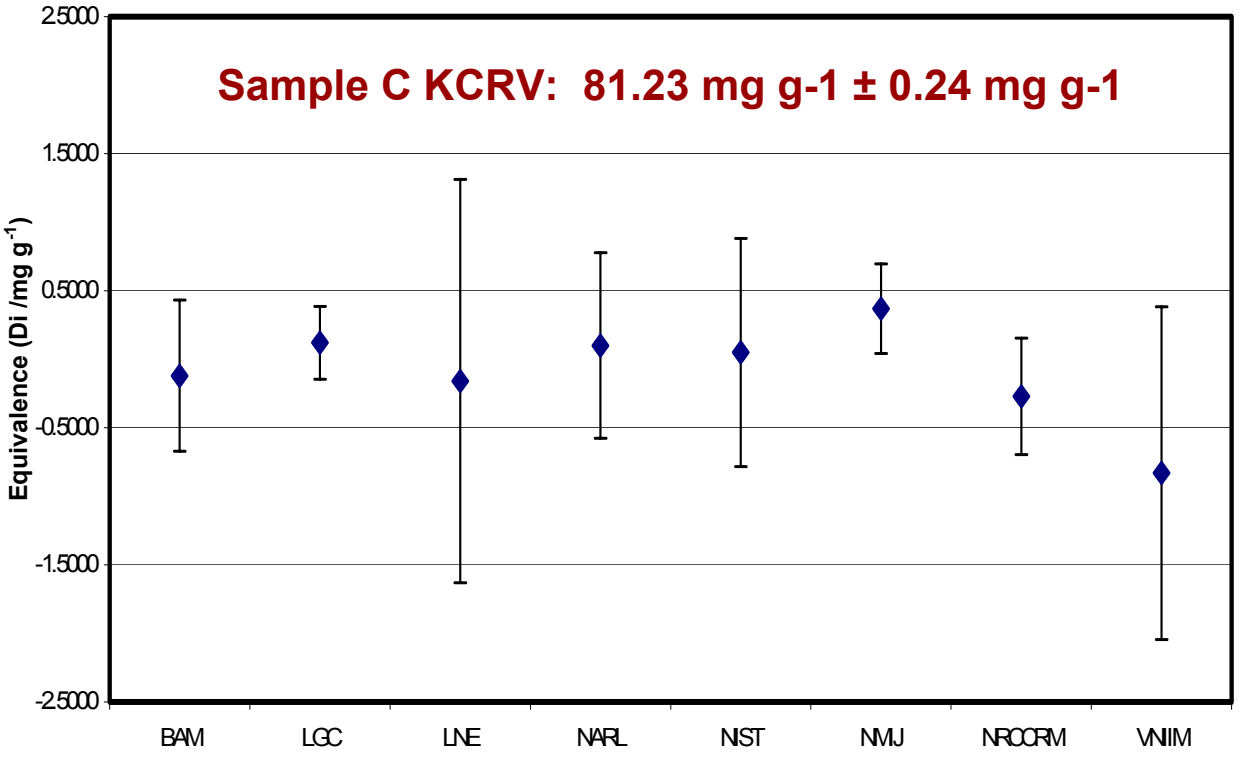
Determination of Ethanol in Aqueous Matrix

CCQM-K27b Commodity level

Study Period: 2002

K27 Draft B Report of 14 Mar 2003

Figure 6 CCQM-K27b
Degrees of Equivalence for Sample C

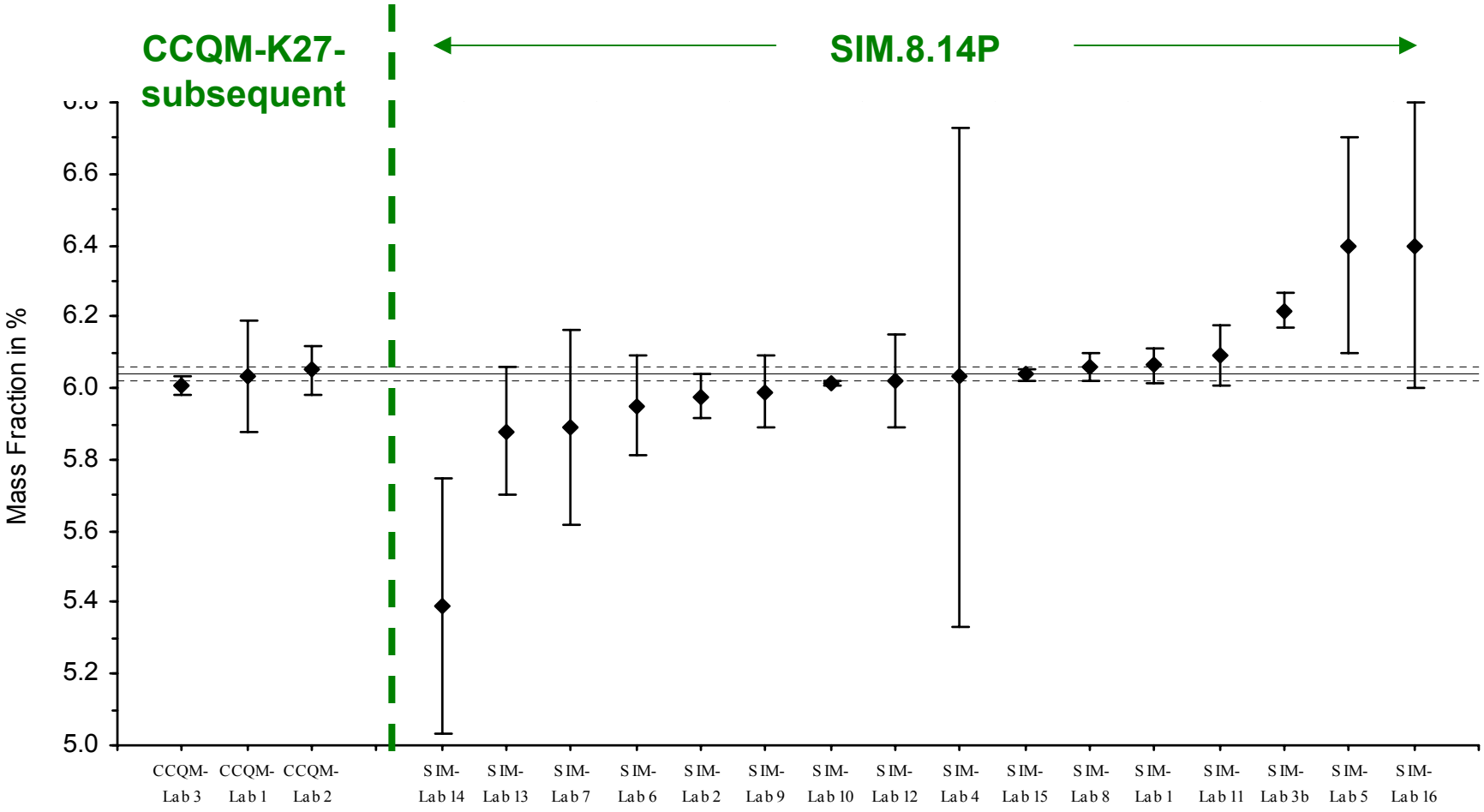


Sample C was a commercial red wine (representing a traded commodity that was stabilised by irradiation to prevent fermentation before opening.

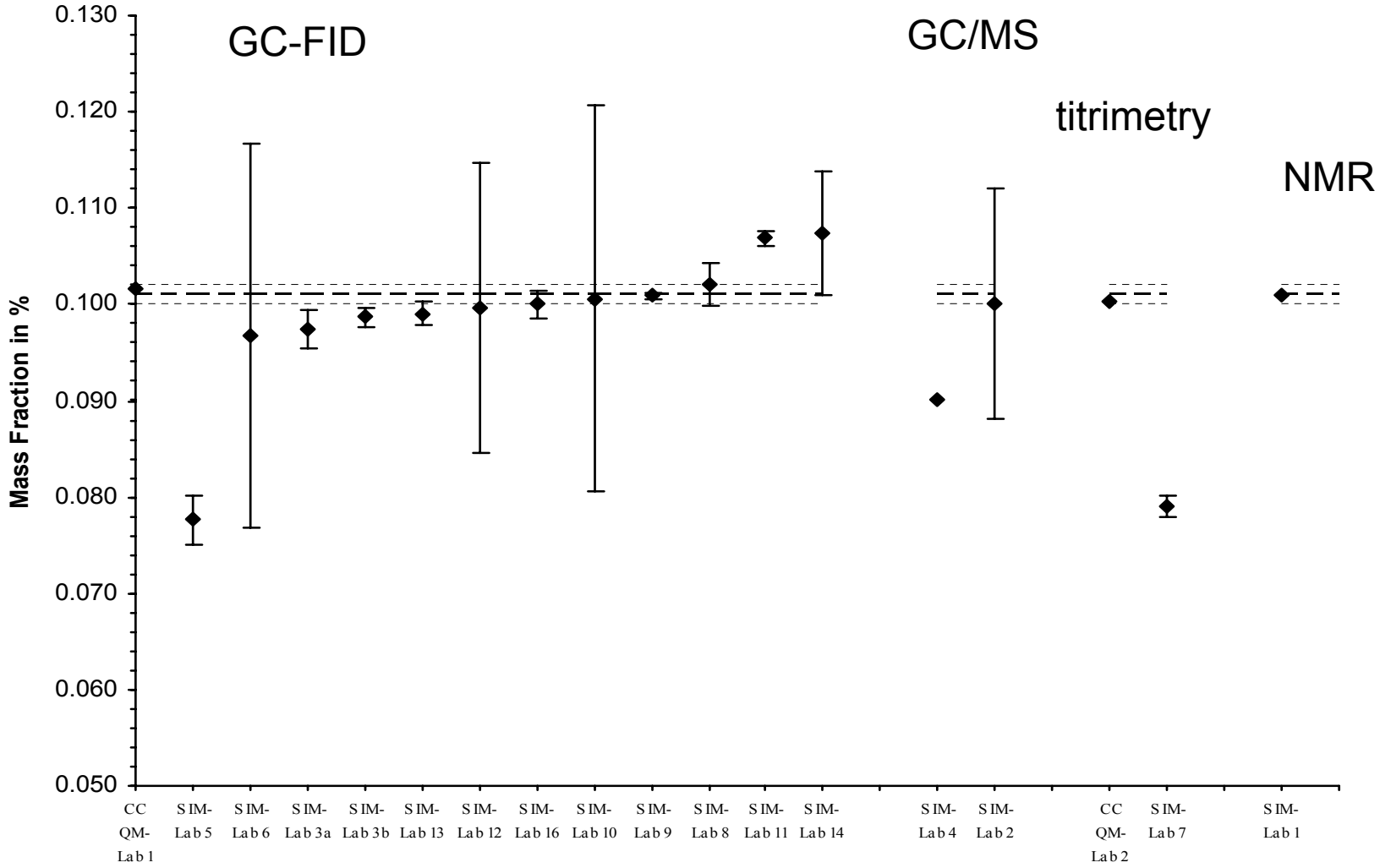
Ethanol in Aqueous Matrix

Sample SIV: nominal concentration 6% ethanol in water

(showing gravimetric value and upper and lower limits of the 95% CI of the gravimetric value based on the CCQM-K27a study)



Sample SII – Results showing gravimetric value and upper and lower limits of the 95% CI of the gravimetric value based on the CCQM-K27a study sorted by method



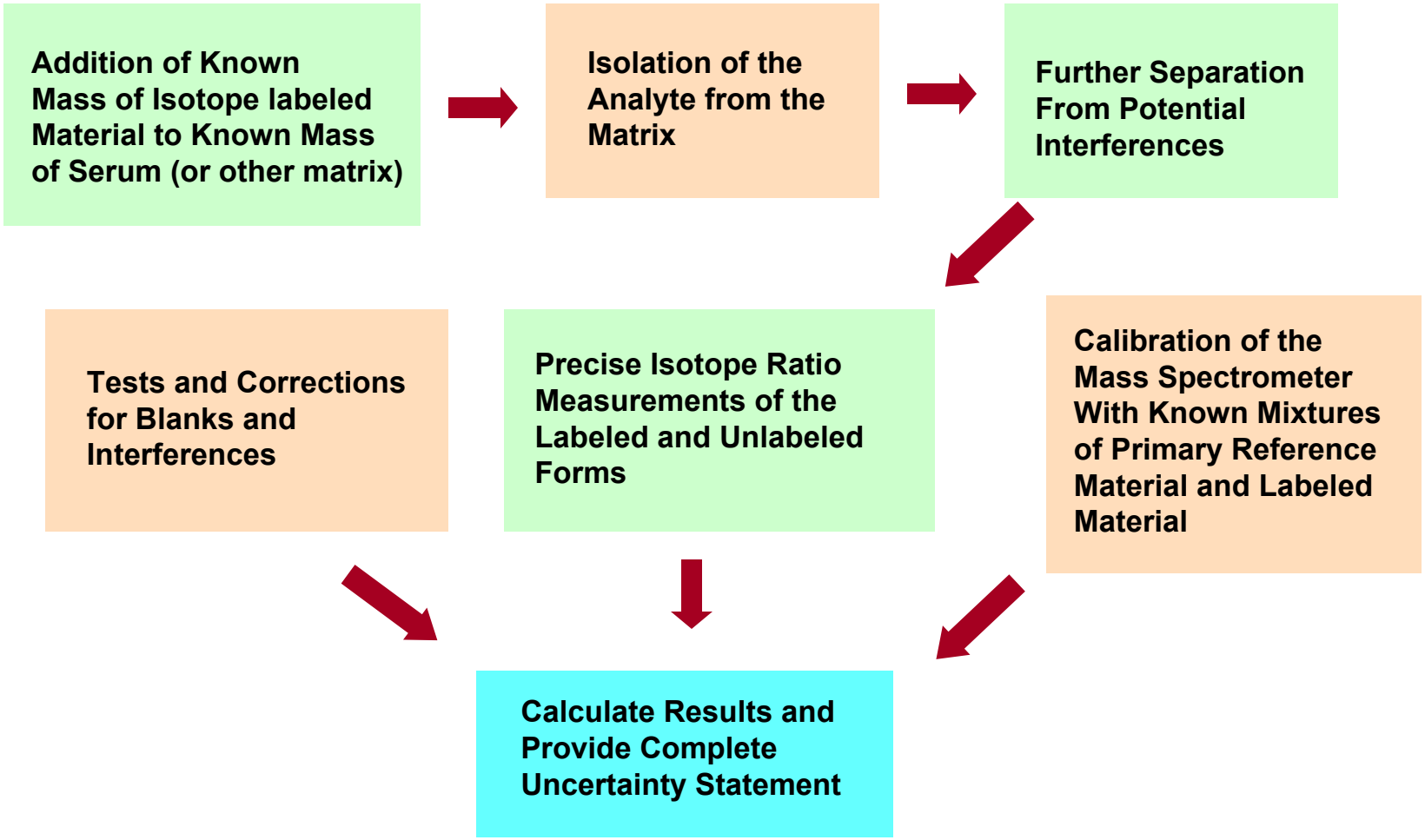
PRINCIPLES OF ORGANIC ISOTOPE DILUTION MASS SPECTROMETRY

A known amount of an isotopically modified version of the compound of interest is added to a matrix containing the compound of interest.

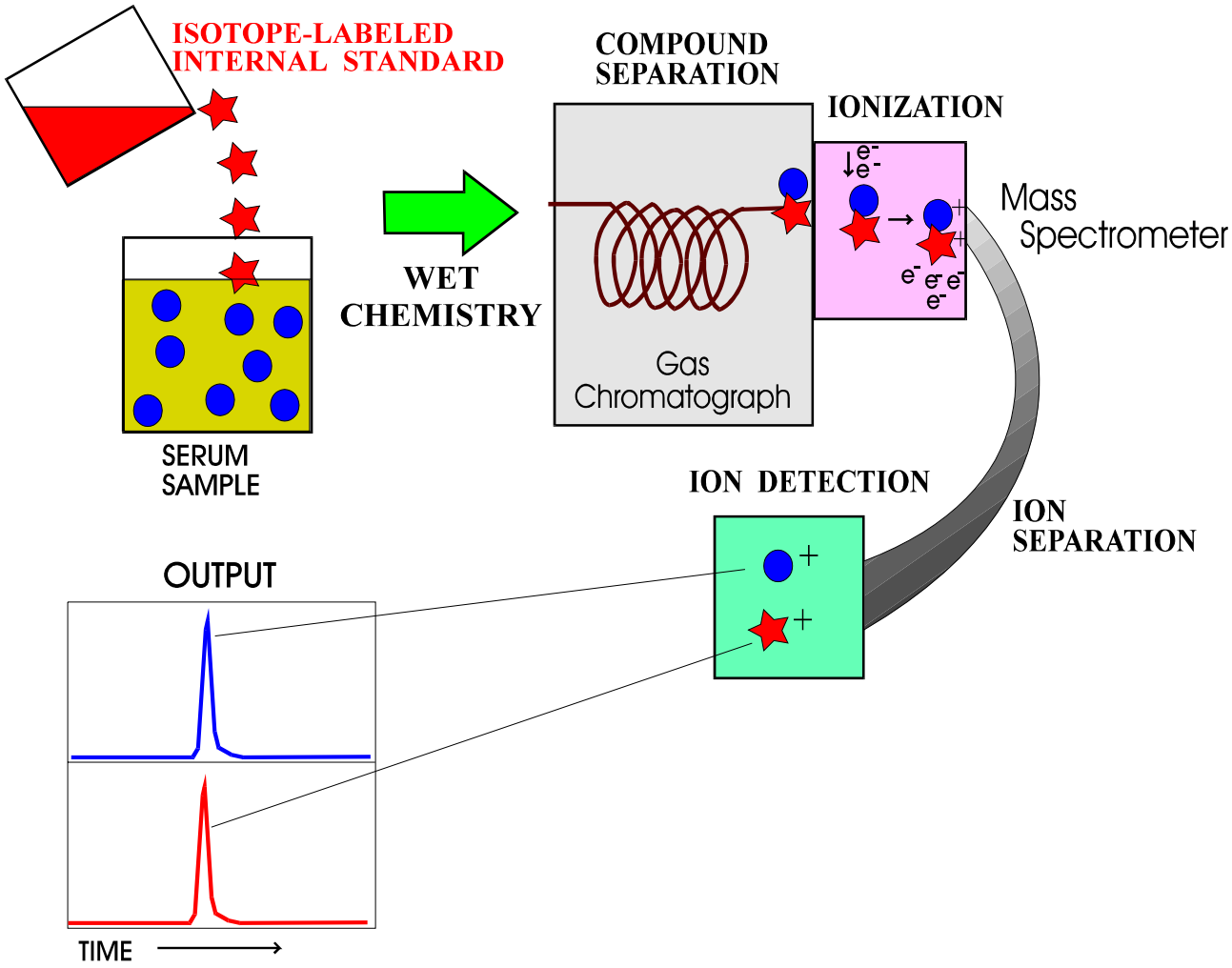
The ratio between the native form and the isotopically modified form is constant throughout the sample preparation and analysis.

A mass spectrometer is used to measure the ratio between the two forms, usually connected with a gas or liquid chromatograph.

Isotope Dilution/Mass Spectrometry-based Definitive Methods



ID-GC/MS



ORGANIC ID-GC/MS METHODS

Requirements:

Mass Spectrometer Capable of Making High Precision Isotope Ratio Measurements of Analytes Eluting from Gas Chromatography Capillary Columns in Narrow Peaks;

A High Purity Reference Compound for Calibration;

A Stable Isotope Labeled Internal Standard;

Meticulous Weighing of Standards and Samples;

Conversion of the Analyte to a Stable Derivative for Gas Chromatography;

Careful Ratio Measurements Following Strict Protocols;

Alternate Measurement Strategies for Detection of Bias

SCOPE OF APPLICABILITY

Target compound must be a discrete chemical form

It must either be stable in the gas phase or be converted to a form that is stable

It must be possible to prepare a stable isotope labeled analog of the target compound

The measured form of the compound must provide ions that are within the range of the mass spectrometer but above significant background

RANGES OF ID-GC/MS APPLICABILITY

ANALYTES: Drugs, Drug Metabolites, Chlorinated Pesticides, PCBs, PAHs, Steroids, Carbohydrates, Amino Acids, Fatty Acids, and many other Natural and Synthetic Compounds. MW 50 - 600 typically.

MATRICES: Biological Fluids and Tissues, Foods, Air Particulates, Sediments, and other Natural and Synthetic Mixtures

CONCENTRATION: *Sub ppb to Low Percent*

LIMITATIONS: Cannot distinguish optical isomers, aliphatic hydrocarbons are difficult because of fragmentation

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PURE CRYSTALLINE MATERIALS

- **Materials of known purity serve as primary standards for calibration**
- **Each material requires a unique approach for purity assessment**
- **Generally a multitude of tests are performed**
  - **Impurities are detected and measured by a variety of tests**
  - **Water content often must be measured**
  - **Outside labs may be used for certain tests (CHN for example)**
  - **Statistics and scientific judgement are used to determine purity and uncertainty**

PRIMARY CALIBRATION COMPOUNDS USED FOR NIST ID-GC/MS OF CLINICAL ANALYTES

SRM	*Certified Purity (mass %)*
911b - Cholesterol	99.8 ± 0.1
912a - Urea	99.9 ± 0.1
913 - Uric Acid	99.7 ± 0.1
914a - Creatinine	99.7 ± 0.3
917a - Glucose	99.7 ± 0.2
1595 - Tripalmitin	99.5 ± 0.2

STABLE ISOTOPE LABELED INTERNAL STANDARDS

Deuterium Labeled:

- Most prone to isotope effects
- Position of label in molecule must be considered
- Significant separation from non-labeled form on GC
- Least expensive

C-13, N-15, O-18

- After deuterium, the most commonly used labels for organic compounds
- Isotope effects and GC separations are minimal
- Can be very expensive

EQUILIBRATION

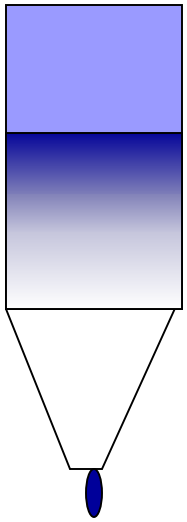
Native analyte may be complexed with matrix components

Labeled internal standard is added uncomplexed

Unlabeled/labeled ratio of material isolated from matrix may not be the same as what was in spiked matrix

Time studies must be performed to determine when equilibration is reached

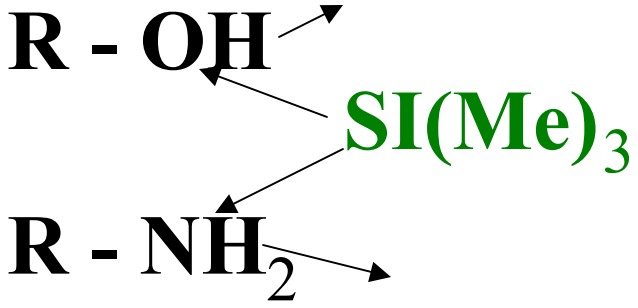
ISOLATION & DERIVATIZATION



SEPARATION FROM MATRIX

DEPENDS ON ANALYTE AND MATRIX

MAY BE SIMPLE OR REQUIRE MULTIPLE STEPS



CONVERSION OF POLAR GROUPS TO NONPOLAR FOR GC (& MS sometimes)

NEED COMPLETE CONVERSION

MAY BE SIMPLE OR COMPLEX REACTIONS

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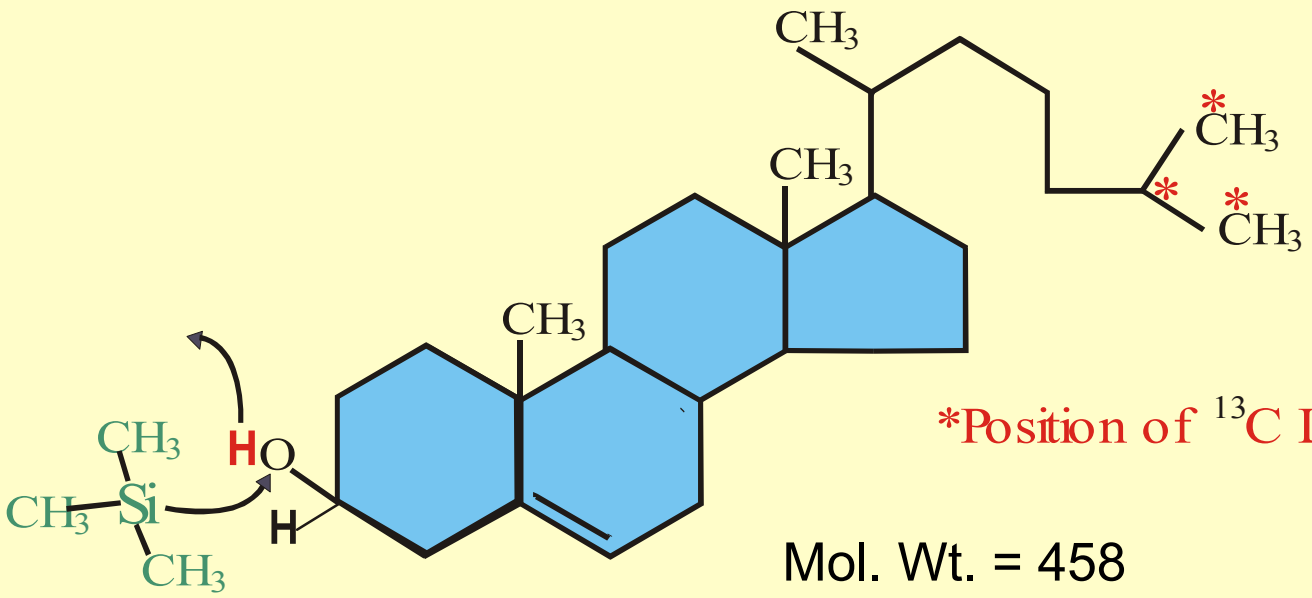
CHOLESTEROL



MEMORY EFFECTS

Substances in chromatographic systems tend to "stick" to active sites

Polar compounds are usually worse than non-polar ones

Subsequent injections may liberate some of this material

In such cases, the ratio measured will be influenced by previous injections

Derivatization completeness, column and injection port condition, carrier gas quality, absence of leaks are important

MASS SPECTROMETER FOR ID-GC/MS PRIMARY METHODS

REQUIREMENTS

Rapid switching between ions and rapid settling

Very precise return to each parameter for each cycle

Good linear dynamic range

Tolerant of pressure changes in the ion source

SUITABILITY OF VARIOUS MASS SPECTROMETERS

Magnetic Sectors

- Demonstrated high precision
- Switching speed can be a problem

Quadrupoles

- Mixed performance
- Newer instruments appear to be better

Ion Traps

- Adequate quantitation not demonstrated
- ID/MS can be a problem

FTMS and TOF have not been tested

MASS SPECTROMETRY
RATIO MEASUREMENTS

Frequent recalibration to monitor instrument performance

Order reversal to test for memory effects

NIST uses bracketing

- **Each sample measurement is surrounded both in ratio and in time by measurements of two calibration standards**
- **Duplicate injections are made to improve statistics and to monitor instrument performance**
- **Process is repeated on a second day with measurement order reversed**

Other calibration schemes may be used

MEASUREMENT PROTOCOL

Acceptance Criteria

Consecutive injections of standards or samples must agree in intensity ratio to within 0.5%. If they do not, it is not a valid measurement for the sample(s) involved.

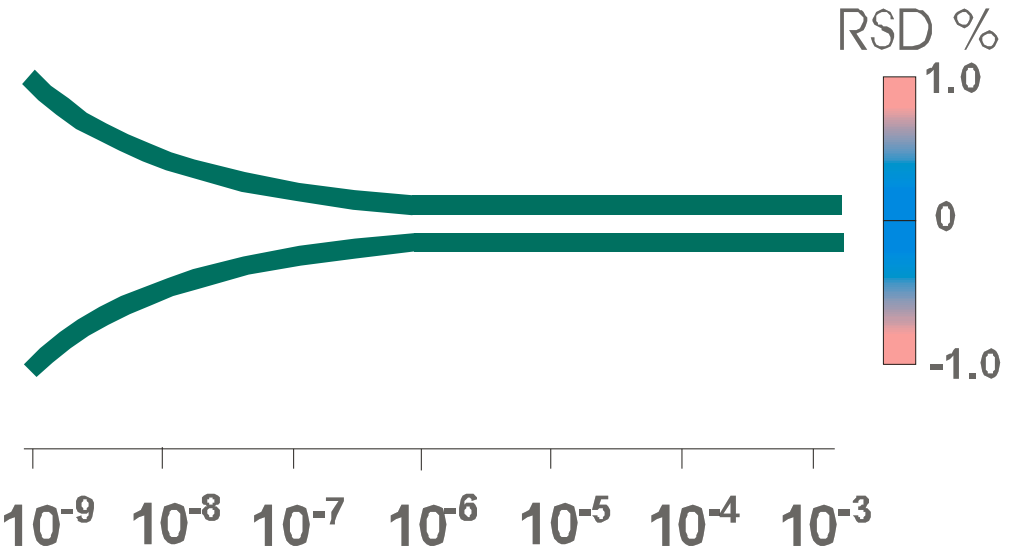
Calculated results from day 1 and day 2 must agree to within 0.5%. If they do not, the measurements are repeated on a third day with all three days' results averaged together.

MEASUREMENT SCHEME

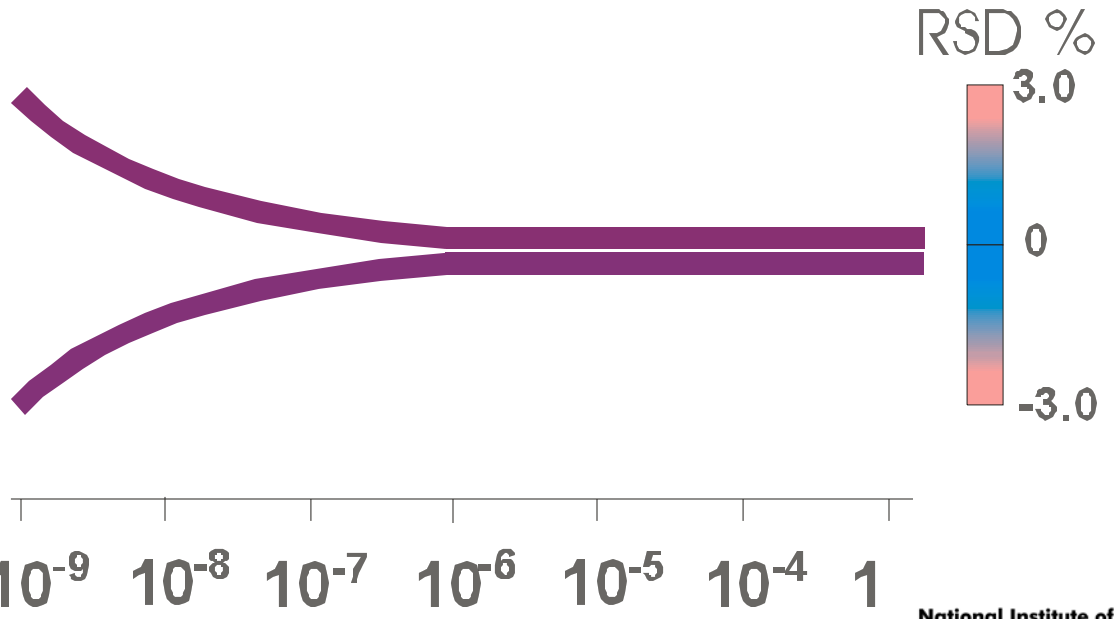
Day 1	*Day 2*
Std A	Std A
Std A	Std A
Sample 1	Sample 2
Sample 1	Sample 2
Std B	Std B
Std B	Std B
Sample 2	Sample 1
Sample 2	Sample 1
Std A	Std A
Std A	Std A

PRECISION OF ID-GC/MS

Repeated Intensity Ratio Measurements



Measured Mass Fractions of Independently Prepared Samples



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CALCULATIONS

1. Calculate the weight ratio of the sample by linear interpolation:

$$W_{sam} = W_{StdL} + \frac{(W_{StdH} - W_{StdL}) \times (I_{sam} - I_{StdL})}{(I_{StdH} - I_{StdL})}$$

Where:

W_{sam} = Weight Ratio of Sample

W_{StdL} = Weight Ratio of Low Standard

W_{StdH} = Weight Ratio of High Standard

I_{sam} = Ion Intensity Ratio of Sample

I_{StdL} = Ion Intensity Ratio of Low Standard

I_{StdH} = Ion Intensity Ratio of High Standard

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CALCULATIONS

2. Calculate the mass fraction of the analyte in the sample

$$F = W_{sam} \times M_{lab}/M_{sam}$$

Where:

F = **Mass fraction of analyte in sample**

M_{lab} = **Mass of labeled material**

M_{sam} = **Mass of sample**

CONFIRMATORY MEASUREMENTS

Even with good chromatography, it is possible for a substance to coelute with the analyte and contribute to one or both of the ions being monitored. This could result in significant bias.

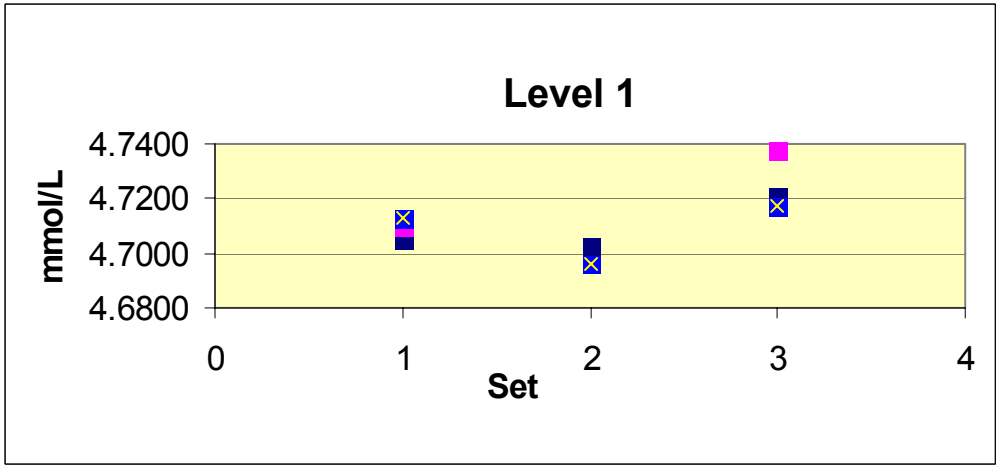
To test for such an interference, measurement conditions are changed. Such changes may involve:

- **A GC column with a different stationary phase**
- **A different mode of ionization (CI)**
- **A different ion from electron impact**

If samples are remeasured using at least two of these options and results agree with the original, it is strong evidence for the absence of significant bias.

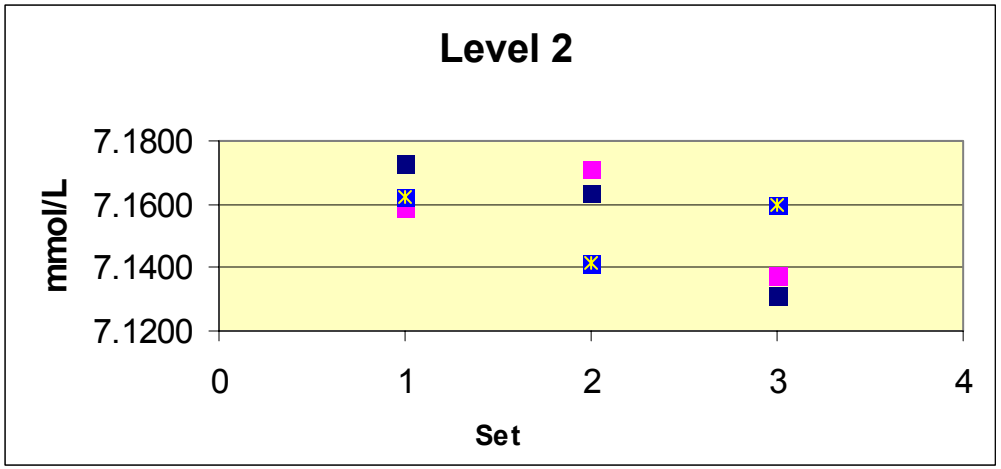
EXAMPLE OF RESULTS

Cholesterol in SRM 1951a
Lipids in Frozen Human Serum



Certified Concentrations

4.7109 ± 0.0116



7.1554 ± 0.0142

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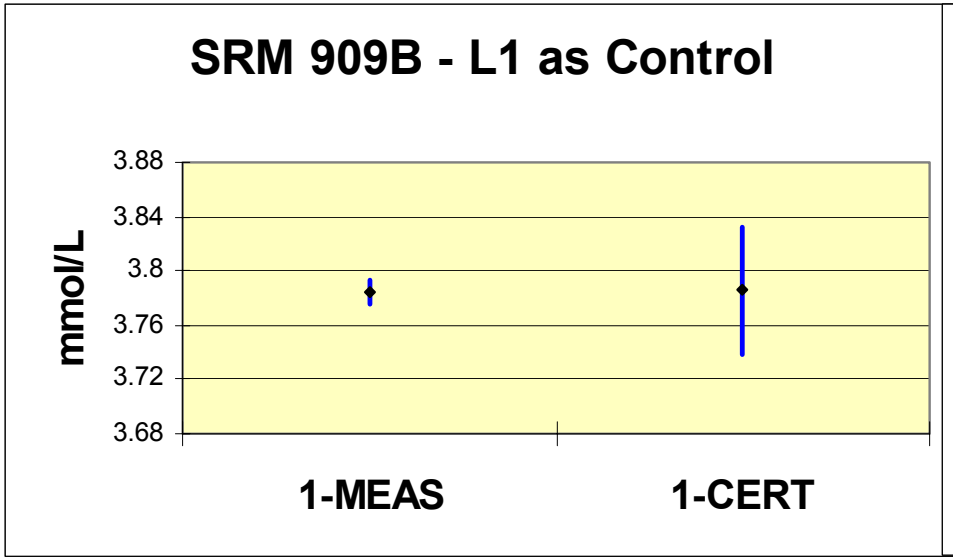
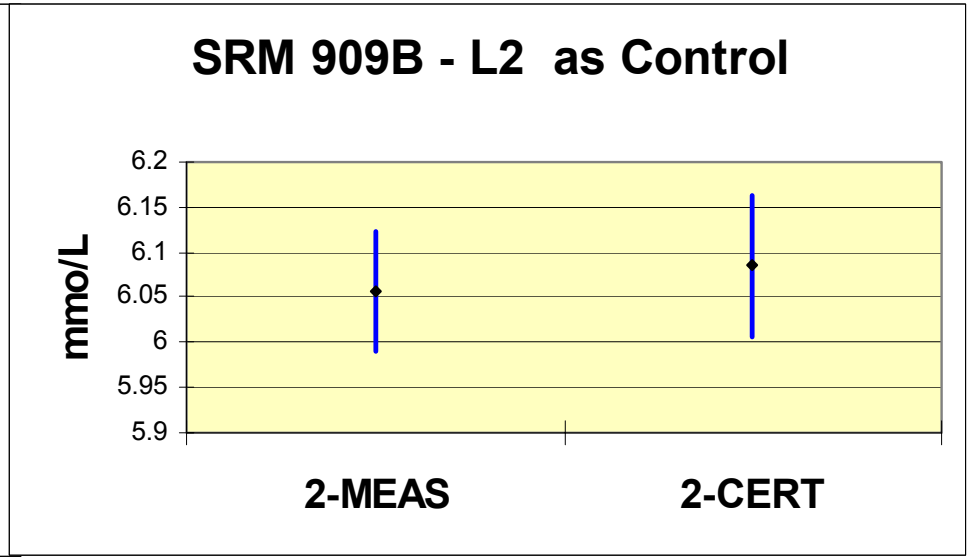
CONTROL MEASUREMENTS





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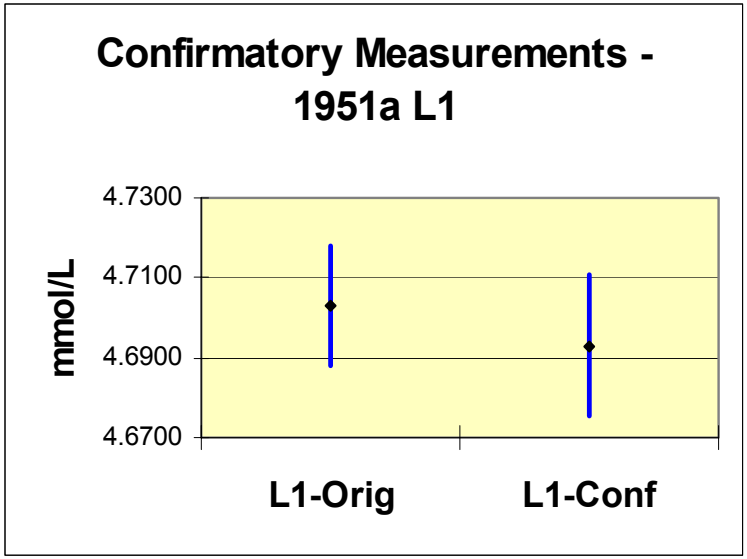
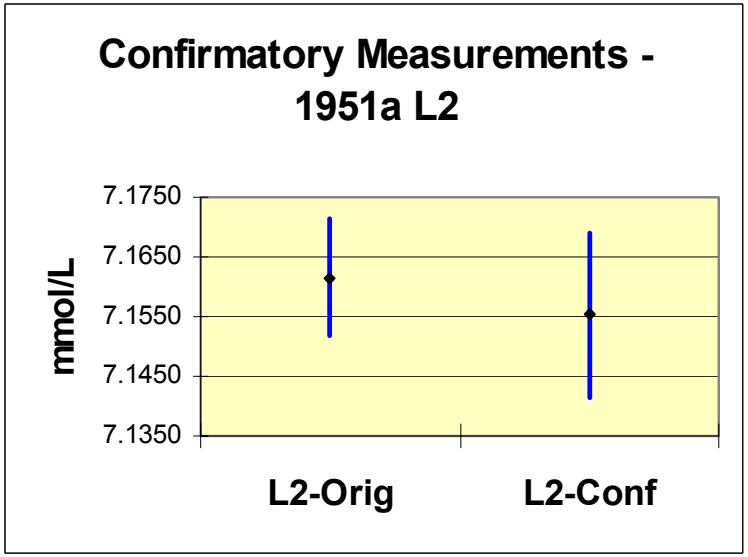
CONFIRMATORY MEASUREMENTS ON SRM 1951a





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NIST ID/MS MEASUREMENTS OF CHOLESTEROL

Type A Sources of Uncertainty

	Uncertainty type		Relative Std
Steps in Process	***A***	***B***	**Uncertainties (%)**
Measurements	**X**		**measured**
Includes:			
Weighing reference standard			
Preparing reference standard solution			
Weighing aliquots of labeled solution			
Weighing aliquots of standard solution			
Weighing samples			
ID/MS ratio measurements			

These steps are all performed multiple times in the process of measuring a material. Therefore variability in these steps will be reflected in the imprecision that is measured

NIST ID/MS MEASUREMENTS OF CHOLESTEROL

Type B Sources of Uncertainty

	Uncertainty type		Relative Std
Steps in Process	***A***	***B***	**Uncertainties (%)**
Purity of reference standard		X	0.05
Hydrolysis of cholesteryl esters		X	0.1
Stability of Cholesterol in base		X	0.1

Complete hydrolysis of cholesteryl oleate was demonstrated. However, other esters may not be completely hydrolyzed, which would result in a low bias.

Recovery of cholesterol from treatment in strong base is > 99%. It is possible that some degradation occurs and differentially for free and esterified cholesterol.

NIST ID/MS MEASUREMENTS OF CHOLESTEROL

Potential Sources of Uncertainty that Do Not Contribute in the NIST Method

Weighing labeled material
Preparing labeled solution
} **Samples and calibration standards are spiked with the same solution**

NIST ID/MS MEASUREMENTS OF CHOLESTEROL

Computation of Expanded Uncertainty

Material K6-A				
	Uncertainty type		*Relative Uncert (%)*	*d.f.*
Steps in Process	*A*	*B*		
Purity of reference standard		X	0.050	inf
Hydrolysis of cholesteryl esters and Equil.		X	0.100	inf
Stability of cholesterol in base		X	0.100	inf
GC/MS measurements	X		0.123	2
Combined rel std uncertainty			0.194	
Calculated degrees of freedom			12.3	
k-factor			2.179	
Relative expanded uncertainty (%)			0.423	
Mean value			2.215	*mg/g*
Abs. expanded uncertainty			0.00938	*mg/g*

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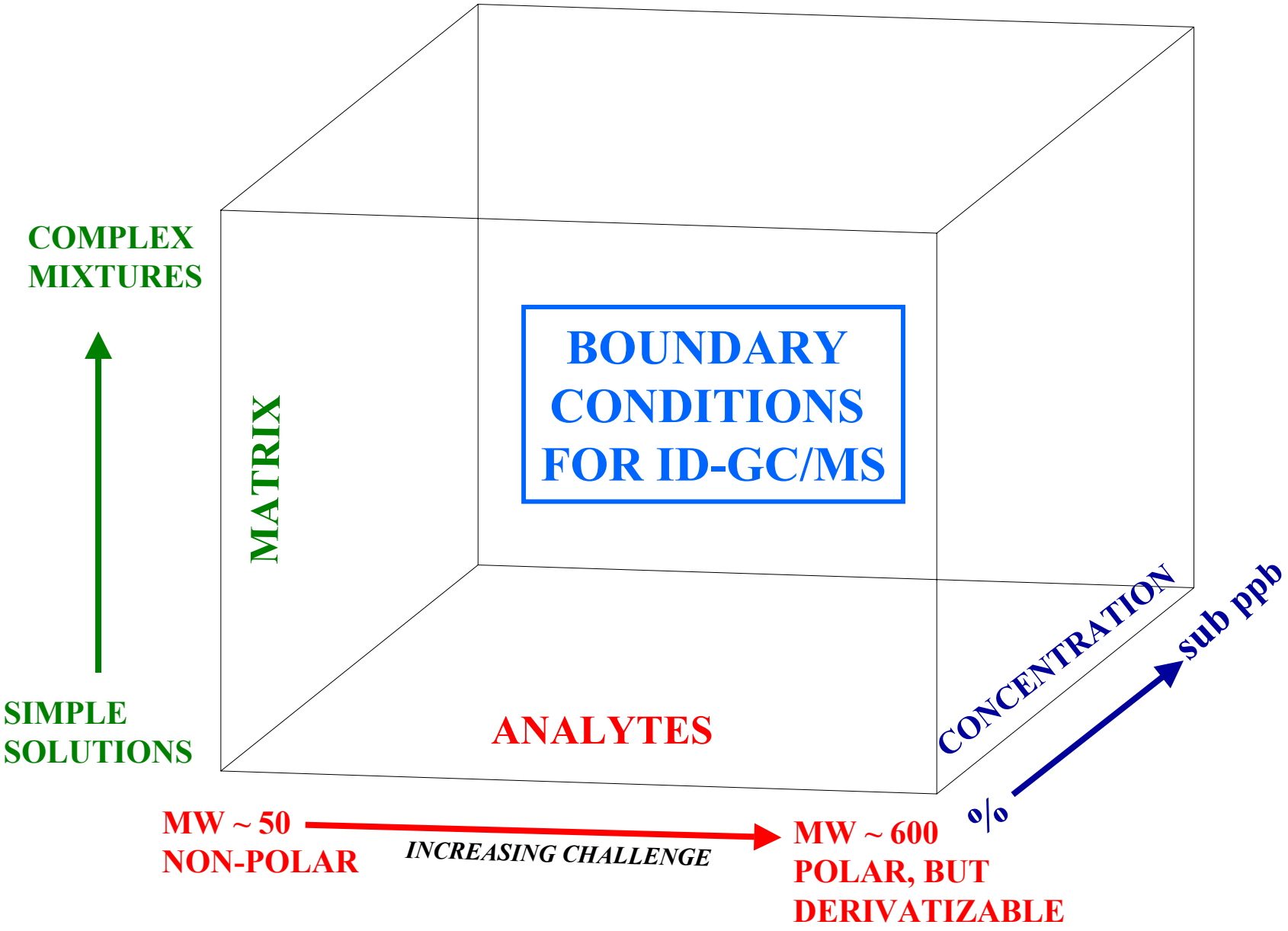
SO HOW FAR DOES THE LIGHT SHINE FROM A KEY COMPARISON UTILIZING ID-GC/MS?

For Cholesterol, probably not very far by itself, but…

If a laboratory can also accurately measure glucose (*highly water soluble)* and creatinine (*polar and low concentration)* in serum by ID-MS, it has demonstrated capability for accurate measurements of other clinical analytes in the same MW and concentration range. Key Comparisons are underway for these two analytes.

Therefore, if a laboratory can demonstrate consistent high quality capabilities for a variety of analytical challenges, those points of light may illuminate the entire playing field.


COMPLEX MIXTURES
SIMPLE SOLUTIONS
MATRIX
BOUNDARY CONDITIONS FOR ID-GC/MS
ANALYTES
CONCENTRATION
sub ppb
%
MW ~ 50
NON-POLAR
INCREASING CHALLENGE
MW ~ 600
POLAR, BUT DERIVATIZABLE

CONCLUSIONS

Primary method techniques are capable of providing very accurate and precise results

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Most Primary methods are very limited in scope

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For organics, have potentially thousands of interfering substances

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For organics, have potentially thousands of interfering substances

Unlikely that one can write a measurement equation that accounts specifically for all sources of uncertainty for organic ID/MS

Contacts for Further Information

Gravimetry	Tom Vetter	thomas.vetter@nist.gov
Titrimetry	Helene Felber	Helene.Felber@empa.ch
Coulometry	Michal Mariassy	mariassy@smu.gov.sk
	Kenneth Pratt	kenneth.pratt@nist.gov
DSC	Laurie Besley	laurie.besley@agal.gov.au
	Reenie Parris	reenie.parris@nist.gov
Inorganic ID/MS	Stephen Long	stephen.long@nist.gov
Organic ID/MS	Michael Welch	michael.welch@nist.gov