

Harmonisation & Standardisation of Chromatography - Mass Spectrometry

R. Greaves

AACB ASM

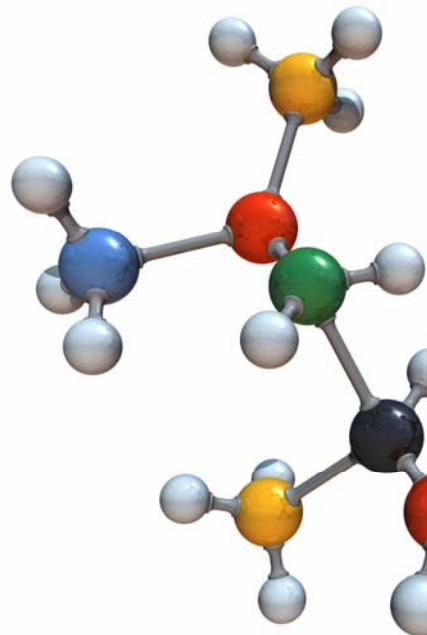
12th October 2011

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Overview

1. Terminology
2. Issues
3. JCTLM database
4. APFCB MS project
5. Reference Intervals
6. Summary

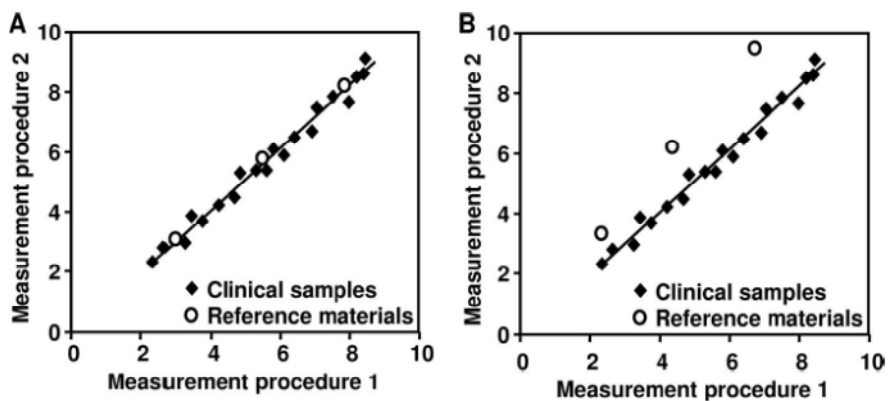


DEFINITIONS

In recent years there has been a concerted effort to improve assay results, particularly in relation to:

Commutability
Traceability
Standardisation
Harmonisation

1. COMMUTABILITY



Not all reference materials have proven commutability

Clinical Chemistry 57:8
1108–1117 (2011)

2. Traceability

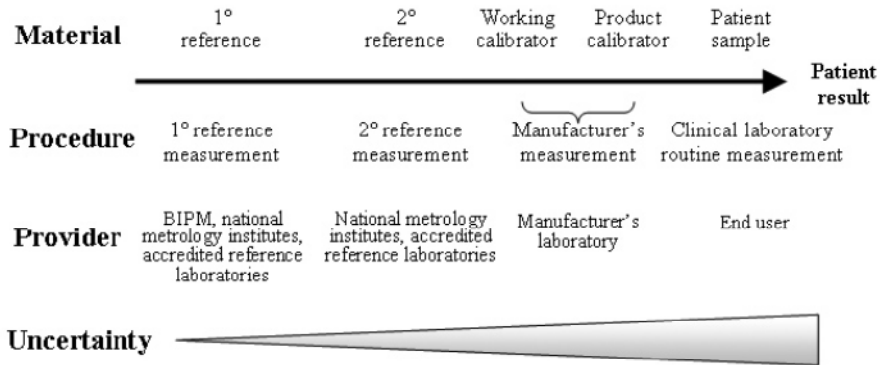
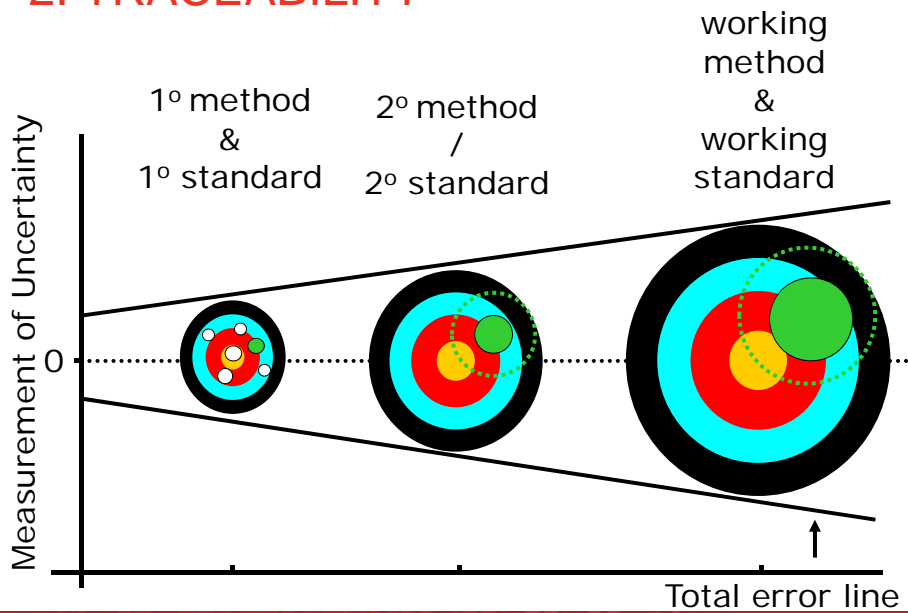


Figure. A generic calibrator traceability flowchart. Based on ISO 17511:2003 Fig 4.2.2.

Clin Biochem Rev Vol 28 August 2007 | 105

2. TRACEABILITY



Standardisation and Harmonisation

- Harmonisation and Standardisation are both trying to achieve the same goals in a systematic way
- **Measurand** = quantity intended to be measured
- **Standardisation** is when you understand the measurand
- **Harmonisation** is when you do not understand the measurand completely
- E.g. The measurands that can be expressed in SI units can be standardised, whereas those that are not can only be harmonised.

4. Harmonisation

Clinical Chemistry 57:8
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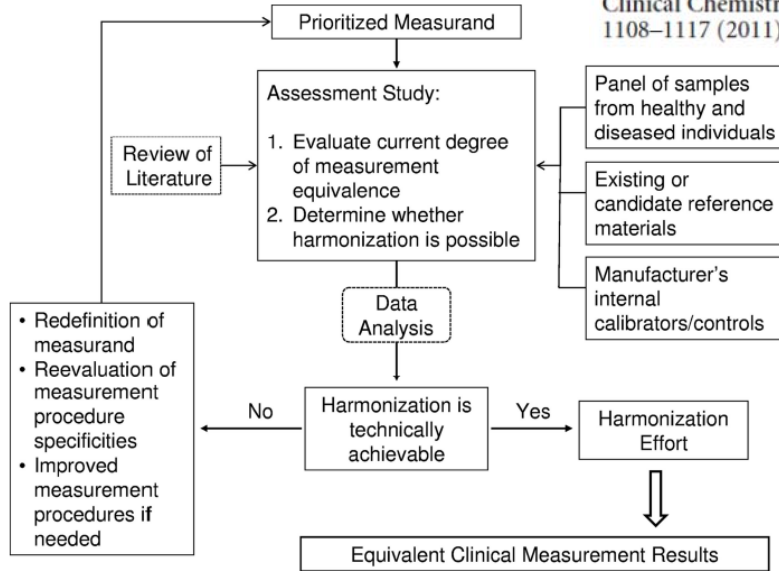
Special Report

Roadmap for Harmonization of Clinical Laboratory Measurement Procedures

W. Greg Miller,^{1*} Gary L. Myers,² Mary Lou Gantzer,³ Stephen E. Kahn,⁴ E. Ralf Schönbrunner,⁵
Linda M. Thienpont,⁶ David M. Bunk,⁷ Robert H. Christenson,⁸ John H. Eckfeldt,⁹ Stanley F. Lo,¹⁰
C. Micha Nübling,¹¹ and Catharine M. Sturgeon¹²

4. Harmonisation: General Process

Clinical Chemistry 57:8
1108–1117 (2011)



ISSUES

Differences in Measurand

Primary Methods

Primary Standards - Acceptability

Quality of Labs assigning Primary Values

Commutability

Primary Standards - Acceptability

External quality assurance target setting with NIST SRM 968d material: performance in the 2010 Royal College of Pathologists of Australasia Quality Assurance Program with retinol, α -tocopherol and β -carotene

“The allowable error of the primary material needs to be tighter than routine methods, and the methods used for assignment of SRM values need to be derived from higher order methods,⁷ which is not apparent from the NIST certificate of analysis”

480 *Annals of Clinical Biochemistry* Volume 48 September 2011

Ronda F Greaves^{1,2,3}, Kirsten E Hoad^{1,4},
Gerald A Woollyard^{1,5}, Trevor A Watmsley^{1,6},
Scott Briscoe^{1,7}, Lambro A Johnson^{1,7},
Wendy D Carter^{1,8} and Jan P Gill^{1,8}

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JCTLM DATABASE

Accurate measurements traceable to SI, being long-term stable, fixed anchor points are essential for several fields

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Joint Commission for Traceability in Laboratory Medicine - (JCTLM)

- EU Directive on Vitro Diagnostic measurements requires traceability to standards of “higher order”
- JCTLM Established by BIPM, IFCC and ILAC
- In co-operation with all stakeholders
- Traceability to SI, but if not (yet) possible to other internationally agreed references (e.g. WHO units for biological activity)
- Aim of JCTLM is to realise and support worldwide reliable comparability and traceability of measurement results in laboratory medicine

JCTLM Database

Reference Laboratories and Methods is developing criteria for reference measurement laboratories

Reviewing reference materials and methods nominated as being of higher order

Database of higher-order reference materials, measurement methods/procedures and services

JCTLM Database Laboratory medicine and *in vitro* diagnostics

Refine search by category: All

Refine search by matrix category: All

Select your requirement:

- Higher-order reference materials
- Reference measurement methods/procedures
- Reference measurement services

Reset Search

<http://www.bipm.org/jctlm/>

Examples of Standardisation

Measurand	Method	Standards	Laboratories
Cholesterol	Yes x 3	Yes x 5	Yes x 13

Based on JCTLM database search 10th August 2011

APFCB MS HARMONISATION PROJECT



Harmonisation: A practical application

- Conference:
Asia Pacific Chromatography Mass Spectrometry Meeting
- Chairs:
Danny Sampson & CS Ho
- Date:
2010 January Hong Kong
- Discussion:
Need for agreement b/w MS assays
- Inaugural meeting:
Saturday 16th January 2010



•“Mass spectrometry has become one of the most important tools in the analytical laboratory, with a wide range of applications” Spectrophotometry March 9, 2011

•Many applications relevant to Clinical Biochemistry

•Ideal to develop reference methods

Inaugural Meeting 16th Jan 2010

Present

- Ronda Greaves
- Danny Sampson
- John Joseph
- James Pitt
- Mike Morris
- Brett McWhinney
- Chris Salonikas
- Michael Lee
- CS Ho
- Kirsten Hoad
- Trish Anderson (apology)

Analytes identified for harmonisation

1. Newborn screening
2. Inborn Errors of Metabolism
3. Immunosuppressants
4. Steroids
5. Vitamins esp. vitamin D
 - Minimum 4 labs needed

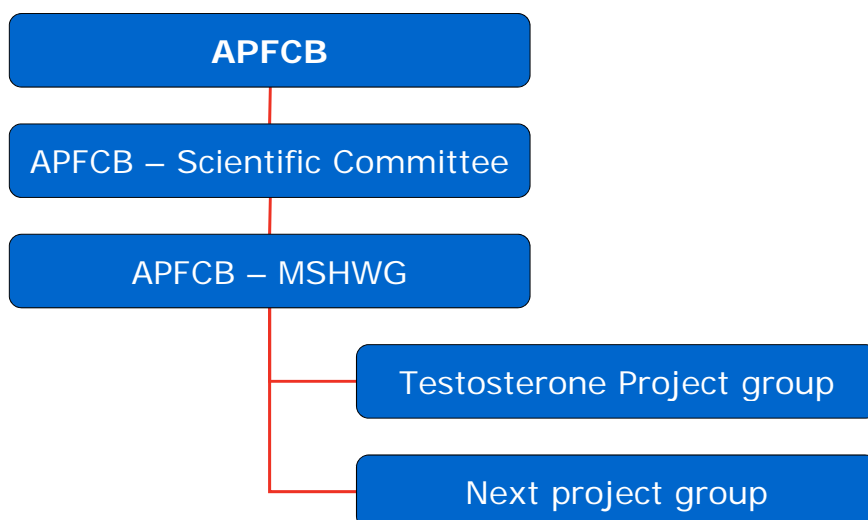
Outcome of Meeting

- Proposed formation of the MS harmonisation working group
- Discussions with AACB (SRAC), HKSCB and APFCB
- Agreed formation under APFCB umbrella
- Encourages participation throughout Asia Pacific Region


Notes:


1. No financial support sort from member societies
2. Specific company support
3. 2008 Roman Lecture – *“Research on a Shoestring”*

4. APFCB MS harmonisation WG



Launched – Seoul 2010


ASIAN AND PACIFIC FEDERATION OF CLINICAL BIOCHEMISTRY
MASS SPECTROMETRY HARMONISATION WORKING GROUP (MSHWG)
Serum Testosterone Project


ASIAN AND PACIFIC FEDERATION OF CLINICAL BIOCHEMISTRY
MASS SPECTROMETRY HARMONISATION WORKING GROUP (MSHWG)
TERMS OF REFERENCE


World Pacific Federation of Chemistry Harmonisation Working Group


Kiyoshi ICHIHARA* and Chana Khan MD*

*Prince of Wales Children's Research Institute, at Prince Alfred Hospital, Australia; *Transgenic Inc., Prince of Wales Hospital, Hong Kong SAR. ichihara@rmit.edu.au

Reference intervals is of high importance for the future clinical laboratories. The proposal to establish a group (MSHWG) was put forward at an inaugural Pacific Conference of Chromatography and Mass Spectrometry meeting in 2008. The first MSHWG meeting included members from the Hong Kong Society of Clinical Chemistry and Industry, under the Asian and Pacific Federation of Clinical Biochemistry.


This reference document was subsequently endorsed. MSHWG is a Working Group of an Executive Committee, and as a Project Group will include scientific initiatives.


MASS SPECTROMETRY HARMONISATION WORKING GROUP (MSHWG)
Serum Testosterone Project


Testosterone

Reference intervals of serum testosterone concentrations in clinical laboratories using tandem mass spectrometry (LC/MS/MS) methods. The first project in testosterone assays, based on the serum testosterone LC/MS/MS methods, harmonise serum testosterone results through harmonised LC/MS/MS reference intervals for serum testosterone.

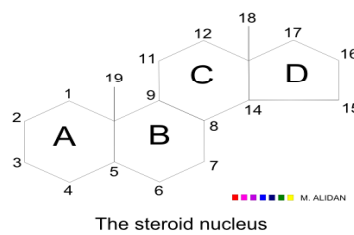
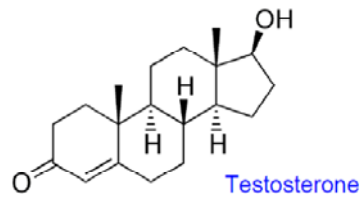
Towards harmonisation of testosterone assays. The first project in testosterone assays, based on the serum testosterone LC/MS/MS methods, harmonise serum testosterone results through harmonised LC/MS/MS reference intervals for serum testosterone.


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Why Testosterone?

- Initially steroids
- Simplified to one analyte
- Small number of labs
- Easier to control as the pilot
- No other group currently looking at testosterone
- Difficult analyte to measure in children and females
- Specific interest of three of the core group



Definition of Testosterone project

“This project is to harmonise the measurement of serum testosterone concentrations in clinical samples using liquid chromatography isotope-dilution tandem mass spectrometry (LCTMS) methods”

Goals

1. To provide detailed information on the different serum testosterone LCTMS methods used in Asian Pacific clinical biochemistry laboratories
2. To harmonise the serum testosterone results through the use of a set of common secondary serum calibrators that have been validated by a reference method
3. To harmonise reference intervals for serum testosterone of different sexes and age groups in the Asian Pacific region

Strategies

- A. Formation of the Project Working Group
- B. Recruitment of participating laboratories
- C. Documentation of serum testosterone LCTMS methods of participating laboratories
- D. Initial assessment of performance – Stage I
- E. Harmonisation of accuracy performance – Stage II
- F. Harmonisation of reference intervals – Stage III

A. Formation of the Project Working Group

Corresponding:

1. Trish Anderson - ASE
2. Brian Cooke – Royal Perth Hospital
3. Jan Gill – RCPA QAP
4. Ronda Greaves – RMIT University
5. Dr CS Ho – Prince of Wales Hospital
6. Kirsten Hoad – Royal Perth Hospital
7. Kiyoshi Ichihara - Yamaguchi University
8. John Joseph – QE2
9. Katsuhiko Kuwa – Japan Meteorology
10. Tze Ping Loh – National University Hospital
11. Brett McWhinney – Royal Brisbane
12. Michael Rennie – PM separations
13. Danny Sampson - RPA
14. Prof Junghan Song – Seoul National University Bundang Hospital
15. Trevor Walmsley - Canterbury Health

Location:

- Hong Kong
- Japan
- South Korea
- NSW
- New Zealand
- Queensland
- Singapore
- Victoria
- Western Australia x 2

Company Support:

- Agilent Technologies
- ASE
- PM Separations
- RCPA QAP Chemical Pathology Program

B. Recruitment of participating laboratories

7 Labs currently with methods:

- Hong Kong
- Korea
- Melbourne
- New Zealand
- Queensland
- Western Australia x 2

C. Documentation of serum testosterone LCTMS methods of participating laboratories

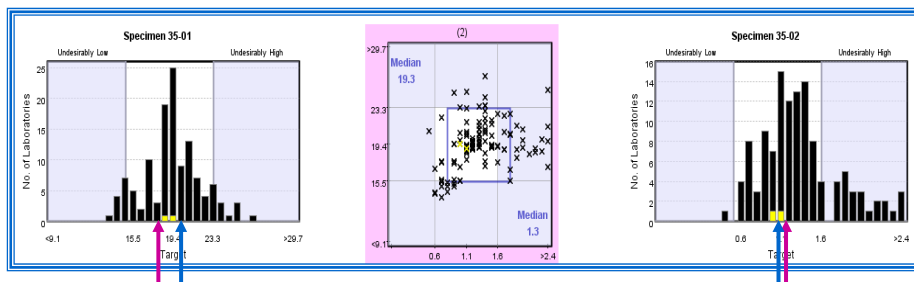
Question	Response Summary
Testosterone standard material:	
Source	1: Lipomed, 2: WEQAS, 3-4: Sigma
Purity	1: > 98.5 %, 2: 99%, 3: >98%
Isotope-labelled testosterone:	D2 Testosterone
Source	Cambridge Isotope Laboratories Inc.
Purity	98%
Labelling positions	1,2
Calibrator matrix	Stripped plasma Foetal Bovine or stripped serum
Source	1:Sigma, 2:WEQAS, 3:Sera Care Life Sciences, 4:In-house

C. Documentation of serum testosterone LCTMS methods of participating laboratories

Question	Response Summary
MRM for quantitation	289 > 109 or 97
MRM for confirmation	289 > 109 or 97
MRM for labelled testosterone	291 > 111 or 99

D. Initial assessment of performance – Stage I

Accuracy, between-batch precision and linearity performance will be evaluated via the RCPA QAP Endocrine program over a 12-month period i.e. 2011



D. MS-HWG: Testosterone Results umol/L

Level	1	2	3	4	5	6
TARGET	1.1	4.8	8.4	12.1	15.8	19.4
lab A	0.9	4.8	8.4	12.1	16.9	19.4
	1.0	5.2	8.4	11.2	16.4	18.7
lab B	1.0	5.3	8.7			21.5
	1.1	5.2	9.0	12.5	17.5	19.4
lab C	1.0	4.8	8.6	12.0	16.9	19.6
	1.1	4.8	8.8	12.6	15.4	19.1
lab D	1.2	5.0	8.8	12.3	16.2	18.7
	1.2	5.0	8.3	12.1	16.2	19.4
mean	1.06	5.01	8.63	12.11	16.50	19.48
SD	0.11	0.20	0.24	0.46	0.67	0.88
CV %	10.0	4.1	2.8	3.8	4.1	4.5

RCPA QAP Cycle 35

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Update of Project Status

1. Formation of the Project Working Group ✓
2. Recruitment of participating laboratories ✓
3. Documentation of serum testosterone LCTMS methods of participating laboratories ✓
4. Initial assessment of performance – Stage I ✓
5. Harmonisation of accuracy performance – Stage II
6. Harmonisation of reference intervals – Stage III

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Stage Ia: Confirm accuracy performance

- Meeting held Tuesday 11th October 2011
- Decision to further test performance of labs using a set of patient samples
- Approximately 40 males and 40 females to be distributed to the 7 labs in 2012 (subject to funding)
- Each sample to be run in duplicate on two separate occasions
- This study outline tbc
- Funding source

Harmonisation of accuracy performance – Stage II

- 5.1. Distribution of set of common secondary serum calibrators will be sent to each participating laboratory
- 5.2. This set of serum calibrators are lyophilized human serum samples with different testosterone concentrations that have been validated by a reputable laboratory using reference gas chromatography isotope dilution mass spectrometry method
- 5.3. Each participating laboratory will report serum testosterone EQA results to the RCPA QAP Endocrine program using this set of calibrators
- 5.4. Accuracy, between-batch precision and linearity performance will be evaluated via the subsequent RCPA QAP Endocrine program cycle
- 5.5. The Project Group will compare the performance of these parameters using data obtained from the 2 cycles
- 5.6. Supply of the calibrators will be for another 2 cycles of RCPA QAP Endocrine program

Harmonisation of reference intervals – Stage III

- 6.1. Each participating laboratory will recruit healthy volunteers as reference individuals
- 6.2. Serum testosterone concentrations of these reference samples will be measured using the set of common calibrators when the RCPA QAP Endocrine performance is in control
- 6.3. Project Group will collate and analyse reference values contributed by all the participating laboratories
- 6.4. Project Group will source the supply of validated serum testosterone calibrators for the participating laboratories so that these harmonised reference intervals can be implemented for routine service

REFERENCE INTERVALS

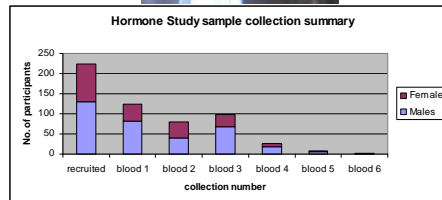
Current Steroid reference intervals based on immunoassays

Measurand different for MS assays

Need to develop these intervals

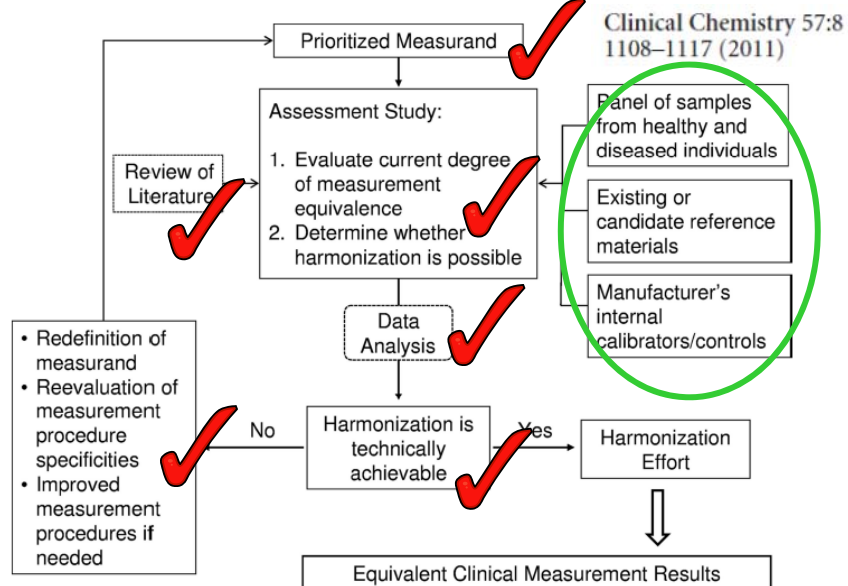
Premature RI – August 2011 audit

- Parents of infants born < 32 weeks gestation were approached for consent either antenatally or soon after delivery
- Whole blood collected from babies on approximately days 1, 4, 7, 14, 21, 28 and 42, coinciding with routine blood sampling



study no.	recruited	blood 1	blood 2	blood 3	blood 4	blood 5	blood 6
Males	131	82	40	69	18	7	2
Females	94	42	41	29	8	1	1
Total	225	124	81	98	26	8	3

Harmonisation: General Process



In Conclusion

- **Achieving harmonisation and standardisation of clinical assays will provide commutability of results between laboratories**
- **This will produce improved ease of result interpretation for the doctor and patient**
- **Unique opportunity to do this early for TMS assays**