Nanoparticle concentration – critical needs and state-of-the-art measurement
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Nanoparticle Reference Materials:
Lessons Learned and the Case for Number Concentration Measurements

Vince Hackley

Materials Measurement Science Division
National Institute of Standards & Technology

NIST role in Standards Development

Mission

Advance <u>measurement science, standards, & technology</u> in ways that enhance U.S. economic security and improve quality of life

NIST supports accurate and compatible measurements by certifying and providing over <u>1200 Reference Materials</u> with well-characterized composition and/or properties.

- Over 32,000 RM units sold each year.
- NIST RMs serve as a mechanism for supporting measurement traceability in the U.S.

http://www.nist.gov/srm/

Scope of Presentation

- ❑ Some basic definitions
- ❑ Impact of nano RMs (NIST AuNP RMs as a case study)
- ❑ Lessons learned – number concentration RMs – challenges and recommendations
- ❑ Concluding remarks

Some Basic Definitions

What is a Reference Material?

Substance whose property values are sufficiently homogeneous, stable, and fit for its intended use in a measurement process

[ISO/Guide 30:2015, 2.11 / VIM:2012, 5.13]

- RM is a generic term
- Properties can be quantitative or qualitative
  - e.g. amount of a substance present is a quantifiable property
  - e.g. identity of substances present is a nominal property

Certified Reference Material (CRM)

RM accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated:

- Uncertainty statement
- Traceability statement (to known references)
- Metrologically valid procedures

"Standard Reference Material"™ is a NIST CRM
- many "NIST Reference Materials" meet VIM/ISO criteria for CRMs

Quality control material – RM used for quality control of a measurement (e.g., proficiency testing)

Value Assignment Terms

certified value

value, assigned to a property of a RM that is accompanied by an uncertainty statement and a statement of metrological traceability, identified as such in the RM certificate

reference value (NIST)

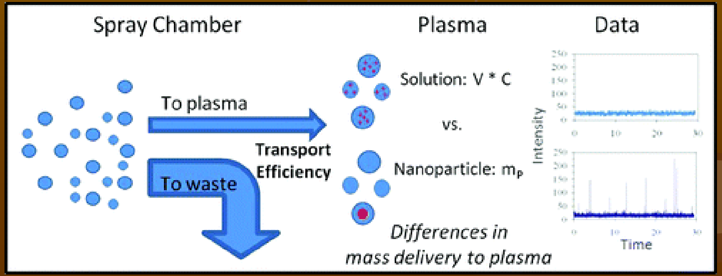
best estimate of the true value, provided on a NIST certificate of analysis or report of investigation, where all known or suspected sources of bias have not been fully investigated by NIST.

information or indicative value

value of a quantity or property, of a RM, which is provided for information only

A RM/CRM may contain more than one type of value assignment

Mean size
Mass concentration



RMs 8012 & 8013

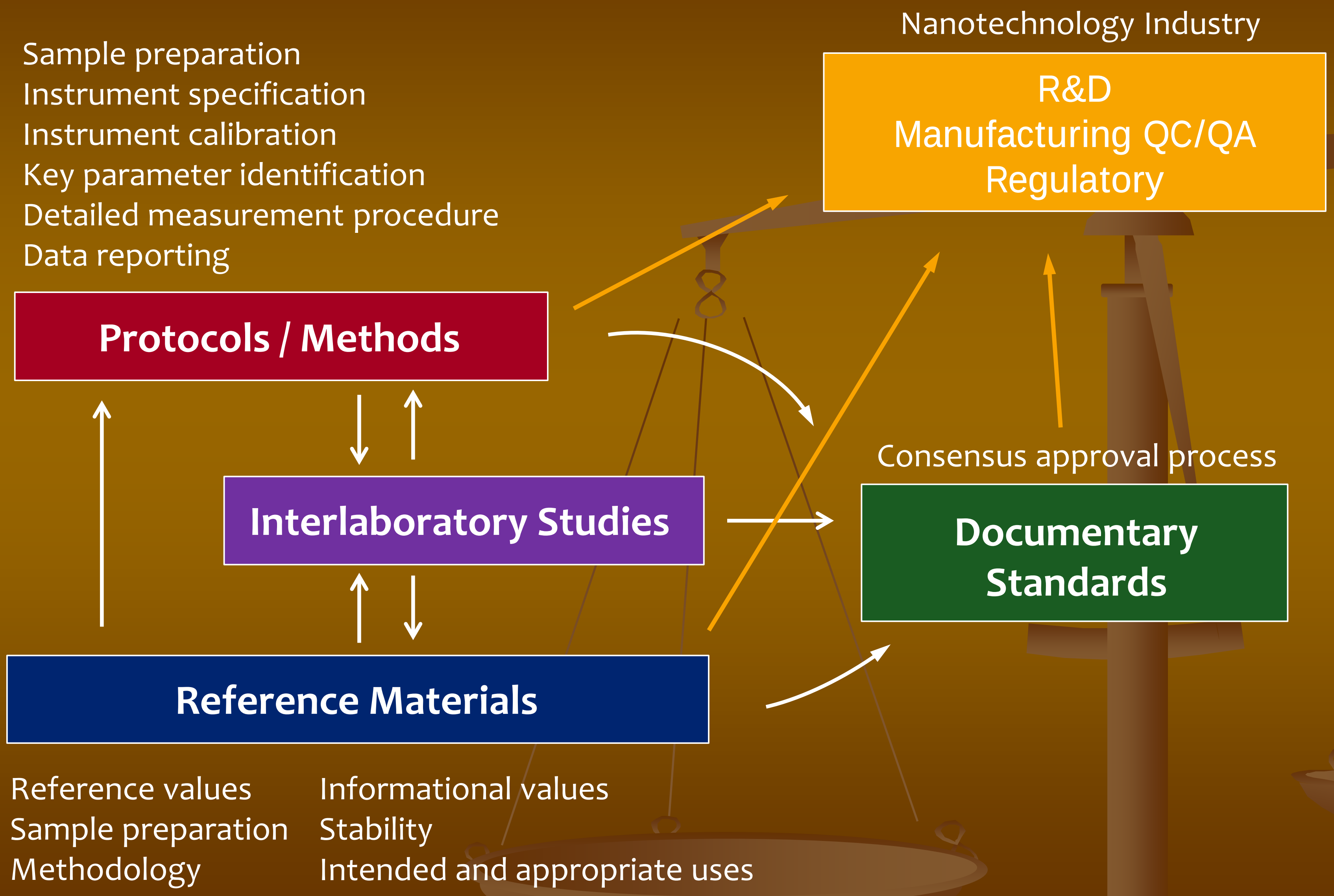
Pace et al., 2011

Impact of Nano-Reference Materials

Primary Drivers for Nano-RM Development

1) Poor reliability of P-C measurements & hazard assessments
2) Poor inter-comparability between labs
3) QC/QA requirements in manufacturing
4) Need to meet regulatory requirements
   - e.g., EC policy defining NMs based on number concentration

Metrology Infrastructure



Impact: Case Study - NIST Gold NP RMs

Released in 2008. Developed in cooperation with US-National Cancer Institute.

Gold NP RMs

>1800 units delivered
>3600 ampoules (2 amps/unit)
53% non-US/ 47% US
- 46% to industry
- 42% to government (incl. 12 NMIs & 6 regulatory agencies)
- 12% to academia/NGOs

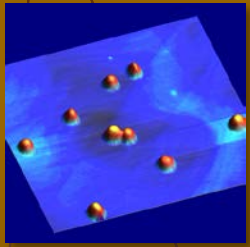
10 , 30 and 60 nm nominal diameter
5 mL, citrate-capped in aqueous suspension
Mean size measured by 6 techniques
Extensive "informational" data included

NIST RMs 8011, 8012, 8013

Protocols

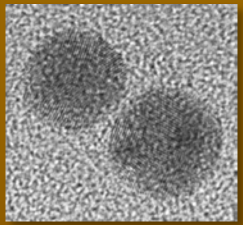
www.ncl.gov

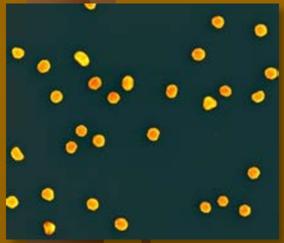
- Size by AFM, TEM, SEM, and DLS
- Electrolytic conductivity
- pH of nanoparticle suspensions
- Mass fraction of particle-bound gold in suspensions
- Mass concentration of gold in rat tissue and blood



AFM, 30 nm

TEM, 10 nm





SEM, 60 nm

J. Grobelski, J. Bonevich, A. Vladar, NIST

Nanotechnology Characterization Laboratory

NIST-NCL collaboration

Documentary Standards

www.astm.org

ASTM International Committee E56 on Nanotechnology

- NIST gold nanoparticle RMs used in three interlaboratory studies linked to standards: E2490-09, E2524-08, E2526-08
- Precision statement generated for E2490 (Sizing by DLS)
- E2859-11 (Sizing by AFM) developed from NIST-NCL protocol

Impact: Case Study - NIST Gold NP RMs

Publications

>90 peer reviewed articles* including 27 on spICP-MS alone

9 white papers + reports + application notes

2 standards (ASTM E2490-09 P&B, ASTM E2859-11)

8 published protocols (NCI/NCL-NIST – Assay Cascade)

*identified as of Fall 2016

Principal Uses (anecdotal & published sources)

- Method validation/instrument calibration
- QC/QA (especially analytical service labs, pharma, materials producers)
- Basic and applied research
- Metrology research
- Method/technique comparisons
- Inter-laboratory comparisons
- Toxicological/biological investigations

Lessons Learned -
Number Concentration Nano RMs

General Challenges for Nano RM Development

- Must address a broad spectrum of research, industry and regulatory needs
- Will be used for many purposes, regardless of the intended use
- Single value assignment may be insufficient; broad spectrum of property data/methods preferred (i.e., multi-parametric RMs)
  - Exception may be “number concentration”
- Prioritization by industry & regulatory communities still lacking
  - Again, exception may be “number concentration”

Challenges for Number Concentration RMs

- NPs suspended in aqueous media are inherently unstable, concentration may change over time
  - How much change is acceptable?
- At very low concentrations (ppb to ppt) losses to container surface can become significant
  - Stability can also be adversely effected
- Value assignment procedure and verification
  - Method selection, limitations, verification
- Applicability to wide range of techniques needed
- Transferability to other materials, matrices
- Application to solid matrices, nano-enabled products
  - Is this possible from a practical standpoint?

Gold as a Candidate for Concentration RMs

- Gold as a core material for RM development
  - high atomic mass, electron and scattering contrast
  - Insoluble under "typical use" conditions
  - Oxidatively stable
  - Low limits of detection (for many methods)
  - Facile surface modification (amine/thiol reactive groups)
  - Generally considered safe

Gold-Based Concentration RMs

- Citrate gold is relatively stable under native conditions
  - Not stable in many salt containing test media (e.g., PBS, serum)
  - Surfactants/capping agents improve stability, but may alter properties
  - Coatings susceptible to degradation over time
- PEGylated AuNPs, if properly formulated, offer a good option
  - Stable in physiological test media
  - Resistant to nonspecific protein adsorption and loss to container wall
  - Long term colloidal stability; can desorb some PEG without loss of stability
  - Will not interfere with spICP-MS (Au mass sensitive only) or TEM
  - Will alter hydrodynamic size, but not number concentration

Value Assignment Considerations

- Methods for number quantification (counting methods) :
  - Single Particle Inductively Coupled Plasma Mass Spectrometry (spICP-MS)
  - Particle Tracking Analysis (PTA)
  - Electrospray-Differential Mobility Analysis w/ Condensation Particle Counter (ES-DMA-CPC)
  - Tunable Resistive Pulse Sensing (TRPS)
  - Resonant Mass Measurement (RMM)
- Ensemble methods include SAXS w/ absolute intensity calibration
  - Must convert mass/vol to number basis, model to invert scattering data
  - Not recommended as a primary method for value assignment
  - Useful as a verification method, transferring RM value

Value Assignment Considerations

- Method limitations

  Lower size limits (Dmin) / concentration range (part/cc):

  - spICP-MS (20-25 nm, Au / $10^3 – 10^6$)
  - PTA/NTP (10-15 nm, Au / $10^6 – 10^{10}$)
  - RMM (35 nm or 450 attograms, Au / $10^4 – 10^9$)
  - TRPS (40 nm, Au? / $10^5 – 10^{12}$)
  - ES-DMA-CPC (3 nm / $10^9 – 10^{13}$)
  - Likely to miss extremely small NPs (<10 nm) using most commercially available single particle methods (other than DMA or ICP-QQQ)
- Need multiple methods to cover the nanoscale/conc. range and for validation & traceability purposes
- Methods assume spherical geometry
    - Error (uncertainty) minimized if particles conform to assumption

Recommendations for NP Concentration RMs

PEG-thiol encapsulated gold NP core as a candidate material

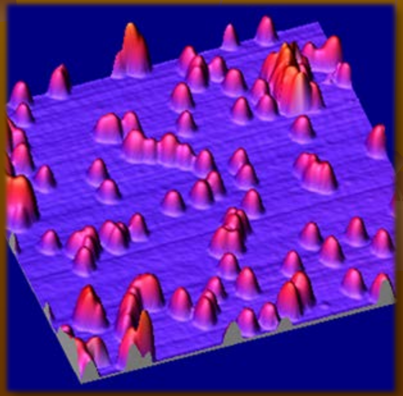
- Core size (diameter) 40-60 nm (smaller sizes ok for some methods, not all)
- High monodispersity (less than 4% COV on diameter to minimize error due to undersize and oversize particles)
- High sphericity (less ≤ 2% by number non-spherical/odd shaped particles)
- Low MW (e.g., 2 kDa) thiolated PEG-based capping agent
- 10-50 mg/L ($\approx 10^{10}$ p/cc) stock Au concentration (must be diluted for use)
- Mean size based on traceable TEM/SEM measurements
- Au concentration by traceable ICP-MS following digestion
- Use multiple particle counting methods for verification, to assess method specific bias and precision

Concluding Statements

- RMs provide confidence in measurements and traceability for regulatory purposes and international commerce
- Lessons learned from prior efforts to develop nano RMs can inform choices and decisions relevant to number concentration standards
- Stability, uniformity and monodispersity, combined with easily detected core materials (e.g., gold) protected with a stable hydrophilic coating, should yield a promising candidate RM
- With multiple techniques available to measure NP concentration, certification should be possible and traceability should be achievable

Contact Information

vince.hackley@nist.gov
Tel. 301-975-5790
NIST, 100 Bureau Drive, Stop 8520
Gaithersburg MD 20899



30 nm gold RM

Supplemental Slides

Specific Challenges in nano RM Development

Material Selection

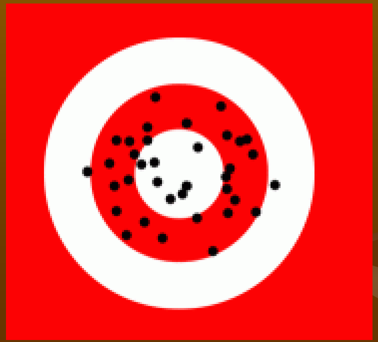
- Availability (commercial sources? single batch?)
- Quality and stability are critical
  - Many factors to consider for quality, how to assess? (shape, size distribution, fraction of odd shapes, presence of under/over sized populations, purity)
  - Sterile and pyrogen free? Are these tested? Pyrogens may not matter for intended use?
  - Difficult to predict stability and shelf life (unless in dry form)
- Fitness for intended use
  - Is composition appropriate for measurement method(s)?
  - Is concentration appropriate? Can it be diluted in use?
  - Are surfactants and/or excipients present? Are they a factor?
  - Heterogeneity? – at what level? ug, mg, g ? (w/r to values)

Specific Challenges in nano RM Development

Value Assignment

- Are appropriate measurement methods available to assign values?
- Are certified values required? Greater work, greater cost
  - Traceability (to SI) possible? Is it required for intended use?
- Is the assigned value method-dependent?
  - Intrinsic or operationally defined property?
- Uncertainty (precision + trueness = accuracy)
  - Are there existing references for bias assessment?
  - What is acceptable accuracy for intended use?
  - What is acceptable precision for intended use?

High trueness
Poor precision



High precision
Poor trueness

Setting Priorities for Nano RM Development

Table III. "Priority" properties of interest for nano-object characterisation during toxicological assessment.

Property	Frequency (#/18 lists)	List source*
Elemental/molecular composition (bulk)	16	a – i, k, l, n – p
Surface area (specific)	14	a – c, e, f – i, k – p
Particle size	13	a, c, e – j, l – p
Morphology/shape/form	13	a – g, i – l, o
Particle size distribution	12	b, c, e – i, k, m, o, p
Surface chemistry	12	a – c, e, h – n, p
Agglomeration/aggregation state	11	b, c, e, f, h, j, k, m, n, p
Crystal structure	10	a, c – e, h, i, k, l, p
Surface charge	9	b, c, e, f, i – k, n
Dispersability (dry/wet)	7	c, f, i, j, l – n
Surface reactivity	6	b, c, e, i, m, p
Particle concentration	4	a, f, k
Solubility (biological)	4	b, i, n, o
Solubility (water)	3	i, j, m
Density	1	i
Porosity (specific)	1	i
Stability	1	b
Surface morphology/structure	1	d

*a = Aitken et al. 2008; b = Bouwmeester et al. 2011; c = Boverhof & David 2010 (adapted from the MINChar initiative, http://www.characterizationmatters.org); d = Bucher et al. 2004; e = Card & Magnuson 2009; f = Crane et al. 2008; g = Fubini et al. 2010; h = Gonzalez et al. 2008; i = NANO Risk Framework 2007; j = NIST 2008; k = Oberdörster et al. 2005 (three separate lists); l = Powers et al. 2006; m = SCENIHR 2009; n = Stone et al. 2010; o = Warheit et al. 2007; p = Warheit 2008.

- 18 published lists of priority measurands for tox assess.
- "composition" appears most frequently (16 times)
- Next 4 in frequency are dimensionally related

Conclusions

- ✓ need consensus on prioritization
- ✓ poorly defined or qualitative measurands lack metrological traceability (need for clarity)
- ✓ better understanding by EHS community of measurement processes will inform their use and avoid artifacts or misinterpretation

Nanoscale reference materials for environmental, health and safety measurements: needs, gaps and opportunities.
Stefaniak et al., *Nanotoxicology*, 2012

Prioritizing Nano-RM Development at NIST

NMs of greatest potential risk/impact based on:

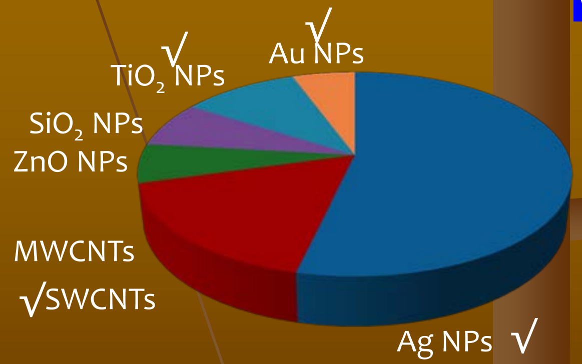
- Production volume
- Use in products or manufacturing processes
- Similar characteristics to known toxicants, e.g., asbestos fiber-like morphologies

ENMs in nanotechnology-enabled consumer products :

Silver	Silicon
Carbon	Titanium
Zinc	Gold

by element

√ = NIST



MWCNT: multi-wall carbon nanotube
SWCNT: single-wall carbon nanotube
NP: nanoparticle

Most products are coatings or composites with “special” properties

- Antimicrobial: Ag
- Strength: SiO_2, MWCNTs
- Optical: ZnO, TiO_2
- Photocatalysis: TiO_2

Source: Project on Emerging Nanotechnologies, Wilson Institute

Availability of Nano RMs

RM: reference material; CRM: Certified RM; SRM: Standard RM (NIST only); NP: nanoparticle: CNT: carbon nanotube

Material Type	Form	Reference Property	Nominal Value(s)	Institution*	Identifier(s)
gold NPs	aqueous suspension	mean diameter	10 nm, 30 nm & 60 nm	NIST	RMs 8011, 8012, 8013
silicon dioxide NPs	aqueous suspension	mean diameter	20 nm	IRMM	ERM-FD100
	aqueous suspension	mean diameter	40 nm	IRMM	ERM-FD304
	aqueous suspension	mean diameters	20 nm and 80 nm mixture	IRMM	ERM-FD102
titanium dioxide NPs	dry powder	specific surface area	55 m^2/g	NIST	SRM 1898
	dry powder	specific surface area	76 m^2/g	AIST/NMIJ	NMIJ-5713a*
	dry powder	specific surface area	11 m^2/g	AIST/NMIJ	NMIJ-5711a*
	dry powder	specific surface area	57 m^2/g	AIST/NMIJ	NMIJ-5712a*
silver NPs	freeze-dried	mean diameter	75 nm	NIST	RM 8017
	freeze-dried	mean diameter	10 nm	NIST	in production
	suspension	particle size distribution	7 nm to 36 nm	BAM	BAM-N001
single-wall CNTs	dry soot	mass fraction	impurity elements	NIST	SRM 2483
	dry soot	mass fraction	impurity elements	NRC	SWCNT-1
	aqueous suspension	length	"long", "medium", "short"	NIST	RM 8281
multiwall CNTs	dry soot	mass fraction	impurity elements	NIST	in production
polystyrene NPs	aqueous suspension	mean diameter	100 nm	NIST	SRM 1963a
	aqueous suspension	mean diameter	60 nm	NIST	SRM 1964
	aqueous suspension	mean diameter	120 nm	AIST/NMIJ	5701-a
	aqueous suspension	particle size	115 nm	NIM	GBW 12019
	aqueous suspension	particle size	84 nm	NIM	GBW (E)120090
	aqueous suspension	particle size	65 nm	NIM	GBW (E)120091
silicon NPs	toluene suspension	mean diameter	2 nm	NIST	RM 8027
nano-alumina	dry powder	specific surface area	445.4 m^2/g	NIM	GBW 13901
	dry powder	specific surface area	359.4 m^2/g	NIM	GBW 13906
	dry powder	specific surface area	515.3 m^2/g	NIM	GBW 13907
cellulose nanocrystal	dry powder	mass fraction	trace metals	NRC	CNC-1
	aqueous suspension	mass fraction	trace metals	NRC	CNCS-1

*NIST (US); AIST: Japanese National Institute of Advanced Industrial Science/National Metrology Institute of Japan
NIM (China): National Institute of Metrology; NRC (Canada): National Research Council
IRMM: Joint Research Center - Institute for Reference Materials and Measurements
BAM: German Federal Institute for Materials Research and Testing