Nanoparticle Reference Materials: Lessons Learned and the Case for Number Concentration Measurements

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Views and opinions expressed are those of the speaker and do not necessarily reflect the official policy or position of any agency of the U.S. government
NIST role in Standards Development

Mission

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

NIST supports accurate and compatible measurements by certifying and providing over 1200 Reference Materials 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
What is a Reference Material?

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


- 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.

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**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
Impact: Case Study - NIST Gold NP RMs


**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

**Protocols**
- 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

**Documentary Standards**

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

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 RM
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 affected
- 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
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
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 (≈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.

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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
Nanoscale reference materials for environmental, health and safety measurements: needs, gaps and opportunities.

Stefaniak et al., Nanotoxicology, 2012

- 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

**Setting Priorities for Nano RM Development**

<table>
<thead>
<tr>
<th>Property</th>
<th>Frequency (#/18 lists)</th>
<th>List source*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elemental/molecular composition (bulk)</td>
<td>16</td>
<td>a – i, k, l, n – p</td>
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<tr>
<td>Surface area (specific)</td>
<td>14</td>
<td>a – c, e, f – i, k – p</td>
</tr>
<tr>
<td>Particle size</td>
<td>13</td>
<td>a, c, e – j, l – p</td>
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<tr>
<td>Morphology/shape/form</td>
<td>13</td>
<td>a – g, i – l, o</td>
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<tr>
<td>Particle size distribution</td>
<td>12</td>
<td>b, c, e – i, k, m, o, p</td>
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<tr>
<td>Surface chemistry</td>
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<td>a – c, c, h – n, p</td>
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<tr>
<td>Agglomeration/aggregation state</td>
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<td>b, c, e, f, h, j, k, m, n, p</td>
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<td>Crystal structure</td>
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<td>a, c – e, h, i, k, l, p</td>
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<tr>
<td>Surface charge</td>
<td>9</td>
<td>b, c, e, f, i – k, n</td>
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<tr>
<td>Dispersability (dry/wet)</td>
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<td>c, f, i, j, l – n</td>
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<td>Surface reactivity</td>
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<td>4</td>
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<td>Solubility (biological)</td>
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<tr>
<td>Porosity (specific)</td>
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<tr>
<td>Stability</td>
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<td>b</td>
</tr>
<tr>
<td>Surface morphology/structure</td>
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</table>

Prioritizing Nano-RM Development at NIST

NMIs 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

ENMIs in nanotechnology-enabled consumer products:

- Silver
- Carbon
- Zinc
- Silicon
- Titanium
- Gold

Most products are coatings or composites with “special” properties:

- Antimicrobial: Ag
- Strength: SiO₂, MWCNTs
- Optical: ZnO, TiO₂
- Photocatalysis: TiO₂

Source: Project on Emerging Nanotechnologies, Wilson Institute
## Availability of Nano RMs

<table>
<thead>
<tr>
<th>Material Type</th>
<th>Form</th>
<th>Reference Property</th>
<th>Nominal Value(s)</th>
<th>Institution*</th>
<th>Identifier(s)</th>
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<tbody>
<tr>
<td>gold NPs</td>
<td>aqueous suspension</td>
<td>mean diameter</td>
<td>10 nm, 30 nm &amp; 60 nm</td>
<td>NIST</td>
<td>RMs 8011, 8012, 8013</td>
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<td>titanium dioxide NPs</td>
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<td>SRM 1898</td>
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<td>freeze-dried</td>
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<td>In production</td>
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<tr>
<td></td>
<td>suspension</td>
<td>particle size distribution</td>
<td>7 nm to 36 nm</td>
<td>BAM</td>
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<td>single-wall CNTs</td>
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<td>mass fraction</td>
<td>impurity elements</td>
<td>NIST</td>
<td>SRM 2483</td>
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<td>SWCNT-1</td>
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* 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