3rd Tri-National Workshop on Standards for Nanotechnology – CENAM Mexico

Enabling Standards for Nanomaterial Characterization: Findings and Summary of the Recent NIST Workshop

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Purpose of Workshop

It is now generally accepted that an urgent need exits for standardization and validation of measurements to assess the chemical, physical and biological properties of nanomaterials

Critical for EHS assessment of nanomaterials, where the lack of validated standards has created a bottleneck to progress in the field

The purpose of the workshop was to assess this need and to promote a community-driven solution

High-Level Goals of Workshop

- Stimulate discussion among stakeholders with respect to nanomaterial characterization issues
- Promote cooperation between US agencies and international stakeholders in addressing critical standardization needs
- Accelerate development and validation of protocols, standards and reference materials

Specific Workshop Objectives

- Provide broad overview of current nanomaterial standards needs and related activities on an international level
- Disseminate preliminary findings of 3 ASTM interlab studies and use this information to establish a baseline for future ILSs
- Demonstrate and discuss a prototype Wiki (Web 2.0 based collaborative on-line site) to facilitate pre-standard development and protocol validation
- Form a "community of interest" to support protocol and reference material development, ILSs via the Web 2.0 infrastructure
- Consensus nomination of initial “projects” for Wiki

Overarching Needs & Issues

- Day 1 morning sessions were used to frame issues, identify overarching needs, and establish context for the meeting.
- Speakers represented US agencies, SDOs, industry and academia, providing a broad spectrum of viewpoints.

Focus on Biological Testing of ENMs

- ENMs interfere with many established biological in vitro assays
  - cause false positive and false negative responses
- Occupational exposure is one of the most important routes for free ENMs
  - methods for occupational risk assessment are generally lacking
  - relevant metrics, suitable RMs, validated exposure tools needed
  - standards for data management also needed
- Reference Materials (RMs) to support nanotoxicology are needed
  - consensus on priority RMs exists (e.g., TiO_2, Au, Ag, SWCNT) , but no consensus on required RM properties or metrics
- Emphasis on need for verifiable science in assessing ENM hazards
  - small differences in size can dramatically influence results
  - protocols need to be validated across multiple labs
  - reproducibility in biological testing possible, but challenging

Industry & Regulatory Perspective

- Knowledge key to enable life-cycle risk assessment and management
  - nano-bio interface poorly understood on fundamental level
  - high purity / well characterized ENMs needed for reliability
  - standardized biological media and assays
  - best metrics for assessing toxicity (mass, size, surface ?)
  - exposure monitoring methods needed for ENMs
  - do current protocols correlate with acute and/or chronic toxicity
- For drug development size matters most
  - RMs required for qualification/calibration of size measurements
  - operator independent reproducibility is a must
  - standards for data management also needed
- Industry standards must be easy to implement
  - minimal processing for RMs
  - methods acceptable by the industrial community
  - complete and clear supporting documentation a must

Industry & Regulatory Perspective

- FDA-recognized test standards improve quality of applications and facilitate regulatory process
  - FDA needs validated standards adopted by scientific community
  - biocompatibility and toxicity assessment standards are high priority
  - medical device perspective: does inclusion of ENM change product risk classification?
- Academic institutions can investigate large libraries of ENMs
  - identify problem areas
  - facilitate RM development
  - application of information technologies can be complementary to standards development

Interlaboratory Studies & Standards Development

- Day 1 afternoon sessions focused on role of ILSs to support protocol validation and standards development.
- ASTM Interlaboratory Testing Program overviewed, findings for E56 sponsored studies presented and discussed
- Roughly 80% of participants in ASTM studies present at workshop
- Broad ranging mediated discussion at day's end

E56 Interlaboratory Studies

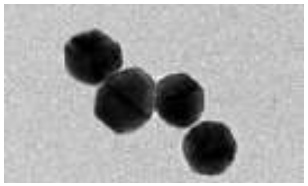
- Physical testing standard: ASTM E2490 (NP sizing by PCS)
  - ILS166 to generate precision statement for standard
  - corollary TEM, SEM, AFM data collected using defined protocols
  - protocols address sample handling, prep, measurement & analysis
  - 5 test materials: 3 NIST Au RMs and 2 G6-PAMAM dendrimers
  - 26 labs participated, 7700+ individual results generated
  - image files collected to assess impact of image analysis software on microscopy results (planned study)
- Preliminary Findings of ILS166
  - greatest variance with smallest particles
  - aliquot effect for PCS observed in 4 of 5 test materials
  - sample prep is a significant issue even with defined protocols

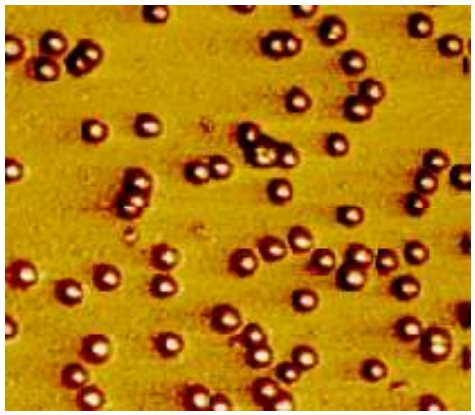
    images show large variation in deposition quality for microscopy
- Outputs
  - report on ILS 166 to be published by ASTM
  - precision statement for E2490 in balloting

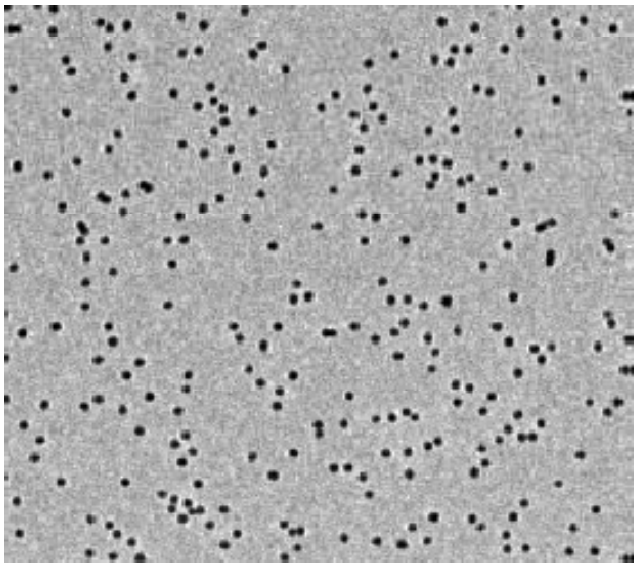
Summary of Reported Test Results

Sample A - 8011	All results in nm (10-9m)						
	NIST	X	s	sr	sR	r	R
TEM	8.9 +/- 0.1	8.47	1.49	0.00	1.49	0.00	4.17
SEM	9.9 +/- 0.1	9.55	1.90	NA	1.90	NA	5.31
AFM	8.5 +/- 0.3	7.64	1.15	0.05	1.15	0.13	3.21
DLS: z-average A1	13.5 +/- 0.1	14.55	3.99	1.84	4.32	5.14	12.09
DLS: z-average A2		13.72	2.36	0.83	2.47	2.33	6.93
DLS: Polydispersity A1		0.34	0.30	0.17	0.34	0.47	0.94
DLS: Polydispersity A2		0.27	0.19	0.08	0.20	0.22	0.57
DLS: Intensity Mean A1		13.84	2.36	0.50	2.40	1.41	6.72
DLS: Intensity Mean A2		14.60	1.57	0.95	1.78	2.65	4.99
DLS: Volume Mean A1		9.69	3.94	0.39	3.96	1.10	11.08
DLS: Volume Mean A2		8.77	4.89	1.29	5.02	3.63	14.06
Other							
SAXS	9.1 +/- 1.8						
DMA	11.3 +/- 0.1						

Au Nanoparticles



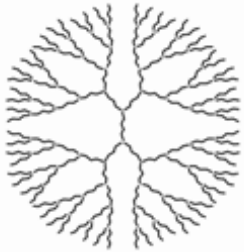




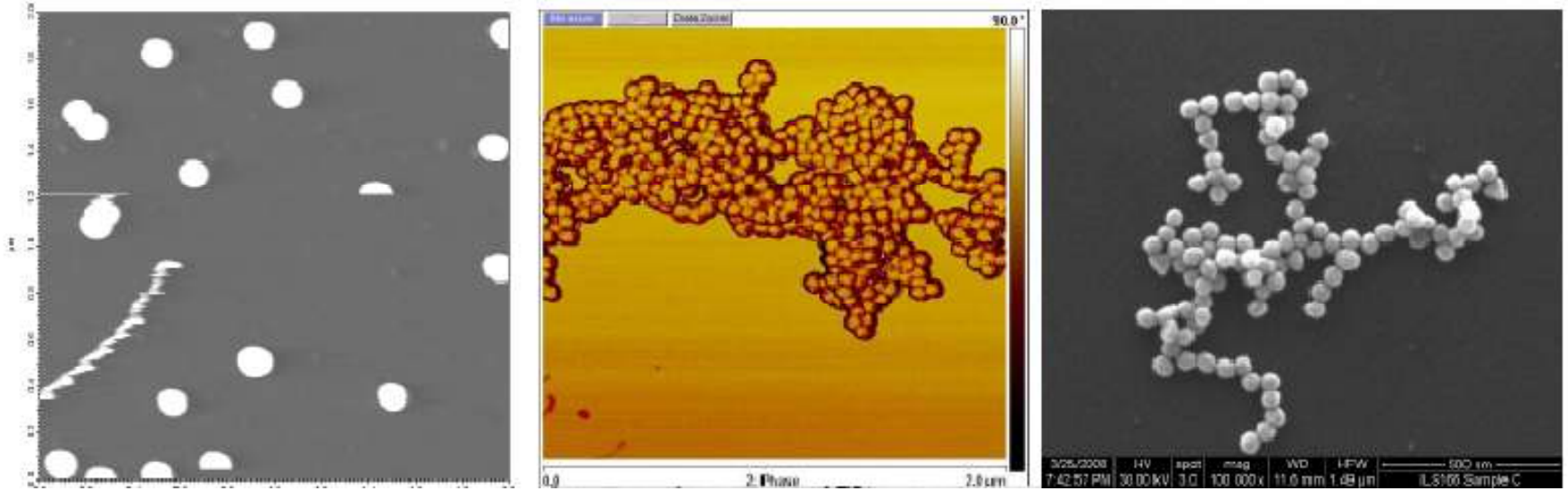
aliquot effect?

Sample D - G6 dendrimer	All results in nm (10-9m)					
	X	s	sr	sR	r	R
DLS: z-average A1	43.31	120.09	12.18	120.58	34.10	337.62
DLS: z-average A2	7.03	0.62	0.46	0.75	1.28	2.09
DLS: Polydispersity A1	0.22	0.30	0.05	0.31	0.13	0.86
DLS: Polydispersity A2	0.14	0.18	0.06	0.18	0.16	0.51
DLS: Intensity Mean A1	7.94	1.34	0.42	1.40	1.18	3.91
DLS: Intensity Mean A2	8.23	2.44	0.60	2.50	1.68	7.00
DLS: Volume Mean A1	7.20	1.18	0.56	1.28	1.56	3.59
DLS: Volume Mean A2	7.43	2.45	0.79	2.55	2.20	7.14

PAMAM Dendrimers



Even with functionalized substrates provided and detailed sample deposition protocols, variation in quality of deposited samples is large.



E56 Interlaboratory Studies

- Biological testing standard: ASTM E2524 (Hemolysis)
  - ILS201 to generate precision statement for standard
  - test protocol required fresh human whole blood (pooled)
  - 4 test materials: 2 NIST Au RMs and 2 G6-PAMAM dendrimers
  - 9 labs participated
  - standard based on NCL protocol
- Conclusions
  - total & plasma-free hemoglobin tests consistent across labs
  - only 1 lab had problem with blood quality as received from vendor
  - overall assay performance based on standard curve and quality controls was good
  - only 2 labs submitted complete data sets
  - most problems occurred where sample modifications were required
  - insufficient results obtained for precision statement
  - plans to repeat study under consideration

E56 Interlaboratory Studies

- Biological testing standard: ASTM E2526 (Cytotoxicity)
  - ILS202 to generate precision statement for standard
  - toxicity to human hepatocarcinoma and porcine renal cells
  - MTT reduction and LDH enzyme leakage methods
  - 6 labs participated; same test materials as ILS201
  - standard based on NCL protocol
  - only cationic dendrimer generally considered to have “toxic” response
- Conclusions
  - only MTT results described (colorimetric assay)
  - only 2 labs observed enough toxic response to estimate IC50 (50% inhibitory concentration) for cationic dendrimer
  - insufficient results obtained for precision statement
  - plans to repeat study under consideration

Lessons Learned from ASTM Studies

- ❑ Careful planning and good communication are key for ILS success
- ❑ Informal testing with fewer labs may be useful prior to formal testing
- ❑ Very good precision attainable with physical (dimensional) tests; bio assays are more challenging in this respect
- ❑ For biological assays, training sets should be incorporated prior to blinded validation studies
- ❑ Test materials with higher toxicity are needed to evaluate cytotoxicity assays
- ❑ Due to complexity of biological assays, procedural schematics would improve results
- ❑ Sample preparation is the single largest source of variation in test results
  - ENM introduction to testing media for biological assays
  - inconsistent deposition for microscopy (artifacts)
  - For PCS, any modification/transfer of sample is a potential source of error

Interagency & International Cooperation on Nano Standards Development

- ❑ Day 2 morning session addressed the need for cooperation between federal agencies and between international stakeholders.
- ❑ Speakers represented NNCO, NIST/NCI, NIEHS, EPA/OECD, APEC

- Standards development is a “tragedy of the commons” – benefits everyone, but in no one’s best interest to invest directly

- Limited pool of qualified and wiling participants to work on standards – resource must be used more efficiently for sustainable progress

- Must be science based – otherwise can entrench inferior technologies

- Focus on collaborative resources and high throughput is needed

- International cooperation on RMs necessary to meet growing demands for nanotechnology in a timely fashion

Facilitating Pre-Standard Development

❑ Day 2 afternoon session addressed the concept of electronic-based and community driven approaches to pre-standard protocol and RM development

❑ Marty Fritts and Raul Cachau from the National Cancer Institute at Frederick

❑ Web 2.0 approach (Wiki) provides greater transparency, can accelerate and complement the formal SD process

- Documenting comments, dissent and resolution
- Organizing and assisting technical discussions
- Linking to authoritative documents, SDOs
- Foster collaborative development and validation of protocols, RMs
- Support “community of interest”

Facilitating Pre-Standard Development

❑ Prototype Wiki developed at NCI's Advanced Biomedical Computing Center

❑ Wiki is a collection of IT tools implemented as a social protocol to enhance collaboration and access to information (e.g., Wikipedia)

- Policy driven site management that addresses: access and permissions, data curatorship, ease of use, user requirements, data archiving, security, and user notification (e.g., RSS)

- Neutral posture, "owned" by no one, meeting place for diverse but intersecting standards activities, flexible rules and structure

- Better inclusion of participants beyond standards committee members, SDOs

❑ Prototype Wiki being vetted by volunteer group, public access early 2009

❑ NNCO, ASTM buy-in to concept, general consensus was positive

Focused Breakout Discussions

- Workshop concluded with 4 breakout discussions
  1. Protocol Development – Biological Interactions
  2. Protocol Development – Physical Property Characterization
  3. Test Materials, Media & Sample Preparation
  4. Interlaboratory Studies & the Testing Network

- Some recommendations for new projects to populate the proposed Wiki
  - Low-Dose Positive and Negative Controls, Benchmarks for bio assays: identify and test candidates, validate with interlab studies
  - Identify tests that predict chronic tox, address mechanisms of tox
  - Abiotic and simulated biological matrices for ENM dispersion and testing: develop dispersion protocols, standardize media recipes
  - RMs and “study” materials: identify candidates, define required characteristics for specific uses, evaluate with interlab studies

Final Recommendations & Conclusions

- ❑ Science necessary to establish relevant biological tests for ENMs is immature
- ❑ Interlab studies are vital part of standards/protocol development and require more attention by the standards community
- ❑ Biological protocols are subject to greater uncertainty relative to physical measurements
  - ≥ 20 % variability in bio assay results considered very good
  - operator bias, assay complexity, serial nature of procedures, use of biological components that are subject to change/adaptation
- ❑ Training sets should be incorporated into all interlab studies that test bio assays
- ❑ Effective communication between organizers and test analysts is critical
  - breakdowns in communication led to poor results or incorrectly handled test components during ASTM studies
- ❑ Well characterized RMs (preferably certified by chartered bodies) should be included in all interlab studies
  - they provide traceability and confidence in results

Final Recommendations & Conclusions

- Workshop participants endorsed the concept of a community guided effort using a Web 2.0 approach for
  - exploration of best laboratory practices
  - harmonization of preliminary stage standards development
  - aiding selection and vetting of candidate RMs
  - greater engagement by the world's EHS and measurement expertise
- Letters of intent would be solicited from US and international agencies and institutions wishing to participate
- Resources for operating the Wiki would be solicited by the NNCO from NSET based on letters of intent
  - NCI would initially host & administer site during developmental phase
- An international team drawn from government, industry and academia has volunteered to help launch the pilot
- Issues regarding permanent hosting, operation and governance would be decided by the community

Workshop Sponsors & Contributors

Food and Drug Administration
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