Environmental Consequences of Nanotechnologies

Nanoparticle Dispersion in Aqueous Media: SOP-T-1

Jessica G. Coleman, Alan J. Kennedy, and Ashley R. Harmon

February 2015

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Environmental Consequences of Nanotechnologies
Scientific Operating Procedure SOP-T-1

Jessica G. Coleman, Alan J. Kennedy, and Ashley R. Harmon

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Abstract

Homogenous dispersions of nanoparticles (NPs) for use in bioassay media for exposures/characterizations are a crucial need in ecotoxicology assays. The present protocol provides guidance and step-by-step methods for: (1) creating a working stock from nanoparticle powder and nanoparticle aqueous suspensions, (2) spiking working stock suspensions into aqueous bioassay media. The protocol also provides guidance on optimization of test media and organism health. Peer-reviewed publications and standards, with adaptations for aquatic bioassays are included. Users are directed to follow the protocol section relevant to their need and parent material (e.g. aqueous NPs or powder NPs). This protocol was developed on procedures created specifically for metal nanoparticles, but may have broader application. In an effort to validate procedural consistency, values associated with reference materials (RM) are included. The standard references include powder-based and aqueous suspensions from the National Institute of Standards and Technology (NIST) and NanoComposix (NC); including aqueous suspension of NIST Reference Material citrate coated 30 nm Au and NC citrate coated 30 nm Ag, and powdered NIST standard reference material 1898 TiO2 nanomaterial. The Ag and Au reference materials were selected to represent moderately soluble and relatively insoluble materials, respectively. If followed correctly, near-homogenous particle dispersions of the reference materials and a best case scenario for dispersion of test materials in aqueous media should be generated based on best procedural guidance available.

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Preface

This procedure was developed under the Engineer Research Development Center (ERDC) Environmental Quality and Technology (EQT) Research Program titled “Environmental Consequences of Nanotechnologies.” Procedures link to the ERDC NanoGRID (Guidance for Risk Informed Deployment) framework for testing the exposure and hazard of nanotechnology Environmental Health and Safety (EHS). The technical monitor was Dr. Elizabeth Ferguson.

The work was coordinated by the Environmental Risk Assessment Branch (EPR) of the Environmental Processes and Engineering Division (EPE) at the U.S. Army Engineer Research and Development Center – Environmental Laboratory (ERDC-EL). Buddy Goatcher was the Branch Chief, CEERD-EP-R, Warren Lorentz was the Acting Division Chief, CEERD-EP-E; and Dr. Elizabeth Ferguson was the Technical Director for Military Environmental Engineering and Sciences. The Deputy Director of ERDC-EL was Dr. Jack Davis and the Director was Dr. Elizabeth Fleming.

COL Jeffrey Eckstein was the Commander and Executive Director of ERDC, and Dr. Jeffery P. Holland was the Director.
## Definitions

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agglomerate</strong></td>
<td>in nanotechnology, an assembly of particles held together by relatively weak forces (for example, Van der Waals or capillary), that may break apart into smaller particles upon processing, for example.</td>
</tr>
<tr>
<td><strong>Nano object</strong></td>
<td>object with at least one dimension between 1-100 nanometer to include sphere, fiber, tube, rod, etc.</td>
</tr>
<tr>
<td><strong>Dispersion Stability</strong></td>
<td>Resisting change or variation in the initial properties of a dispersion over time (ISO 13097, 2013)</td>
</tr>
<tr>
<td><strong>Working stock</strong></td>
<td>dispersion of NP concentrate from manufacturer in aqueous suspension for bioassay media spiking/characterization.</td>
</tr>
<tr>
<td><strong>Bioassay aqueous media</strong></td>
<td>aqueous media utilized in biological testing, bioassays, characterization, etc. Media may include, but is not limited to, moderately hard water, hard water, ultrapure water, de-ionized water, cell media, etc.</td>
</tr>
<tr>
<td><strong>Clump</strong></td>
<td>assemblage of particles which are either rigidly joined or loosely coherent (ISO 14887, 2000)</td>
</tr>
</tbody>
</table>
## Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
</tr>
<tr>
<td>FFF</td>
<td>field flow fractionation</td>
</tr>
<tr>
<td>MHRW</td>
<td>moderately hard reconstituted water</td>
</tr>
<tr>
<td>NM</td>
<td>nano material</td>
</tr>
<tr>
<td>DI</td>
<td>de-ionized</td>
</tr>
<tr>
<td>SOP</td>
<td>standard operating procedure</td>
</tr>
<tr>
<td>SRM</td>
<td>standard reference material generated by NIST</td>
</tr>
<tr>
<td>RM</td>
<td>reference material</td>
</tr>
<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>inductively coupled plasma mass spectrometry</td>
</tr>
<tr>
<td>mL</td>
<td>milliliter</td>
</tr>
<tr>
<td>L</td>
<td>liter</td>
</tr>
<tr>
<td>W</td>
<td>watts</td>
</tr>
<tr>
<td>S</td>
<td>seconds</td>
</tr>
<tr>
<td>&lt;T&gt;</td>
<td>test materials</td>
</tr>
<tr>
<td>&lt;R&gt;</td>
<td>designates reference materials</td>
</tr>
</tbody>
</table>
1 Introduction

The Scientific Operating Procedure (SOP) described herein for assessing the properties of nanotechnologies was developed for two purposes: 1) creation of an aqueous working stock from NP powder and NP suspension and 2) methods for spiking NP working stocks into aqueous bioassay media. The SOPs in this series will guide users in best practice material preparation techniques. This protocol was developed specifically on procedures created for metal NPs, but may have broader applications. The present SOP combines best laboratory practices available from the literature and professional experience of the Engineer Research Development Center (ERDC) research scientists, developed under the ERDC Nanomaterials Focus Area.

This SOP describes guidance on how to create well-dispersed NP working stock suspensions, utilizing both bath and probe sonication techniques. Methods are also provided which describe how to spike working stocks into aqueous bioassay media with recommendations for optimizing media and test organism health. Methods include associated nanomaterial values from internal testing for protocol calibration.
2 Scope

The present protocol provides two procedural sections. The two sections can be utilized separately or in tandem based on needs. Sections are divided as: 1) generation of working stock from powder or aqueous suspension of NP concentrates 2) spiking of working stock into aqueous bioassay media for biological testing and characterization. The protocol is applicable to metal powder and solution-based nanomaterial concentrates. Although the methods are based on metal NPs, broader application to non-metal NPs may exist. The protocol also includes media and organism health optimization recommendations for bioassays. Users should refer to the section of the protocol which relates to their parent starting material (Figure 1).

Nanomaterials were purchased and utilized as internal reference materials (RM) in the present protocol methods. These materials included NIST Standard Reference Material™ (SRM) 1898 Titanium Dioxide Nanomaterial (TiO₂), NIST Reference Material 8012 citrate-stabilized aqueous gold (Au) 30 nm, and NanoComposix (NC) aqueous citrate-coated 30 nm silver (Ag). The values generated from internal protocol application to the RMs are included below. For protocol calibration of powder-based NPs, users are directed to test NIST SRM 1898 Titanium Dioxide Nanomaterial (TiO₂). For solution-based NPs, users are directed to test NIST RM 8012 citrate-stabilized 30 nm Au and NC citrate coated 30 nm Ag.
Figure 1. Conceptual SOP Flow Chart.

Starting material

Material Phase

Powder

Aqueous suspension

Required materials (Section 4.1.1)

Preparation method (Section 5.1.1.1)

Dispersion method (Section 5.2.1)

QA/QC & Reporting (Section 6)

Spiking into bioassay media

Required Materials (Section 4.2)

Spiking Method (Section 5.3)

QA/QC Reporting (Section 6)

Required materials (Section 4.1.2)

Preparation method (Section 5.1.1.2)

Dispersion method (Section 5.2.2)

QA/QC & Reporting (Section 6)
3 Background

Creating a repeatable, homogenous dispersion of nanoparticles for use in biological exposures/characterizations is crucial in aqueous phase toxicology testing and characterization. In an effort to optimize consistent nanoparticle spiking methods into aqueous biological media for laboratory use, ERDC has leveraged previous publications and protocols (ISO 2000; Kennedy et al. 2012; Handy et al. 2012; Taurozzi et al. 2010, 2011, 2012, 2013; OECD 2012; Prospect 2010) in conjunction with lab-based assessments to create a guide that provides users with step-by-step procedures and guidance to obtain homogenous particle distributions in working stocks and environmentally relevant media. Nanoparticles received from a manufacturer or incorporated into a technology may be in a powder or aqueous suspension form. To address the issue of consistent preparation for either form, the present protocol was divided into two sections. Users will follow the steps and procedures associated with the parent material characteristics.

Producing a consistent dispersion can be challenging, and is material and medium dependent. Therefore, several research organizations such as the NIST, Organization for Economic Co-operative and Development (OECD), and the International Organization for Standards (ISO) have invested in generating protocols and guidelines for dispersing nanomaterials. The intention of this protocol was not to duplicate available standards, but rather leverage protocols from available resources while modifying procedures to meet Army and toxicology needs. The present protocol utilizes the methods outlined in ISO 2000, Taurozzi 2012, and Taurozzi 2013 for pre-dispersion of NP powder for stock generation. Aqueous NP stock generation, mixing, dilutions, and media spiking methods/recommendations are leveraged from ISO 2000, Jiang et al. 2009, Kennedy 2009, Kennedy 2010, Romer 2011, Taurozzi 2011, Kennedy 2012, Handy et al. 2012, Tejamaya et al. 2012, Harmon et al. 2014.

Sonication has the potential to alter NP characteristics; therefore, it is important to keep the intensity and time required to sonicate to the minimum to reach desired dispersion results (Taurozzi 2010, 2011, 2013). Furthermore, Taurozzi 2010 provides step by step guidance to directly measure the acoustic energy delivered to sonicated media through
calorimetry. The present protocol recommends the use of the calibration specifically outlined in Taurozzi 2010 (p. 10) for probe sonicators prior to sample preparation. Low intensity (e.g., bath) sonication is recommended, followed by higher intensity probe sonication if needed. For probe sonication, users are directed to refer to Table 1 (Taurozzi 2012) for optimized starting points to begin their sample preparation.

ISO published a specific, stepwise procedure for sample preparation of dispersing powder material into a liquid (ISO 14887, 2000). ISO 14887 provides steps on how to effectively de-agglomerate and wet material “clumps” into a useable form for dispersion. Clumps are defined as an assemblage of particles, which are either rigidly joined or loosely coherent (ISO 2000). The procedure also provides a list of agents useful for dispersing wetted materials, or “test paste” into a liquid. For preparation of powder materials, which may be heterogeneous in nature, use guidelines provided in ISO 14887 for the creation of test “paste,” followed by the methods in Taurozzi et al. 2013. The use of dispersion agents in biological testing media is not recommended unless such agents are environmentally relevant (e.g., NOM). However, this does not preclude use of dispersion agents if application relevant. In that case, dispersant-only controls are recommended to ensure that agent does not have an adverse biological effect. Refer to ISO 14887 for a list of dispersion agents and procedures if materials require the addition of agents other than test media/water.

During preparation, outside factors can affect results. Characteristics that can affect dispersions include, but are not limited to, sample volume, media characteristics, pH, and particle characteristics (i.e., coatings, size, solution, etc). For consistent results, glassware that is used to create the working stock should be identical to that used to calibrate the probe sonicator. In bath sonication, it is recommended to use a 100 mL glass beaker or similar size container.
4 Materials and Apparatus

4.1 NP Powder and Aqueous Suspension Working Stocks

4.1.1 Materials for NP Powder Working Stock

- 100mL glass beaker or similar\(^1\)
- High purity de-ionized water
- Ultrasonic probe sonicator with a standard ½ inch (1.3 cm) diameter horn fitted with a removable flat tip
- Shallow container (2-3 inches deep) containing ice
- Bath sonicator
- Weighing dish (aluminum)
- Stainless steel spatula
- Analytical balance
- Lab jack for lifting sample
- Volumetric pipettes
- Clamp or other locking device for holding cylinder in water
- pH meter

4.1.2 Materials for NP Aqueous Suspension Working Stock

- Aqueous suspension of nanoparticle concentrate
- Refrigerator (materials to be kept in dark storage at 4 °C if not designated by manufacturer)
- Amber glass or aluminum foil
- Ultrasonic bath sonicator
- Clamp, or other locking device for holding cylinder in water
- pH meter

4.2 Materials for Aqueous Bioassay Media Spiking

- Aqueous bioassay test media (i.e., MHRW, DI water, etc)
- 100 ml glass volumetric cylinder
- 100 ml volumetric flask
- Transfer pipettes
- Ultrasonic bath sonicator
- Working stock

\(^1\) Use same glass vessel for probe calibration
- Volumetric pipettes
- Clamp or other locking device for holding cylinder in water

### 4.3 Apparatus

The present method may require two forms of sonicators: (1) a probe sonicator with a standard ½ inch (1.3 cm) diameter horn fitted with a removable flat tip and (2) a bath sonicator. User must perform a probe calibration sequence prior to use; therefore, a temperature probe fitted with a data logger is required. Particle sizing capability (such as dynamic light scattering) is required for confirmation of stability and dispersion size.
5 Procedure

5.1 Procedural overview

1. Procedures for creation of working stock from NP aqueous suspensions or powders are provided as guidelines. Users should follow the section which best represents their parent material. The RM values provide a calibration for the method to ensure procedures are correctly executed in section 5.2.

2. Spiking of a working stock into an aqueous media for biological testing and characterization are included in section 5.3.

3. Procedures for bioassay media optimization and particle stability assessments are included in section 5.4.

4. Guidelines for assessing organism health through reference toxicant testing are included in section 5.5.

5.1.1 NP Powder Preparation for Analysis

Powder materials should be visually assessed to determine if a test paste is needed (see definition of “clump,” ISO 2000) prior to sonication for homogenous dispersions. If material clumping is observed, follow the procedures outlined in ISO 2000 for creation of test paste prior to pre-dispersion of NPs. If powder does not exhibit clumping, follow the pre-dispersion techniques outlined in section 5.2.1 which are referenced from Taurozzi 2013.

5.1.2 NP Aqueous Suspension Preparation for Analysis

Nanoparticle concentrate in suspension from manufacturer will be homogenized utilizing bath sonication to evenly disperse NPs prior to spiking aqueous media. Methods have been adapted from Kennedy 2010 and 2012, Coleman 2013, Taurozzi 2012. The NP solutions are to be stored according to manufacturer specifications. In the absence of specifications, store concentrate at 4°C in the dark.

5.2 Analysis: NP Working Stocks

In the text below “<T>” designates “Test materials” and “<R>” designates “Reference materials;” for powder test materials, “<R>” is applicable to SRM 1898 for NP powder; for aqueous suspended test materials, “<R>” is applicable to NC 30Ag/RM 8012 (30 Au) for NP aqueous concentrates,
unless otherwise specified. The pH of a water or solution can effect NP dispersions, therefore, it is recommended that pH be monitored and kept consistent for best results.

### 5.2.1 Starting Material: NP powder

1. Using an analytical balance and an aluminum dish, weigh an adequate mass of dry powder under a fume hood to achieve the desired concentration into ultrapure water (<T>, <R>). Concentration can impact dispersions; in the absence of a target concentration, users are directed to refer to the table below (referenced from Taurozzi 2012, [http://dx.doi.org/10.6028/NIST.SP.1200-30](http://dx.doi.org/10.6028/NIST.SP.1200-30)). For SRM 1898, use 0.5 mg/mL into 50mL.

<table>
<thead>
<tr>
<th>Mass (g)</th>
<th>Concentration in 50 mL volume (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.025</td>
<td>0.5</td>
</tr>
<tr>
<td>0.05</td>
<td>1</td>
</tr>
<tr>
<td>0.5</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
</tr>
</tbody>
</table>

2. Add ultra-pure water in a glass beaker with NP powder to /flask under fume hood (<T>, <R>).

3. Degas the bath sonicator for 5 minutes, then sonicate the powdered material in water for 15 minutes to fully wet/submerge the material (<T>, <R>). The RM will go to step 4. For test material, allow to settle for one hour then measure size with DLS. If visual settling of particles is observed, this may indicate a need for probe sonication. If no settling is visually observed, and size after bath equals expected NP size, then there is no need for probe sonication and protocol can be stopped here (<T>). Avoid heating the sample.¹

4. Calibrate probe sonicator as described in Taurozzi 2010 (p. 10) to ensure energy delivery is consistent.

5. Place the glass beaker onto a lab jack and insert the ultrasonic probe tip 2.5 cm below the surface of the solution (<T>, <R>).

¹ Ice bath may be used to avoid sample heating.
6. Place the glass beaker into an ice bath so that ice level is at or above that of the solution (<T>, <R>).
7. Select a sonicator setting that yields a delivered power of approximately 50 W (<T>, <R>).
8. Operate the sonicator at this delivered power level for 15 minutes, using an 80% pulsed operation mode (<T>, <R>).
9. After sonication, test particle size via DLS. For particle sizes for SRM 1898, refer to Taurozzi 2012 for DLS value and pH ranges (<T>, <R>).
10. Immediate use is recommended, if spiking into aqueous bioassay media, see section 5.3 (<T>, <R>).

5.2.2 Starting Material: NP Aqueous Concentrate

1. Gently invert solution in capped container three times (do not shake as air bubbles may introduce instability to the particle dispersion) (<T>, <R>).
2. Insert clean pipette tip, syringe needle or Pasteur pipette with bulb and gently introduce mixing by slowly drawing up sample three times (<T>, <R>).
3. Bath sonication for 5 minutes at an operating frequency of 40kHz may be needed to disperse loosely agglomerated materials such as those containing carbon nano tubes, low concentration metal oxides, or similar. Conversely, sonication may degrade or cause agglomeration to occur in some metallic particles, especially those not capped/coated. The RM 8012 does not require sonication for dispersion. Users must assess their individual material to determine whether sonication is needed for suspension in an aqueous concentrate. (<T>, <R>).
4. Insert clean pipette tip, syringe needle or Pasteur pipette with bulb and gently introduce mixing by slowly drawing up sample three times (<T>, <R>).
5. Measure particle size of material utilizing the DLS. For reference, Table 2 lists ERDC’s value for the RMs in non-adjusted ultra-pure water. If homogenization of stock is all that is required, stop here. If spiking concentrate into an aqueous media, continue to section 5.3.
Table 2. Reference material DLS values. Note that pH of water was not altered (measured pH=7.8). *Not sonicated.

<table>
<thead>
<tr>
<th>Reference Material in ultra-pure water</th>
<th>ERDC DLS Value (n=3) Mean Particle Size ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO$_2$</td>
<td>283 ± 2</td>
</tr>
<tr>
<td>NC Ag 30 nm</td>
<td>35 ± 0.6</td>
</tr>
<tr>
<td>NIST Au 30 nm</td>
<td>37 ± 1.7*</td>
</tr>
</tbody>
</table>

5.3 Analysis: Spiking into Aqueous Bioassay Media

1. Acquire desired volume of the stock prepared in section 5.2.1 or 5.2.2 at known concentration (e.g., 10 mg/L).
2. Fill glass volumetric flask of desired size half way using aqueous bioassay media (may be scaled up or down depending on amount of media needed).
3. Use clamp or locking device to secure and suspend cylinder in bath sonicator.
4. Degas bath sonicator for 5 minutes, then use at an operating frequency of 40 kHz.
5. Pipette desired volume of stock into cylinder containing media while sonicating at a drip rate of 3 drops per minute. Separatory funnel can be used for large volumes.
6. Fill cylinder using aqueous bioassay media; this now represents the spiked aqueous treatment for bioassays.
7. Operate the bath sonicator at the specified power level for 15 minutes, ensuring that the sample is not warmed beyond room temperature by the bath. A chiller unit can be added to the bath if the temperature increases more than 5 °C.
8. After sonication, DLS may be utilized to rapidly assess dispersion quality; although multiple lines of evidence and techniques for confirming dispersion are recommended. If the particle counts are below DLS detection limits, it is recommended that the starting concentration be increased several fold (e.g., 2X, 4X, 8X) to allow characterization; the concentration can then be serially diluted to the desired starting concentration for bioassay testing.
9. An aqueous bioassay treatment must be used on the day of preparation for toxicological studies.
10. Serial dilutions (e.g., 50% series) can be performed by removing half the volume from the homogenized volumetric flask and slowly adding fresh bioassay media to the flask while bath sonicating. Aqueous bioassay media should be added slowly (e.g., 5 drops per second using a separatory funnel) to reduce change of destabilizing particles. Continue to sonicate for 15
minutes and repeat until the desired number of test concentrations is created. Each dilution should also be sonicated for 15 minutes post creation. Total mass (e.g., ICP-MS), dissolved mass, and effective diameter (e.g., DLS) should be characterized in each concentration until below method/instrument reporting limits.

5.4 Analysis: Bioassay Media Optimization

1. A time point study is recommended to assess particle stability in bioassay media prior to utilizing in a toxicological study. The following dilutions should be made beginning with the user’s highest target concentration following the dispersion preparations above. Dilutions include 100%, 50%, and 10% of the NP spiked bioassay media.
2. Dilutions should be sonicated as described in 5.3.1.10. Post sonication, 2 mL samples at time points of 0, 2, 8, and 24 hours are to be collected.
3. Analyze the 2 mL samples utilizing a DLS to assess particle stability through size measurements. Determine if particles are within an acceptable size range for the bioassay being conducted.
4. If applicable, an additional 2 mL aliquot can be sampled with stability time points and assessed for total NP concentration with ICP-MS.

5.5 Organism Health Optimization: Reference Toxicity Test

1. Organism health should be assessed before toxicological testing. Reference toxicants are case specific, although recommendations are included below. For nano Ag or similar, Silver Nitrate (AgNO₃) can be utilized. For nano Copper (Cu) or similar, Copper Sulfate (CuSO₄) can be tested.
2. Begin a reference toxicity test with the highest test target concentration at 100%, followed by a 50%, and 10% dilution created from following 5.4.1.1. Tests should include a control and a minimum of three replicates per treatment.
3. Add the test species to the dilutions (recommended organism addition of n=10 per dilution). Assess organism survival at 24 hours to obtain an Lc₅₀ value. Compare Lc₅₀ values to case specific test needs.
6 Reporting

6.1 Analysis of Results: Reference Materials

Dynamic light scattering (weighted as intensity of light scattered) was utilized to confirm particle size (n=3). Particle size ranges are provided according to ERDC DLS (90 Plus/BI-MAS, Brookhaven Instruments Corp, USA).

6.2 Key Results Provided

The present protocol provides two step-by-step methods for: (1) creating a working stock from nanoparticle powder and nanoparticle aqueous suspensions, (2) spiking working stock suspensions into aqueous phase bioassay media. These results are validated by confirmation with RM NP powder or aqueous suspension working stocks sized through DLS confirmation. Recommendations for optimizing media preparation and test organisms are also provided to improve experimental validity.

6.3 QA/QC Considerations

During preparation, outside factors can affect results. Characteristics which can affect dispersions include, but are not limited to, sample volume, media characteristics, pH, and particle characteristics (i.e. coatings, size, solution, etc). For consistent results, glassware which is used to create the working stock should be identical to that which is used to calibrate the probe sonicator.
References


Taurozzi, J. S., V. A. Hackley, and M. R. Wiesner. 2011 Preparation of a nanoscale TiO2 aqueous dispersion for toxicological or environmental testing Ver. 1.1. *National Institute of Standards and Technology*


Homogenous dispersions of nanoparticles (NPs) for use in bioassay media for exposures/characterizations are a crucial need in ecotoxicology assays. The present protocol provides step-by-step methods for: (1) creating a working stock from nanoparticle powder and nanoparticle aqueous suspensions, (2) spiking working stock suspensions into aqueous bioassay media. The protocol also provides guidance on optimization of test media and organism health. Peer-reviewed publications and standards, with adaptations for aquatic bioassays are included. Users are directed to follow the protocol section relevant to their need and parent material (e.g. aqueous NPs or powder NPs). This protocol was developed on procedures created specifically for metal nanoparticles, but may have broader application. In an effort to validate procedural consistency, values associated with reference materials (RM) are included. The standard references include powder-based and aqueous suspensions from the National Institute of Standards and Technology (NIST) and NanoComposix (NC); including aqueous suspension of NIST 30 nm Au and NC citrate-coated 30 nM Ag, and powdered NIST standard reference material 1898 TiO2. The Ag and Au reference materials were selected to represent moderately soluble and relatively insoluble materials, respectively. Values generated by the user testing the SRM materials should fall within the defined acceptability criteria ranges. If followed correctly, near-homogenous particle dispersions of the reference materials and a best case scenario for dispersion of test materials in aqueous media should be generated based on best procedural guidance available.