NIST - NCL Joint Assay Protocol, PCC-8

Determination of Gold in Rat Tissue with Inductively Coupled Plasma Mass Spectrometry

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This protocol assumes an intermediate level of scientific competency with regard to techniques, instrumentation, and safety procedures. Rudimentary assay details have been omitted for the sake of brevity.

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1. Introduction

A new class of pharmaceutical candidate based on gold nanoparticles has entered the small animal phase of clinical study. The content of gold in the blood and tissue of study animals must be determined. This technical procedure defines the analytical protocols for the determination of gold in the tissues of rats used in a clinical study.

2. Reagents and Equipment[1]

CAUTION: PERSONAL PROTECTION EQUIPMENT SUCH AS SAFETY GOGGLES, LAB COAT, AND RUBBER GLOVES (LATEX OR NITRILE) MUST BE USED WHEN OPERATING UNDER THIS PROTOCOL.

2.1 Reagents

- 2.1.1 Optima grade nitric acid (HNO_3) (Fisher Scientific)
- 2.1.2 Optima grade hydrochloric acid (HCl) (Fisher Scientific)
- 2.1.3 National Institute of Standards and Technology (NIST, Gaithersburg, MD) SRM 3121 Gold Spectrometric Solution as a calibrant for the measurement of gold
- 2.1.4 NIST SRM 3124a, Indium Spectrometric Solution, as an internal standard
- 2.1.5 NIST RM 8012, Gold Nanoparticles, Nominal 30 nm Diameter, for quality assurance
- 2.1.6 High-Purity Standards (Charleston, SC) ICP-MS Calibration Standard, Cat # ICP-MSCS, or equivalent, as a calibrant for semi-quantitative measurement of gold in samples

2.2 Equipment

- 2.2.1 An Agilent 7500cs (Santa Clara, CA) inductively coupled plasma mass spectrometer (ICP-MS) equipped with a Peltier-cooled, inert sample introduction system. Perform the set up and optimization of the ICP-MS daily in accordance with the procedure listed in Appendix A.

[1] Certain commercial equipment, instruments, or materials are identified in this protocol in order to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

2.2.2 A four-place analytical balance, e.g., a Mettler (Columbus, OH) model AT 261 DeltaRange analytical balance, for weighing in the preparation of samples and standards. Verify the calibration of the balance in accordance with the procedure listed in Appendix B.

2.2.3 A microwave digestion system, e.g. a CEM (Matthews, NC) model MARSXpress microwave systems equipped with 55 mL PFA microwave vessels for the digestion of samples in accordance with the procedure listed in Appendix C.

2.2.4 High-purity water generation system, e.g. a Continental Water Systems (Sydney, Australia) Modulab ModuPure Plus for generating 18 MΩ de-ionized water.

3. Digestion of Tissue

3.1 Take frozen tissue samples, up to 0.5 g each, out of the -20 ºC freezer and let the samples thaw at room temperature for approximately 2 h. Take one extra gold-free rat tissue sample and mark it "CM". This tissue sample will be used to prepare matrix-matched calibration standards.

3.2 Using a Teflon-coated spatula, transfer the entire tissue sample into a tared microwave vessel. Accurately weigh the tissue sample by difference (*tissue_mass*)[2].

3.3 Prepare 4 samples of controls by transferring approximately 0.25 g each of RM 8012 into 4 cleaned and tared microwave vessels. Accurately weigh by difference the mass of RM 8012 in each microwave cell *(RM8012_mass*).

3.4 Set aside 6 cleaned microwave vessels for the preparation of 6 procedure blanks.

3.5 Add 8 mL HNO_3 and 2 mL HCl into each microwave vessel described in steps 3.2 through 3.4.

3.6 Cap each microwave vessel. Install the microwave vessels in the turntable. If all positions of the turntable are not utilized, arrange the vessels symmetrically. Program the microwave with Microwave Program 1 listed in Table 1.

[2] The italic words in parenthesis denote a variable that will be used in the calculation.

3.7 Let the microwave vessels cool to near room temperature inside the microwave oven at the completion of the digestion program. Take the microwave turntable out of the oven and place the turntable with the cells in a hood. Slowly open each microwave vessel to allow gentle release of the gas of the digestion.

3.8 Inspect the content of each vessel. If fatty droplets are present in any vessel, go to step 3.9. If digests in all vessels are clear, go to step 3.10.

3.9 Re-cap each vessel, and reload vessels onto the turntable. Digest the samples with the Microwave Program 2 listed in Table 2.

3.10 Quantitatively transfer the contents of each microwave vessel to a pre-weighed 60-mL low density polyethylene (LDPE) bottle. Dilute the contents with deionized water to approximately 50 g, and accurately weigh the contents of the bottle by difference (*digest*).

Table 1. Microwave Program 1 Settings for the Digestion of Tissue Samples.

Step	Power (W)	Power Setting (%)	Ramp Time (min)	T (°C)	Hold Time (min)
1	1200	100	45:00	120	20:00
2	0	0	0	0	30:00
3	1200	100	45:00	195	25:00
4	0	0	0	0	25:00

Table 2. Microwave Program 2 Settings for the Digestion of Tissue Samples.

Step	Power (W)	Power Setting (%)	Ramp Time (min)	T (°C)	Hold Time (min)
1	1200	100	45:00	120	20:00
2	1200	100	25:00	195	25:00
3	0	0	0	0	25:00

4. Preparation of Analytical Portions

4.1 Prepare a diluent containing 1.5 % (v/v) HNO_3 and 4 % (v/v) HCl as follows: (1) using a 100 mL graduated cylinder, transfer 30 mL HNO_3 and 80 mL HCl into a 2 L Teflon or polyethylene bottle; (2) using a 2 L graduated cylinder, add 1890 mL of deionized water to the bottle; cap the bottle and homogenize the contents by shaking.

4.2 Prepare approximately 100 mL of 15 ng/g indium solution by serial dilution from NIST SRM 3124a, using the diluent prepared in step 4.1.

4.3 Prepare the analytical portion of each sample except the one marked "CM" by transferring approximately 1.0 mL of each sample digest into a tared 30-mL LDPE bottle. Accurately weigh the contents by difference (*digest_fraction*). Dilute the contents to approximately 25 g with the diluent from step 4.1 and accurately re-weigh to obtain the mass of the final solution (*dilution1*).

4.4 Prepare calibration solutions from the sample marked "CM":

4.4.1 Accurately prepare a stock solution containing approximately 6.4 µg/g Au by serial dilution from SRM 3121 using the diluent described in step 4.1.

4.4.2 Transfer 0 mL, 0.33 mL, 0.66 mL, 1.00 mL, 1.30 mL, and 1.60 mL of the 6.4 µg/g Au standard into six pre-weighed 30-mL LDPE bottles. Accurately weigh the contents of each bottle by difference. Add 1.0 mL of the digested "CM" (see step 3.1) to each of the six bottles. Dilute the content with the diluent from step 4.1 to approximately 25 g. Accurately weigh the contents of each of the six bottles by difference.

4.5 Prepare semi-quantitative calibration solution: Accurately transfer 2.000 mL High-Purity Standard ICP-MS Calibration Solution into a tared 1000 mL polyethylene bottle. Add 15 mL of HNO_3. Accurately dilute the content to 1000 g ± 1 g. Label the bottle as "20 ng/g multi-element standard".

5. ICP-MS Measurements

5.1 Semi-quantitative determination of gold in samples:

5.1.1 Prepare a method file on the Agilent 7500cs ICP-MS instrument according to the parameters in Table 3.

5.1.2 Prepare a sequence file by using the diluent prepared in step 4.1 as the blank and the 20 ng/g multi-element standard as the calibrant. Determine gold in each sample prepared in step 4.3.

5.1.3 Evaluate the concentration of gold in each sample solution, and arrange samples in increasing concentration of gold for quantitative measurement.

Table 3. Method for Semi-quantitative Measurements

Acquisition Mode: Spectrum Analysis

Number of Masses: 102

Mass	Element	Det.Mode	Integration /Point	Time[s] /Mass
7	Li	Auto	0.1	0.1
9	Be	Auto	0.1	0.1
10- 11	B	Auto	0.1	0.1
23	Na	Auto	0.1	0.1
24	Mg	Auto	0.1	0.1
26	Mg	Auto	0.1	0.1
27	Al	Auto	0.1	0.1
28	Si	Auto	0.1	0.1
31	P	Auto	0.1	0.1
32	S	Auto	0.1	0.1
34	S	Auto	0.1	0.1
39	K	Auto	0.1	0.1
43- 44	Ca	Auto	0.1	0.1
45	Sc	Auto	0.1	0.1
47- 48	Ti	Auto	0.1	0.1
51	V	Auto	0.1	0.1
52- 53	Cr	Auto	0.1	0.1
55	Mn	Auto	0.1	0.1
56- 57	Fe	Auto	0.1	0.1
58	Ni	Auto	0.1	0.1
59	Co	Auto	0.1	0.1
60	Ni	Auto	0.1	0.1
63	Cu	Auto	0.1	0.1
64	Zn	Auto	0.1	0.1
65	Cu	Auto	0.1	0.1
66	Zn	Auto	0.1	0.1
68	Zn	Auto	0.1	0.1
69	Ga	Auto	0.1	0.1
71	Ga	Auto	0.1	0.1
72	Ge	Auto	0.1	0.1
74	Ge	Auto	0.1	0.1
75	As	Auto	0.1	0.1
78	Se	Auto	0.1	0.1
79	Br	Auto	0.1	0.1
82	Se	Auto	0.1	0.1
85	Rb	Auto	0.1	0.1
86	Sr	Auto	0.1	0.1
88	Sr	Auto	0.1	0.1
89	Y	Auto	0.1	0.1
90- 91	Zr	Auto	0.1	0.1
93	Nb	Auto	0.1	0.1
95	Mo	Auto	0.1	0.1
98	Mo	Auto	0.1	0.1
101-102	Ru	Auto	0.1	0.1

Mass	Element	Det.Mode	Integration /Point	Time[s] /Mass
103	Rh	Auto	0.1	0.1
105	Pd	Auto	0.1	0.1
107	Ag	Auto	0.1	0.1
108	Pd	Auto	0.1	0.1
109	Ag	Auto	0.1	0.1
111	Cd	Auto	0.1	0.1
114	Cd	Auto	0.1	0.1
115	In	Auto	0.1	0.1
116	Sn	Auto	0.1	0.1
120	Sn	Auto	0.1	0.1
121	Sb	Auto	0.1	0.1
123	Sb	Auto	0.1	0.1
125	Te	Auto	0.1	0.1
127	I	Auto	0.1	0.1
133	Cs	Auto	0.1	0.1
137-138	Ba	Auto	0.1	0.1
139	La	Auto	0.1	0.1
140	Ce	Auto	0.1	0.1
141	Pr	Auto	0.1	0.1
146	Nd	Auto	0.1	0.1
147	Sm	Auto	0.1	0.1
153	Eu	Auto	0.1	0.1
157	Gd	Auto	0.1	0.1
159	Tb	Auto	0.1	0.1
163	Dy	Auto	0.1	0.1
165	Ho	Auto	0.1	0.1
166	Er	Auto	0.1	0.1
169	Tm	Auto	0.1	0.1
172	Yb	Auto	0.1	0.1
175	Lu	Auto	0.1	0.1
178	Hf	Auto	0.1	0.1
181	Ta	Auto	0.1	0.1
182-183	W	Auto	0.1	0.1
185	Re	Auto	0.1	0.1
189	Os	Auto	0.1	0.1
193	Ir	Auto	0.1	0.1
195	Pt	Auto	0.1	0.1
197	Au	Auto	0.1	0.1
201-202	Hg	Auto	0.1	0.1
205	Tl	Auto	0.1	0.1
206-207	(Pb)	Auto	0.1	0.1
208	Pb	Auto	0.1	0.1
209	Bi	Auto	0.1	0.1
232	Th	Auto	0.1	0.1
234		Auto	0.1	0.1
235	U	Auto	0.1	0.1
238	U	Auto	0.1	0.1

Number of Points per Mass	1
Acquisition Time	26.1200 [s]
Number of repetitions	10
Total Acquisition Time	261 [s]

Set Peristaltic Pump Program

--- Before Acquisition ---	
Uptake Speed	0.50 rps
Uptake Time	30 s
Stabilization Time	30 s
--- After Acquisition (Probe Rinse) ---	
Rinse Speed	0.00 rps
Rinse on rinse port (Sample)	0 s
Rinse on rinse port (STD)	0 s
--- After Acquisition (Rinse) ---	
Rinse Vial 1	1
Rinse Speed	0.00 rps
Rinse on rinse vial (Step 1)	0 s
Rinse on rinse port (Step 1)	0 s
Rinse Vial 2	2
Rinse Speed	0.30 rps
Rinse on rinse vial (Step 2)	75 s
Rinse on rinse port (Step 2)	0 s
Rinse Vial 3	3
Rinse Speed	0.00 rps
Rinse on rinse vial (Step 3)	0 s
Rinse on rinse port (Step 3)	0 s
Execute Pre-emptive rinse	Off
Pre-emptive Time	20 s
Terminate a Rinse Step at the end of Acq.	Off

5.2 Quantitative determination of gold in sample:

5.2.1 Prepare a method file on the Agilent ICP-MS instrument according to the parameters in Table 4.

5.2.2 Set up the peristaltic pump of Agilent ICP-MS instrument for three-channel pumping, and merge the sample channel with the internal standard channel using a T-connector supplied by Agilent as shown in Figure 1. Place the internal standard pick-up in the 15 ng/g In solution from step 4.2.

5.2.3 Prepare a sequence file in the following order:

a. calibration solutions prepared in step 4.4.2 in order of increasing concentration of gold

b. five rinse solutions using the diluent from step 4.1.

c. samples from step 4.3 in order of increasing gold concentration (using the semiquantitative results obtained in 5.1.3).

d. five rinse solutions using the diluent from step 4.1.

e. calibration solutions prepared in step 4.4.2 in order of increasing concentration of gold.

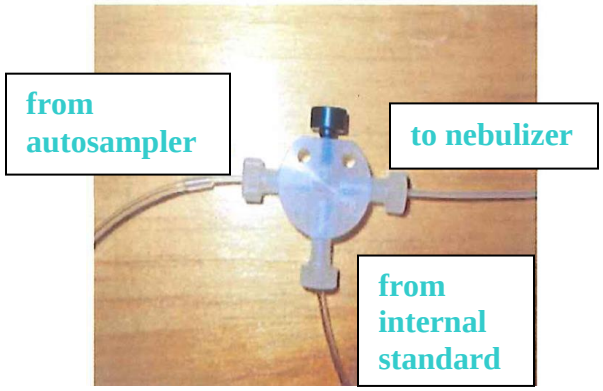

Figure 1. T for introduction of internal standard.

Table 4. Method for Quantitative Determination of Gold

Acquisition Mode	Spectrum Analysis
Number of Masses	2

Mass	Element	Det.Mode	Integration/Point	Time [s]/Mass
115	In	Auto	0.6	1.8
197	Au	Auto	0.6	1.8

Number of Points per Mass	3
Acquisition Time	4.3000 [s]
Number of repetitions	10
Total Acquisition Time	43.0000 [s]

Set Peristaltic Pump Program

--- Before Acquisition ---	
Uptake Speed	0.50 rps
Uptake Time	40 s
Stabilization Time	30 s
--- After Acquisition (Probe Rinse) ---	
Rinse Speed	0.00 rps
Rinse on rinse port(Sample)	0 s
Rinse on rinse port(STD)	0 s
--- After Acquisition (Rinse) ---	
Rinse Vial 1	1
Rinse Speed	0.00 rps
Rinse on rinse vial (Step 1)	0 s
Rinse on rinse port (Step 1)	0 s
Rinse Vial 2	2
Rinse Speed	0.50 rps
Rinse on rinse via l (Step 2)	60 s
Rinse on rinse port (Step 2)	0 s
Rinse Vial 3	2
Rinse Speed	0.10 rps
Rinse on rinse vial (Step 3)	10 s
Rinse on rinse port (Step 3)	0 s
Execute Pre-emptive rinse	Off
Pre-emptive Time	20 s
Terminate a Rinse Step at the end of Acq.	Off

6. Calculations

6.1 Correct for internal standard by dividing the intensity of Au at mass 197 by the intensity of In at mass 115 for each solution.

6.2 Calculate the slope and the intercept of the calibration curve

6.2.1 Take the average of each calibrant analyzed at the beginning and the end of the run.

6.2.2 Enter the exact concentration of Au in each calibrant into column A of a Microsoft Excel spreadsheet. Enter the corresponding average calculated above into column B.

6.2.3 Calculate the slope of the calibration curve, i.e., enter in cell C1 "=slope(B1:B6,A1:A6)". Calculate the intercept of the calibration curve, i.e., enter in cell C2 "=intercept(B1:B6,A1:A6)"

6.2.4 Calculate the concentration of Au in each measurement portion of the solution using Equation 1:

$$[Au] = \frac{Intensity - Intercept}{Slope} \tag{1}$$

where *Intensity* refers to the internal standard corrected intensity calculated in step 6.1.; *Intercept* and *Slope* refer to those calculated in step 6.2.3.

6.2.5 Calculate Au in tissue (*μg/g*) using Equation 2:

$$Au\_in\_tissue = \frac{[Au] \times dilution1 \times digest}{digest\_fraction \times tissue\_mass} \tag{2}$$

where *digest* is the mass of the digest in step 3.10.; *digest_fraction* is the mass of 1 mL digest in step 4.3.; *dilution1* is the mass of the diluted *digest_fraction* in step 4.3.; and *tissue_mass* is the mass of the tissue sample in step 3.2.

6.2.6 Calculate the measured value of Au in each sample of RM 8012 (*μg/g*) using Equation 3:

$$Au\_in\_RM8012 = \frac{[Au] \times dilution1 \times digest}{digest\_fraction \times RM8012\_mass} \tag{3}$$

where *digest* is the mass of the digest in step 3.10.; *digest_fraction* is the mass of 1 mL digest in step 4.3.; *dilution1* is the mass of the diluted *digest_fraction* in step 4.3.; and *RM8012_mass* is the mass of each sample in step 3.3.

7. Abbreviations

Au	gold
C	Celsius
Ce	cerium
Co	cobalt
g	gram
h	hour
H	hydrogen
HCl	hydrochloric acid
He	helium
HNO3	nitric acid
ICP-MS	inductively coupled plasma-mass spectrometry
In	indium
LDPE	low density polyethylene
Li	lithium
mg	milligram
Mg	magnesium
mL	milliliter
MΩ	megaohm
µg	microgram
ng	nanogram
NIST	National Institute of Standards and Technology
Pa	Pascal
RM	reference material
SRM	Standard Reference Material
Tl	thallium
v/v	volume/volume
Y	yttrium

Appendix A. Setup and Optimization Procedure for Agilent 7500cs ICP Mass Spectrometer

Background and scope: The ICP-MS must be optimized before an analysis to ensure that the operating condition of the instrument is fit for the purpose of the measurement. This procedure describes the daily setup and optimization of the Agilent 7500cs ICP-MS.

Equipment: Agilent 7500cs

Procedure:

1. Turn on the water re-circulator.
2. Wait until the pressure of the mass spectrometer decreases to below 8×10^{-5} Pa. Record the vacuum pressure in the 7500cs instrument log book.
3. Clamp down the drain and sample tubing on the peristaltic pump. Ignite the plasma and check the plasma condition through the observation window on top of the instrument lid. Turn off the RF power if there is an abnormal discharge of the plasma. Record the plasma-on pressure of the vacuum in the log book if the plasma is running normally.
4. Let the instrument warm up for about 0.5 h before proceeding to the optimization steps below.
5. Open the tune window in the operating system software and aspirate the tune solution containing 1 ng/g each of Li, Mg, Y, Ce, Tl, and Co. Tune the instrument in "no gas" mode to meet the criteria set in Table A taken from the instrument Tuning and Applications Handbook **(1)**. Record the Li intensity in the log book and proceed to tune the instrument in H_2 and He modes. After tuning, run the instrument in multimode. Print and file a multi-tune report in the Tune File folder **(1)**.
6. If the criteria in Table A are not met, the instrument needs to be cleaned. Consult the instrument Hardware Manual for instructions on cleaning the sampler, skimmer, Ω-lens, and collision cell **(2)**. Repeat steps 1-5 after cleaning.

Table A. Daily Performance Criteria

Measurand	Specification
Li	> 5000 c/s
Background (m/z 5)	< 200 c/s
CeO+ /Ce+	< 2 %
Ce++/Ce+	< 5 %

References:

1. Agilent 7500 Series ICP-MS Tuning & Application Handbook. G3270-90130, Agilent Technology, Inc, 2006.
2. Agilent 7500 Series ICP-MS Hardware Manual, G3270-90103, Agilent Technology, Inc, 2006.

Appendix B. Procedure for Use of Balances

Background and Scope: All balances are serviced annually to determine whether balances are functioning within manufacturer specifications and to perform any adjustments required to meet the appropriate specifications. Calibration certificates are available for each balance. The general procedure for day-to-day use of the balances is described herein.

Each analyst has access to one or more sets of weights (aluminum or stainless steel, Type II, ANSI/ASTM 4) for which the statements of accuracy are available. These "check" masses were verified by the manufacturer using mass standards traceable to NIST.

Equipment: analytical balances, check masses

Procedure:

1. Inspect the level and clean the balance if necessary. Turn off power before cleaning. Turn power on and allow balance to stabilize.
2. Weigh two check masses to verify the accuracy and linearity of the balance. If you will be weighing by difference (or using the tare function) you may choose to determine the mass of the standard weight in the same fashion.
3. For balances that have a calibration function available using internal weights, you may choose to perform that calibration and then weigh the check masses.
4. Record the date, mass identification, mass reading, and your initials in the balance log book.
5. If the observed masses differ from the previously verified mass values by more than 0.1% for masses $\geq$ 50 mg the balance is placed out of service (the balance is unplugged and clearly marked). For masses <50 mg, acceptance criteria are based on the specifications of the balance used.

Uncertainty: The uncertainty for each measurement will depend on masses determined and mode of use, e.g., direct weighing or weighing by difference. See the manufacturer's specifications for each balance. Repeated weighing of a sample provides an immediate measure of precision. Although balance inaccuracy is seldom a significant source of overall analytical uncertainty,

errors from electrostatics, inappropriate drying, or buoyancy corrections may need to be assessed and recorded by the analyst.

Appendix C. Microwave Digestion

Background and Scope: This is the general procedure used for microwave digestion of natural matrix materials and includes digestion of any control materials typically used in an analysis procedure. The selection of sample masses, acids, and the determination of the appropriate digestion conditions will depend on the type of sample to be digested and are the responsibility of the analyst. Only qualified (authorized) staff with the appropriate expertise should perform this procedure.

Equipment: Microwave oven, sample turntables, associated appropriately identified pre-cleaned quartz or Teflon digestion vessels, and Nalgene plastic (or appropriate) bottles.

Procedure:

1. Select the appropriate sample mass for the digestion based on matrix type, analyte levels, and detection method to be used.
2. Weigh each sample (analytical samples and controls). Record all masses transferred into the digestion vessels.
3. Where the addition of internal standards or enriched isotope spikes are required, record the amounts of each added to each vessel.
4. Select acid(s) for digestion based on the matrix and vessel type; transfer acid(s) to the digestion vessels containing the samples and record amounts added and lot numbers of each acid.
5. Prepare reagent blanks by adding the same amounts of acids, and any internal standards or spikes used, to empty digestion vessels.
    a. Selection of the number of blanks to be included is determined by the analyst, based on the analytes determined, detection method used and other relevant considerations such as the expected blank to sample ratio.
6. Select the microwave digestion program and record all appropriate parameters which could include power, pressure, temperature, ramping schemes, hold times, and cool down times.
7. If required, evaporate acids from cooled digests using a microwave-based evaporation system or by quantitatively transferring each digest to appropriate labeled beaker ware and

laboratory hood and evaporate via a hot-plate. Re-dissolve digests by adding an appropriate dilute acid.

8. Quantitatively transfer each cooled digest to the appropriate labeled plastic bottle and dilute as appropriate for the detection technique; record labels, volumes or masses and reagents used for dilution.

Uncertainty: The uncertainties associated with this procedure include those associated with the use of analytical balances. Other uncertainties associated with this procedure include incomplete digestion, analyte losses, or with contributions from analyte blanks.