A POLONIUM-210 RAPID EMERGENCY RESPONSE BIOASSAY METHOD: UPDATE

David P. Saunders, PhD, Supriyadi Sadi, PhD, and Robert L. Jones, PhD

Inorganic and Radiation Analytical Toxicology Branch

RRMC 2015

10/27/2015

National Center for Environmental Health

Division of Laboratory Sciences

Po-210 Method Requirements

- FAST – To be able to analyze hundreds of samples per day with limited equipment availability
- SIMPLE – To be able to expand this radio-chemical procedure to other staff for round-the-clock operations, or to other labs that have the available equipment, using Just In Time Training and Validation Samples
- SENSITIVE – To be able to quantify activities down to levels of interest for medical management – 1/3 of a Child or Pregnant Woman Clinical Decision Guide level

Clinical Decision Guide Levels

- NCRP report No. 161
- Maximum, once in a lifetime intake
- Two CDG levels
  - Adult
  - Child (0-18 years) / Pregnant Women
- Po-210 levels at 5 days post intake
  - Adult = 64 Bq/L
  - Child (0-18 years) / Pregnant women = 12.8 Bq/L

Brief Recap of Po-210 Method

- Clean metal planchet
- Pipet into a 50 mL polypropylene tube
  - 10 mL of urine
  - Po-209 tracer at the Child/Pregnant Women CDG level
  - 450 μL concentrated HCl
  - 100 mg of ascorbic acid
- Melt pressure relief hole into tube

Brief Recap of Po-210 Method cont.

- Place sample in 99°C shaking (~250 Hz, ~3/4" travel) water bath for 1 hour digestion and auto deposition of Po-210 and Po-209 tracer
- Remove, dispose of solution
- Rinse the planchet and pat dry
- Count sample for 1 hour

Summary of Method Validation Parameters

Precision	1.83E+01 – 5.48E+02 Bq/L
Accuracy	1.83E+01 – 5.48E+02 Bq/L
Linearity	9.13E+00 – 1.02E+03 Bq/L
Range	6.45E-01 – 1.02E+03 Bq/L
Recovery	95.97%
Limit of Detection	6.14E-01 Bq/L

Previous Conclusions

- A rapid method has been developed to determine Po-210 in urine at clinically relevant levels.
- Due to method validation studies slight changes have been made
  - Planchet – Copper
  - Container – Flat tube
  - Ascorbic Acid – Not added
- Limit of detection will be reanalyzed with the above changes in place

RRMC 2012 Conclusion

- RRMC/Radiobioassay community members were concerned we would not recover all the Po-210 from actual, radiation emergency response human urine samples.
- We hoped that somewhat elevated temperatures, agitation, and increased digestion/plating time might help us.
- But we also knew there was literature that indicated the need for robust digestion.
  - "THE IMPORTANCE OF ACID DIGESTION OF URINE PRIOR TO SPONTANEOUS DEPOSITION OF ^{210}Po", Fellman, et al, Health Physics, 1989

Post RRMC 2012 Decision

- Obtain urine that has had Po-210 incorporated into it by a biological process as similar as possible to the human process.
- Perform further validation studies with this urine.
- If these studies indicate need for more robust dissolution, develop a fast, robust dissolution method.
- Validate new method (if new method is needed).

OBTAIN URINE

- Identified funding in FY14. Issued a Purchase Order to Lovelace Respiratory Research Institute (LRRI) on 9/11/2014. Unable to fully execute it based on their Institutional Animal Care & Use Committee (IACUC) approval, since it was to use Beagles (companion animals).
- Switched to mini pigs. IACUC approvals required from CDC in addition to LRRI's. CDC's IACUC approval came on 4/15/15.
- We received the urine on June 17th, 2015.

PERFORM FURTHER VALIDATION STUDIES

- But 1st, we had to verify LRRI had fulfilled the terms of the contract. LSC analyses of about half the samples. ✓
- Compare the Method As previously Written (MAW) to MAW with additional pre-dissolution steps.

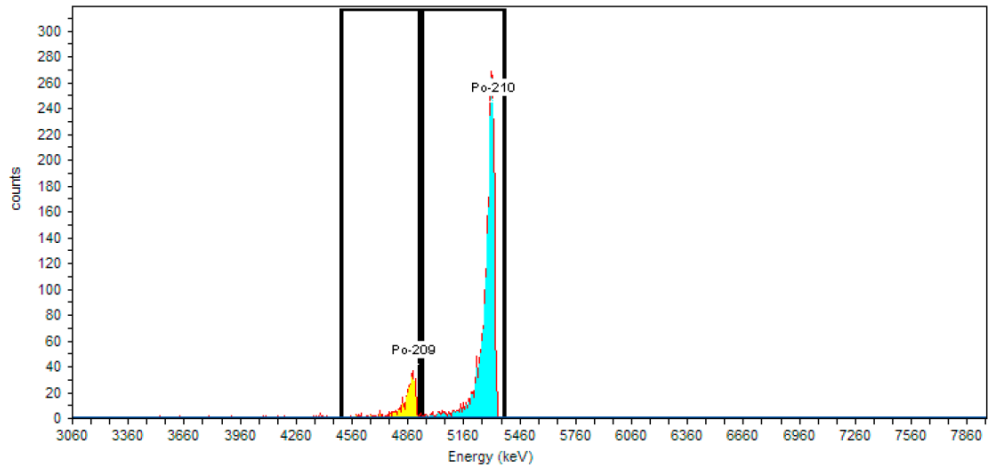
Results for sample analyzed using method as written

Tracer Name: Po-209(SS)
Tracer Activity: 711.89 mBq / mL x (Vol.) 0.77 mL = 547.45 mBq
Tracer Ref. Date: 9/17/2014 5:14:48PM
Tracer Nuclide: Po-209
Tracer Recovery: 95.32%

Acquisition

Detector: D051, SN: 50-027 BB5, ID: 51
Acquisition Start Date: 6/23/2015 6:00:05PM
Live Time: 60.00 min.
Real Time: 60.00 min.
Background Date: 10/5/2015 4:24:37PM
Bkgd Info:

Energy Calibration: 2015.06.22 Detector 51
Efficiency Calibration:2015.06.22 Detector 51
Calibration Date: 6/22/2015 6:53:18PM
Energy Cal: Gain = 4.7983 keV / Ch
Offset = 3,055.87 keV
Quadratic = 0.0000 keV / Ch²
Efficiency: 23.12% +/- 0.77% TPU(2 sigma)



General Analysis

Analysis Method: Interactive ROI Analysis
Decay Correction:6/23/2015 5:54:54PM
MDA Constants: Kα = 1.64 , Kβ = 1.64
Nuclide Library: Polonium
MDA Source: Background

Nuclide Summary (ROI)

Nuclide	Peak Energy keV	ROI Start keV	ROI End keV	FWHM keV	B.R. %	Gross Counts	Bkgd Counts	Net Counts	Activity Bq/L	1.00Sigma TPU Bq/L	Critical Level Bq/L	MDA Bq/L
Po-209	4888.998	4500.167	4917.620	43.6	99.7	431.00	0.0000	431.00	5.218E+001	2.663E+000	0.000E+000	3.437E-001
Po-210	5321.000	4936.813	5378.258	43.2	100.0	3,143.00	0.0000	3143.00	3.961E+002	2.991E+001	0.000E+000	3.411E-001

Tracer recovery: 95%

Activity result: 413 Bq/L (corrected to 6/15/15)

Compare to LRRI LSC result: 4400 Bq/L (6/15/15)

Not looking good…

PERFORM FURTHER VALIDATION STUDIES (cont.)

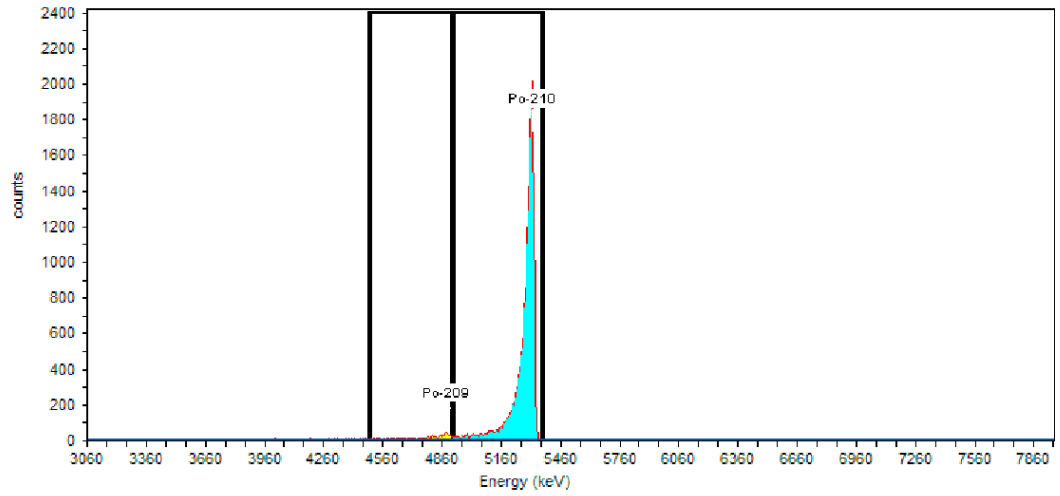
- Sample analyzed using robust hot plate Nitric/Hydrochloric pre-dissolution, drying, reconstitution into 0.5 M HCl in DI water, then MAW

Tracer Name: Po-209(SS)
Tracer Activity: 711.89 mBq / mL x (Vol.) 0.77 mL = 547.45 mBq
Tracer Ref. Date: 9/17/2014 5:14:48PM
Tracer Nuclide: Po-209
Tracer Recovery: 200.92%

Acquisition

Detector: D052, SN: 54-019I3, ID: 52
Acquisition Start Date: 6/23/2015 6:00:05PM
Live Time: 60.00 min.
Real Time: 60.00 min.
Background Date: 10/31/2014 11:37:01AM
Bkgd Info: Sample: BKG052; Det: D052; Spectrum #1; 10/31/2014 11:37:01 AM; Live Time: 2880.000(min.); ID: 52

Energy Calibration: 2015.06.22 Detector 52
Efficiency Calibration:2015.06.22 Detector 52
Calibration Date: 6/22/2015 6:54:51PM
Energy Cal: Gain = 4.7983 keV / Ch
Offset = 3,055.87 keV
Quadratic = 0.0000 keV / Ch²
Efficiency: 20.28% +/- 0.77% TPU(2 sigma)



General Analysis

Analysis Method: Interactive ROI Analysis
Decay Correction:6/23/2015 5:54:54PM
MDA Constants: Kα = 1.64 , Kβ = 1.64
Nuclide Library: Polonium
MDA Source: Background

Nuclide Summary (ROI)

Nuclide	Peak Energy keV	ROI Start keV	ROI End keV	FWHM keV	B.R. %	Gross Counts	Bkgd Counts	Net Counts	Activity Bq/L	1.00Sigma TPU Bq/L	Critical Level Bq/L	MDA Bq/L
Po-209	4879.233	4490.570	4908.023	26.0	99.7	797.00	0.0000	797.00	1.100E+002	4.422E+000	0.000E+000	1.859E-001
Po-210	5321.000	4917.620	5368.662	43.3	100.0	23,203.00	0.0208	23202.98	1.581E+003	1.063E+002	1.635E-002	2.171E-001

Tracer recovery: 201%

Recalculate using 96%

Activity result: 3440 Bq/L (corrected to 6/15/15)

Compare to LRRI LSC result: 4400 Bq/L (6/15/15)

Much better agreement.

Our method obviously needs pre-dissolution

PERFORM FURTHER VALIDATION STUDIES (cont.)

- Sample analyzed using CEM Discover SPD Microwave, Nitric pre-dissolution, drying, reconstitution into 0.5 M HCl in DI water, then MAW

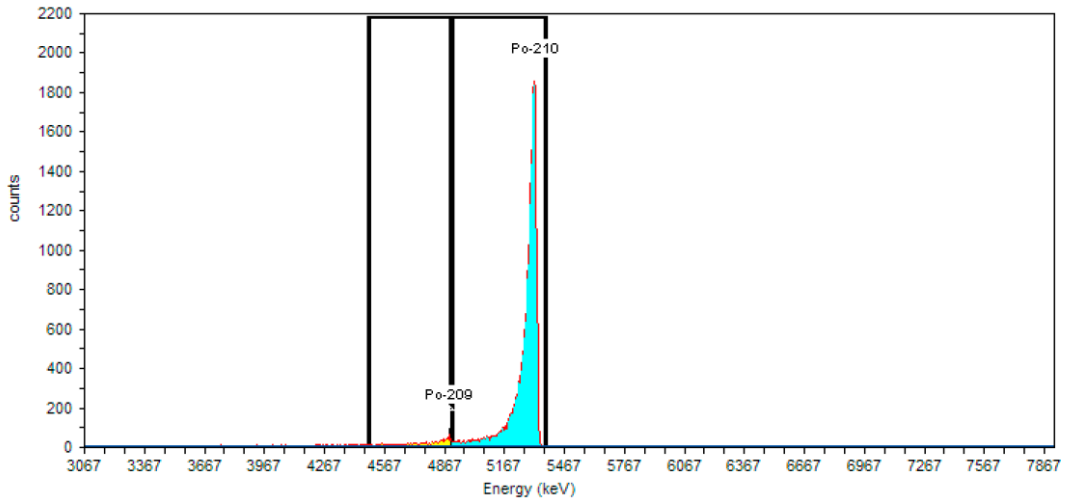
Tracer Name: Po-209(SS)
Tracer Activity: 711.89 mBq / mL x (Vol.) 0.77 mL = 547.45 mBq
Tracer Ref. Date: 9/17/2014 5:14:48PM

Tracer Nuclide: Po-209
Tracer Recovery: 244.91%

Acquisition

Detector: D053, SN: 54-028 N2, ID: 53
Acquisition Start Date: 6/23/2015 6:00:06PM
Live Time: 60.00 min.
Real Time: 60.00 min.
Background Date: 10/31/2014 11:37:04AM
Bkgd Info: Sample: BKG053; Det: D053; Spectrum #1; 10/31/2014 11:37:04 AM; Live Time: 2880.000(min.); ID: 53

Energy Calibration: 2015.06.22 Detector 53
Efficiency Calibration:2015.06.22 Detector 53
Calibration Date: 6/22/2015 6:56:28PM
Energy Cal: Gain = 4.8067 keV / Ch
Offset = 3,062.28 keV
Quadratic = 0.0000 keV / Ch^2
Efficiency: 22.57% +/- 0.83% TPU(2 sigma)



General Analysis

Analysis Method: Interactive ROI Analysis
Decay Correction:6/23/2015 5:54:54PM
MDA Constants: Kα = 1.64 , Kβ = 1.64

Nuclide Library: Polonium
MDA Source: Background

Nuclide Summary (ROI)

Nuclide	Peak Energy keV	ROI Start keV	ROI End keV	FWHM keV	B.R. %	Gross Counts	Bkgd Counts	Net Counts	Activity Bq/L	1.00Sigma TPU Bq/L	Critical Level Bq/L	MDA Bq/L
Po-209	4888.805	4485.046	4898.418	0.0	99.7	1,081.00	0.0000	1081.00	1.341E+002	4.773E+000	0.000E+000	1.370E-001
Po-210	5320.996	4908.032	5374.277	46.7	100.0	24,294.00	0.0208	24293.98	1.221E+003	7.865E+001	1.205E-002	1.601E-001

Tracer recovery: 245%

Recalculate using 96%

Activity result: 3247 Bq/L (corrected to 6/15/15)

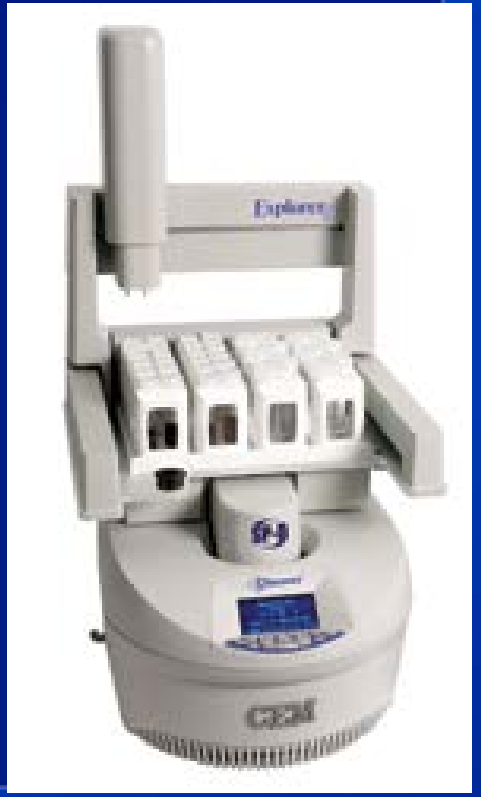
Compare to LRRI LSC result: 4400 Bq/L (6/15/15)

Again, much better agreement.

And again, our method obviously needs pre-dissolution

DEVELOP ROBUST DISSOLUTION METHOD

- CEM Discover SPD microwave with CEM Explorer sample changer
- Tried various solvents and digestion Profiles (times, temperatures & pressures)
  - We need it to be fast:
    - Thoroughly digest sample in short time
    - Produce digestate that can go directly to high-recovery autoplating process
  - We need high throughput:
    - Our branch has 4 of these microwave systems
    - If needed, we can look into a more traditional 40 position microwave



DEVELOP ROBUST DISSOLUTION METHOD (cont.)

- Hydrochloric acid dissolution – straight to autoplating from microwave?

Tried:

- Various HCl concentration from 10 to 75% (% volume of conc. HCl)
- Various temperatures from 125 to 240 °C
- Various pressures from 300 to 600 PSI
- Various hold times from 4 to 15 minutes.

DEVELOP ROBUST DISSOLUTION METHOD (cont.)

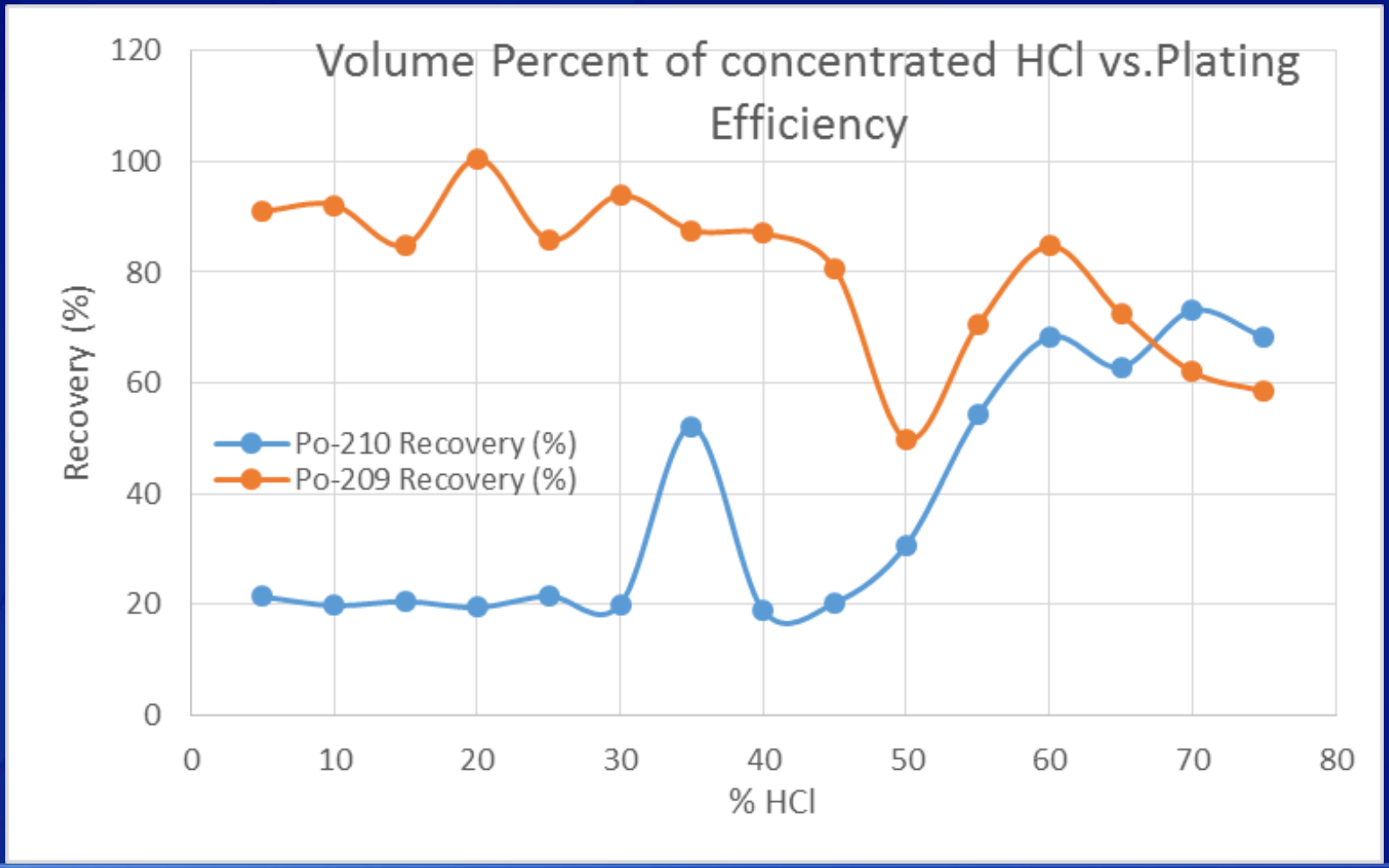
- Hydrochloric acid dissolution – straight to autoplating from microwave?

Results:

- Somewhat lower recoveries than hotplate dissolution for both Po-209 and Po-210
- Digestion pressures varied widely
- Microwave was often unable to reach goal temperatures
- Dissolution times varied from 5 to 26 minutes
- Significant portions of samples were sometimes lost
- Note that these analyses were performed on what we determined to be an ~ 128 Bq/L level of Po-210 in the samples by dilution of the pig urine samples into blank human urine.

DEVELOP ROBUST DISSOLUTION METHOD (cont.)

- Hydrochloric acid dissolution – up to 75% HCl
  - 45% and above had green residue, indicating dissolution of Cu planchet



DEVELOP ROBUST DISSOLUTION METHOD (cont.)

- Dr. Sadi decided to experiment on the hotplate, using 10% HCl with hydrogen peroxide (H2O2) for digestion. Looked promising.
- Then tried this in the microwave:
  - Added Hydroxylamine Sulfate (HAS) to help digestion and control microwave temperatures and pressures.
  - Neutralized remaining H_2O_2 with Oxalic Acid ($H_2C_2O_4$) after digestion for plating.
  - Tried Hydroxylamine Chloride (HAC) instead of HAS.

DEVELOP ROBUST DISSOLUTION METHOD (cont.)

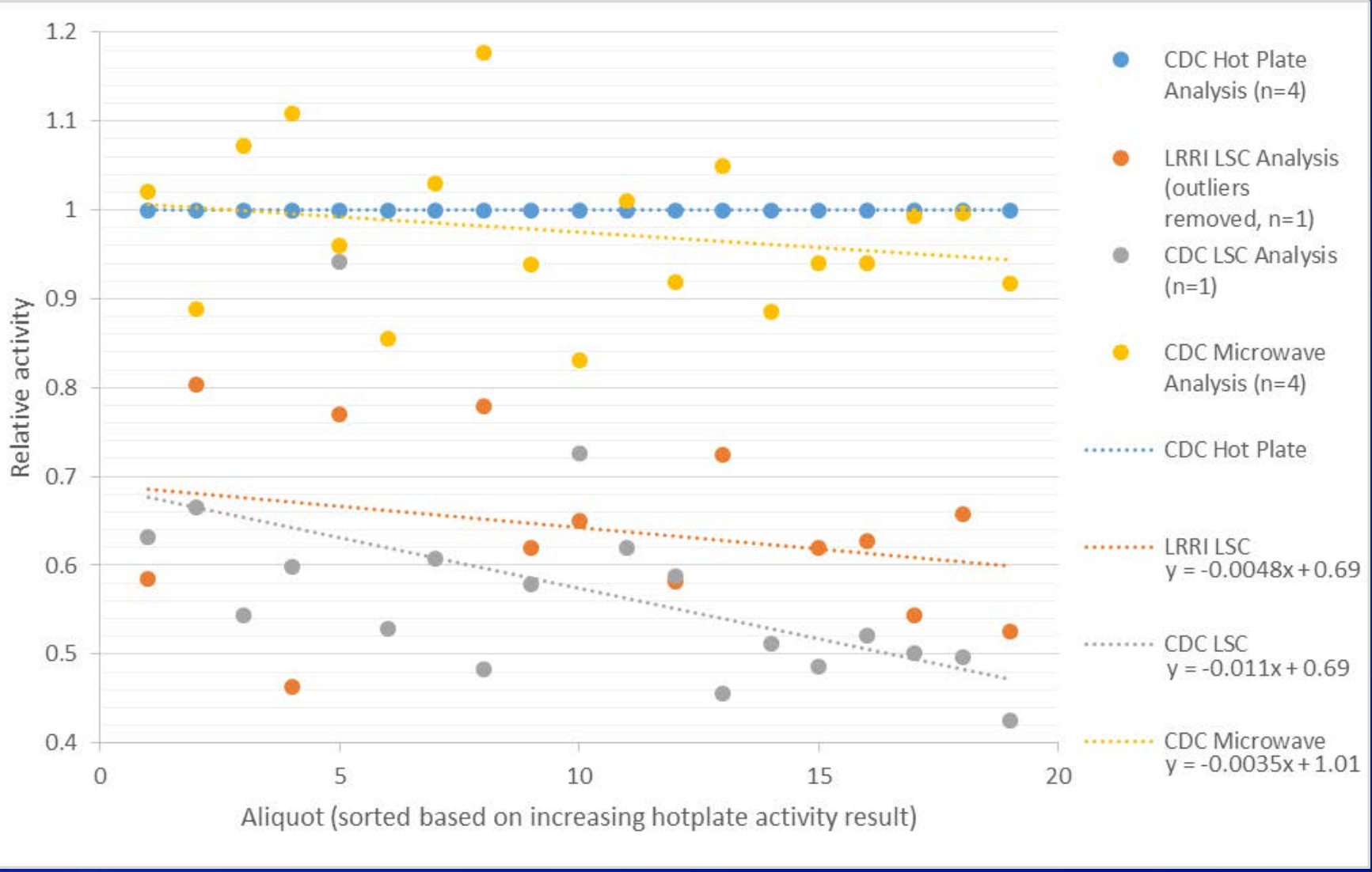
- H2O2 experiments yielded the following optimized results
  - 5 mL sample
  - 385 µL Po-209 tracer (17 Bq/L, for 1 C/P CDG)
  - 556 µL HCl
  - 250 µL 25% w/v HAS
  - 1 mL 30% H_2O_2
  - 1 mL 10% $H_2C_2O_4$
  - HAC caused excessive bubbling and sample loss while in the sample changer queue for digestion.

DEVELOP ROBUST DISSOLUTION METHOD (cont.)

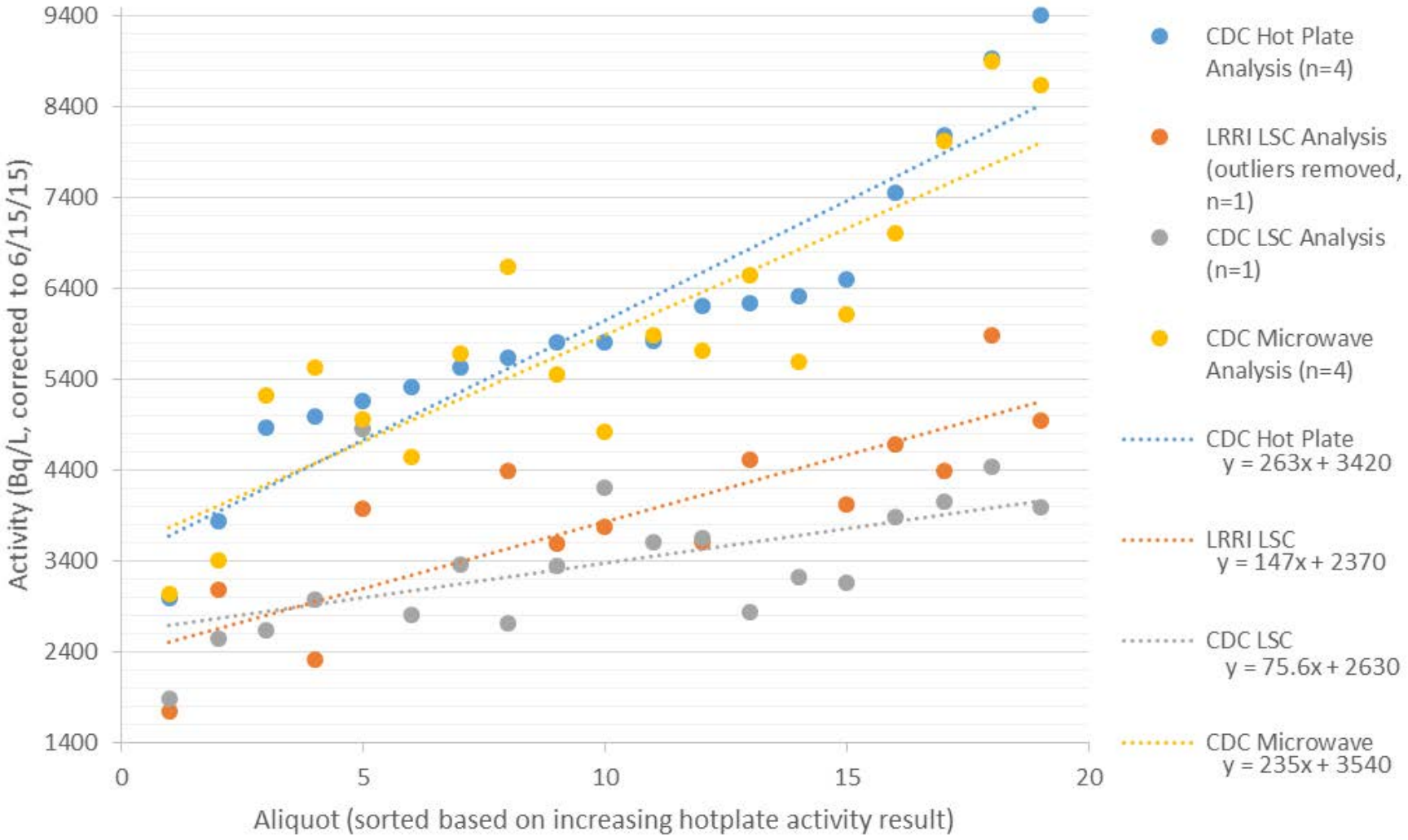
Applied optimized results to analysis of all pig urine aliquots

- Microwave digestion time consistent, averaging ~4.5 minutes with cool down time of ~0.5 to 1 minute.
- Ultimate temperatures and pressures ~160 to 180 °C and ~400 to 600 psi, respectively, with no apparent loss of sample.
- Average Po-209 tracer recovery was 96.5% vs. hotplate digestion recovery of 94.4%.
- Po-210 recovery vs. hotplate digestion was 97.6% (hotplate dissolution assumed to be 100%).
- Throughput per (20 hour) day with 4 microwaves is 800 samples.
- Note - all of these analyses were performed on pig urine diluted into human urine, based on preliminary LSC results, to an activity level of approximately 128 Bq/L.

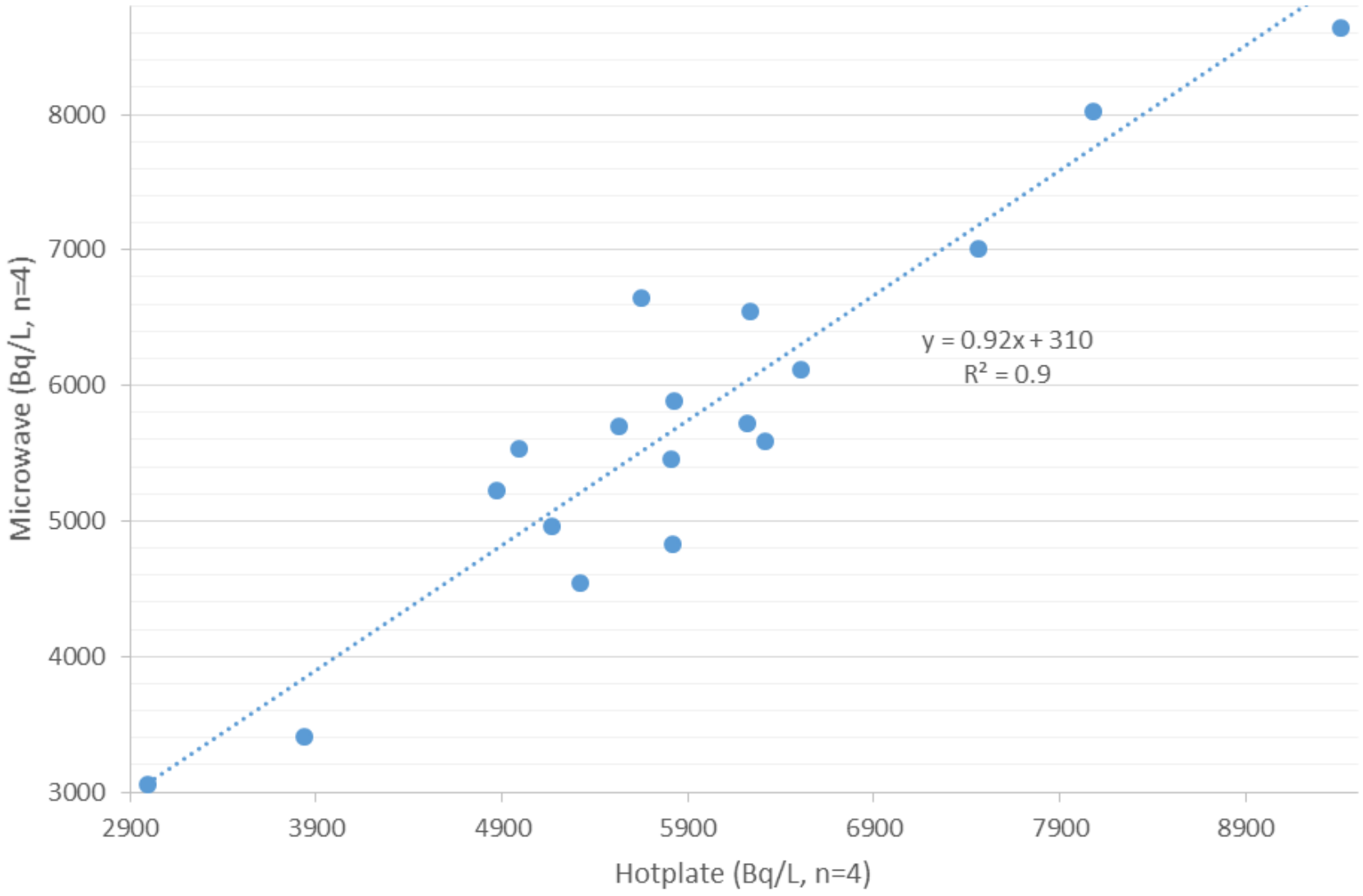
All pig urine results, relative to hotplate



Pig urine results, all methods



Hotplate vs. Microwave pig urine results



Pig urine results

- Hotplate vs. microwave R^2 is 0.897.
  - CDC/DLS criteria for method comparison equivalency is an R^2 value $\geq$ 0.95. 0.9 to 0.95 requires Branch Chief approval.
  - Note - these samples were analyzed after dilution to a target level of 128 Bq/L, based on initial LSC results, since high calibration level will be 2 adult CDGs, or 128 Bq/L (based on calculations using NCRP Report #161 information for Po-210).
  - What we see here is evidently a dilution effect, possibly due to differences between the methods' dissolution abilities for the pig urine and the human urine diluent.
- All method plots indicate better agreement at lower dilutions/activities, and overall acceptable accuracy and precision relative to the hotplate results.
  - This method will be suitable for undiluted samples (and diluted samples up to some point) for our radiation emergency response needs based on published/calculated CDG information.

NEXT

VALIDATE THE NEW METHOD…

Contact

David P. Saunders, PhD

Centers for Disease Control and Prevention
4770 Buford Hwy
Mailstop F-50
Atlanta, GA 30341-3724
DSaunders@cdc.gov

"The findings and conclusions in this study are those of the authors and do not necessarily represent the views of the U.S. Department of Health and Human Services, or the U.S. Centers for Disease Control and Prevention. Use of trade names and commercial sources is for identification only and does not constitute endorsement by the U.S. Department of Health and Human Services, or the U.S. Centers for Disease Control and Prevention."

Questions - Discussion

For more information please contact Centers for Disease Control and Prevention

1600 Clifton Road NE, Atlanta, GA 30333
Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
Visit: www.cdc.gov | Contact CDC at: 1-800-CDC-INFO or www.cdc.gov/info