A POLONIUM-210 RAPID EMERGENCY RESPONSE BIOASSAY METHOD: UPDATE

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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
<table>
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<th>Summary of Method Validation Parameters</th>
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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.
  
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.

**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 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 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

Tracer Nuclide: Po-209
Tracer Recovery: 244.91%

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 = 4.3676 keV / Ch
Offset = 3.062 28 keV
Quadratic = 0.0000 keV / Ch²
Efficiency: 22.57% +/- 0.83% TPU(2 sigma)
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
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.
Hydrochloric acid dissolution – straight to autoplateing 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
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 H2O2 with Oxalic Acid (H2C2O4) after digestion for plating.
- Tried Hydroxylamine Chloride (HAC) instead of HAS.
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% H₂O₂
- 1 mL 10% H₂C₂O₄

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

![Graph showing the relationship between aliquot and activity (Bq/L), corrected to 6/15/15). The graph includes data from various methods: CDC Hot Plate Analysis (n=4), LRRI LSC Analysis (outliers removed, n=1), CDC LSC Analysis (n=1), and CDC Microwave Analysis (n=4). The linear equations for each method are as follows:

- CDC Hot Plate Analysis: \( y = 263x + 3420 \)
- LRRI LSC Analysis: \( y = 147x + 2370 \)
- CDC LSC Analysis: \( y = 75.6x + 2630 \)
- CDC Microwave Analysis: \( y = 235x + 3540 \)
Hotplate vs. Microwave pig urine results

\[ y = 0.92x + 310 \]

\[ R^2 = 0.9 \]
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...
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Questions - Discussion

For more information please contact Centers for Disease Control and Prevention

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