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Integrating LRN-C Ricinidine Testing with LRN-B Ricin Assay for Environmental Evaluation
DISCLAIMER

• Any statements made by the presenter are his own and do not reflect the stance of anyone else living or dead and therefore are worth slightly less than a couple of dingo kidneys. He does however know where his towel is. So there you have it.
Ricin vs Ricinine

Ricin LRN-B

Ricinine LRN-C

\[
\text{H}_3\text{C}\text{-O-}\text{C} = \text{N}
\]

\[
\text{O-}\text{C} = \text{O}
\]

\[
\text{N}\text{-CH}_3
\]
Ricin vs Ricinine

**Ricin**
- Small sample volume
- Large Molecule- 64kDa*
- 5% of castor bean is ricin*
- Prep Time
  - 2 hours (can be prepped prior to sample arrival)
- Run Time
  - 2 hours
- Difficulty with glass, some make-up powders, bleach residues, silicas
- Not a very forgiving assay
- Accepted by FBI and others

**Ricinine**
- Small sample volume
- Small Molecule- molecular weight 164*
- 1% of castor bean*
- Prep time
  - 10 to 15 minutes
- Run Time
  - 10 minutes
- No known interferences (see next steps later)
- Requires additional education

*CDC Training Slides
Working Together

Finding a common goal in rapid response to a possible hazard (chemical or bio) is in the best interests of all parties.

Both groups are looking at the possibility of the same toxin, but take different routes to get there.

How can I help the other group should be part of the plan.

A quick confirmation run in the LRN-C lab would be a huge advantage to the LRN-B group as they do their analysis, or it could be utilized as a quick screen.
What can we offer each other?

- Bio has a ready source of testing materials that are part of an ongoing proficiency program.
- Chem has a rapid, accurate test
- Chem should have different if not fewer possibilities for interferences
- Looks like some common ground to build on
- So how do we start
Getting Started

• Convince your LRN-B and Select Agent RO that you are of sound mind
  – This may be harder for some of us than others but I did it so I believe in you

• Lay out a ground work for what your plan and end goals are
  – Each step should be thought out and discussed with at least:
    • LRN B+C
    • Select Agent RO
    • QA officer (you are dealing with someone’s PT materials after all)
  – Are you looking to make this a routine part of your testing for Environmental samples or a confirmation of an LRN-B result
So Here We Go

• Take a representative sample of powder
  – Dependent on available volume
• Mix with 1mL of diluted Internal Standard (1 mL conc with 5mL in H2O)
• Vortex for 20 seconds
• Centrifuge Filter (200-300uL)
• Transfer to sample vial
• Run using Ricinine/Abrine method
• Amaze your friends and fellow scientists in about 10 minutes
• Casually mock anyone taking 6 or more hours to find out what you already know
Powder Volume
Add ISD
Transfer for Filtration
Transfer to Vial
Run
But Does it Work?

• To date the NC CTAT unit has performed this with 100% Accuracy on 5 Proficiency testing rounds.
• We are running only the samples our BT Lab is clearing of pathogens so not every BT proficiency sample is being analyzed.
• As we are not weighing the samples were are not reporting a concentration, only present or not detectable.
• The BT proficiency has been reported to be only crushed bean, 1% Ricinine, but Ricin contains 20% Ricinine.*

*CDC Training Slides
Next Steps

• Finding out who can see the lowest
  – Running smaller or less concentrated samples until one of us fails to report a positive response
• Check analysis using leftover PBS from LRN-B analysis
• Evaluate for possible interferences
• Abrin? 75 times more toxic
  – I would propose that Abrine should work but there is currently no BT comparison
  – We would like to crush a rosary pea and test it as a starting point for the LRN-C
• Try this on samples from the various stages of extraction for both Ricin and Abrin
• Getting understanding in the law enforcement arena of the validity of this method of testing
Conclusion

• That is it
• Easy
• As
• Pie*

* Store bought pre cooked frozen pie, since I can’t bake for squat
Thanks

• I would like to take the time to say thanks to my staff at the CTAT
  – Bernard Barrameda
  – Lindsey Parker
  – David Whitt

• Thanks to the RO and BT coordinator, Royden Saah

• Thanks to my Admin for letting run with this
  – Dr. Dee Pettit and Dr. Scott Zimmerman

• And thanks to the folks at CDC for their support and encouragement