HMBANA Symposium 2019

Detection of illicit drugs in donor human milk screened by HMBANA guidelines

History of drug testing in Colorado

- January 1st, 2014: Recreational marijuana is legalized in Colorado (Amendment 64)
- July 23rd, 2015: MMB Board of Directors approves in-house toxicology testing
- September 2015: Initial validation testing begins
- October 2015: Validation complete– all pools of donor human milk dispensed by MMB is tested for
  - Amphetamines
  - Cocaine
  - Opiates
  - PCP
  - THC

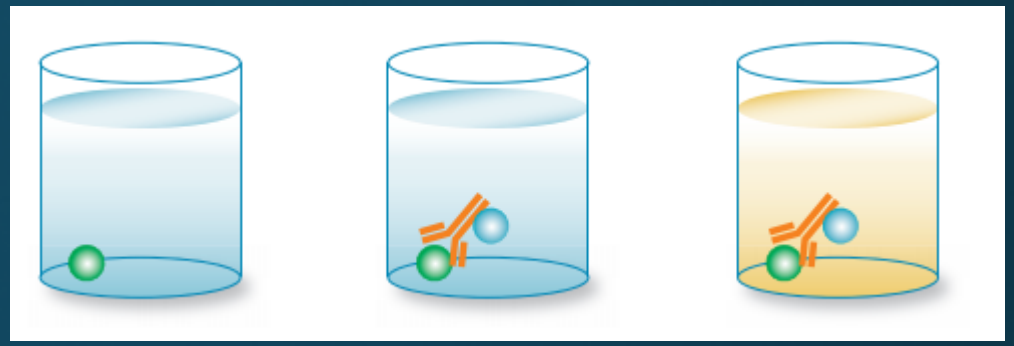
Testing development

- Commercially available kits already exist
  - Approved for human milk
  - But no *controls* for human milk
- Initial testing was intended to set acceptable ranges
  - Once implemented, adsorption value would need to be within range to approve donor milk for dispensation
  - But to be truly reliable, milk-specific controls would be needed

Testing development

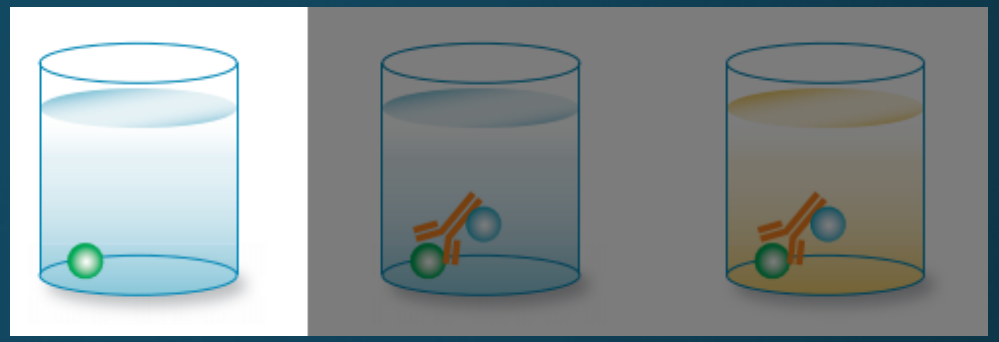
- Selected Neogen as kit manufacturer
  - Breadth of toxicology kit availability
  - Relative ease of ELISA testing for techs with minimal lab experience
- Contacted UTAK Laboratories to manufacture positive and negative controls
  - Created custom spiked donor milk set to the minimum detection levels according to Neogen package insert

Test Methodology



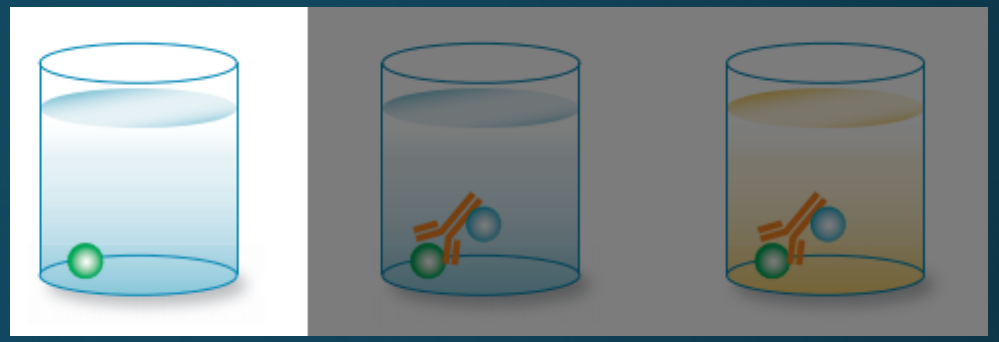
- ELISA
  - Enzyme Linked ImmunoSorbent Assay
  - Step 1: Binding
    - Competition for binding sites between drugs in a sample versus drug-enzyme conjugate supplied in the kit
  - Step 2: Detection
    - Substrate will detect any bound conjugate and trigger a color change
  - Step 3: Reading
    - Color change is halted and wavelength is detected using microplate reader

Test Methodology

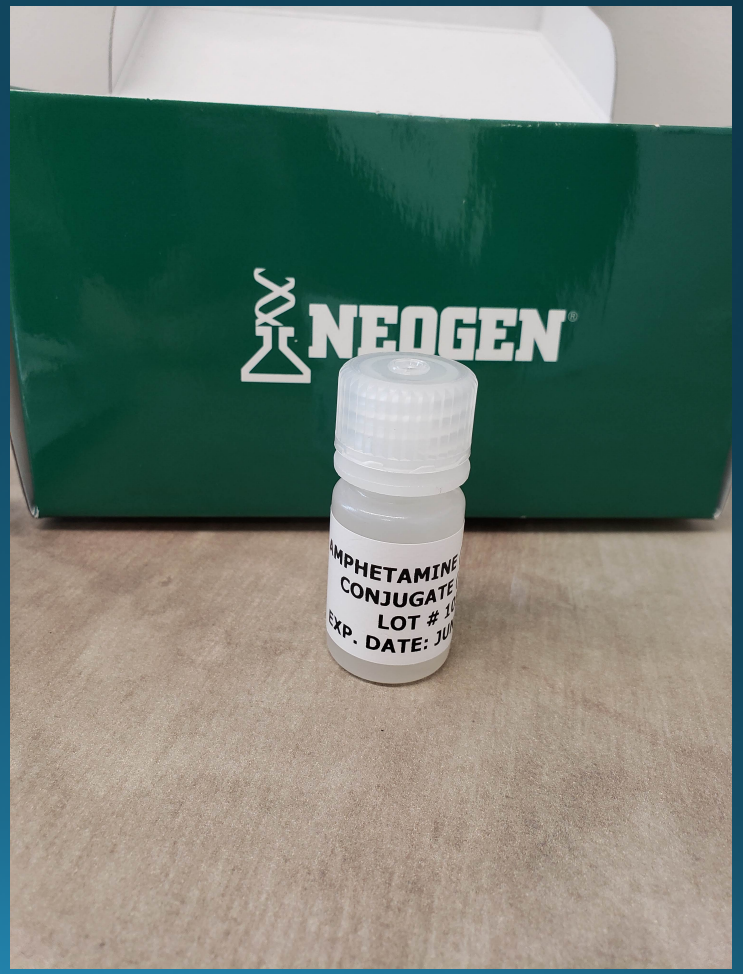


- Dilute donor milk samples with EIA Buffer
  - 20 µL milk to 80 µL buffer
  - Gently pipette up and down to mix
- Pipette 10µL of diluted milk to one microplate well
  - Do not touch the bottom of the well– this is where the anti-drug antibody is bound

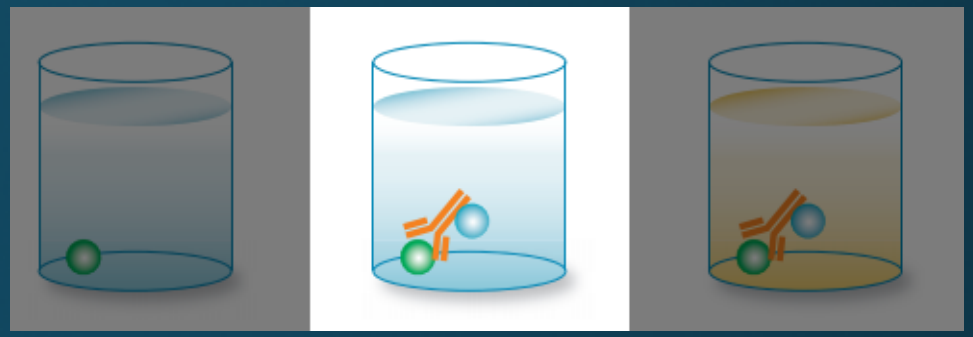
Test Methodology



- Pipette 100µL of drug conjugate to each well being tested
- Mix by gently shaking the plate
- Cover plate and let incubate at room temperature for 45 minutes

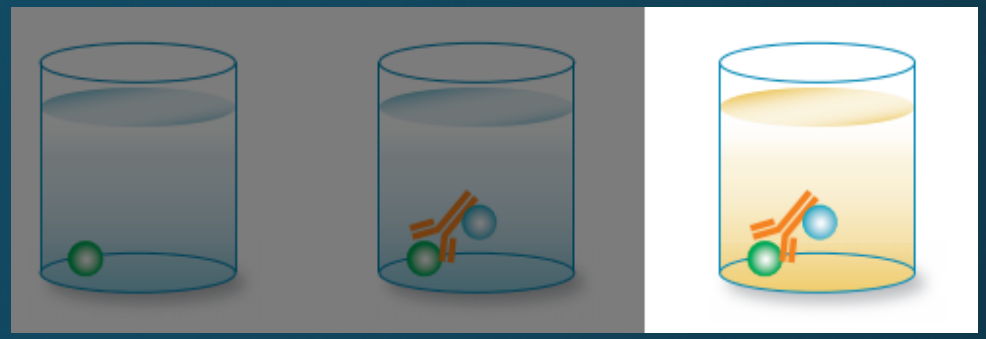


Test Methodology



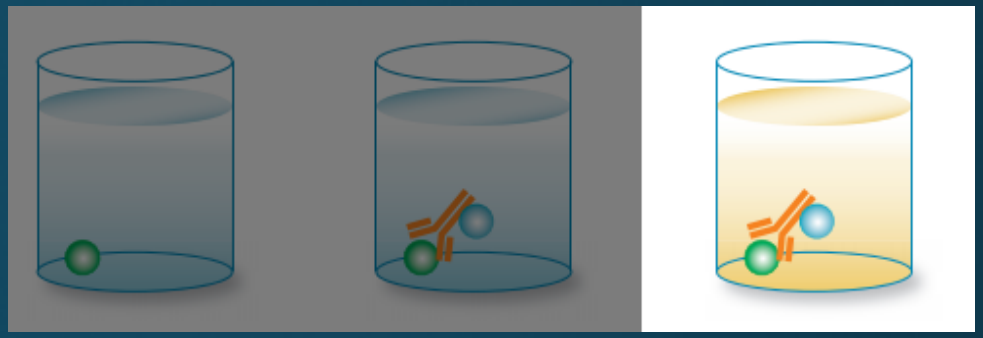
- Wash plate using Wash Buffer
  - We use ELx50 Biotek Plate Washer
  - Automated washing requires 5 washing cycles
- Pipette 100µL of Substrate (TMB chromogen)
  - TMB detects Horseradish Peroxidase, which is part of the Conjugate
- Cover plate and let incubate at room temperature for 30 minutes
  - Wells will turn blue as TMB is oxidized

Test Methodology



- Pipette 100µL of Acid Stop Solution into all testing wells and mix gently
- Color will change from blue to yellow
- If drugs have filled all the binding sites, the liquid will be clear or very pale
  - Substrate does not detect drugs
- If conjugate fills the sites (because no drugs are there to compete for them), the liquid will be yellow

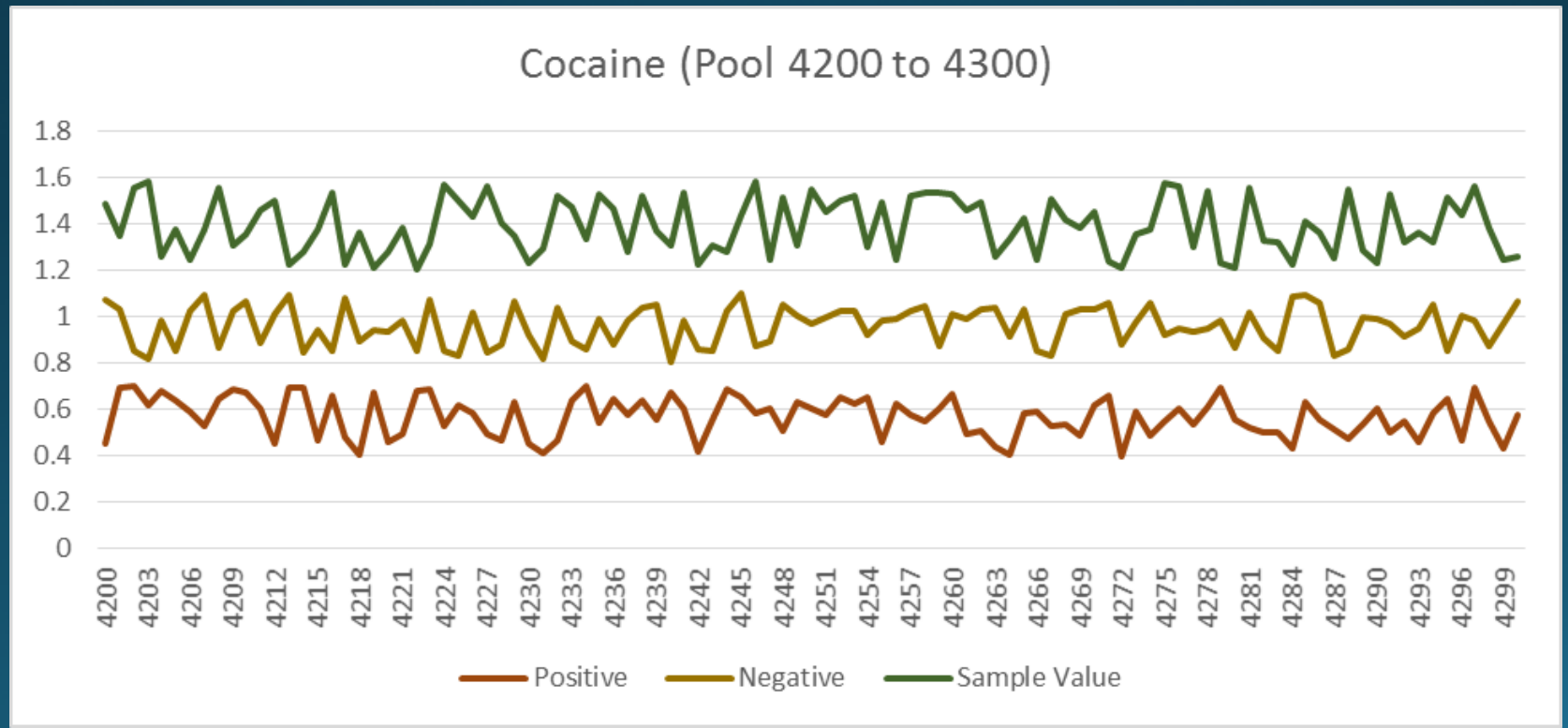
Test Methodology



- Shades of yellow are difficult to distinguish; can be read on a microplate reader to determine precise wavelength
- BioTek ELx800 Plate Reader
  - Comes with Gen5 Software to operate the machine and save results
  - Reads plate at 450 nm
  - Compare absorbance value with controls

Results

- Positive control values have the lowest optical density.
- Negative control values represent the lowest acceptable threshold for sample optical density



Results

- At first donation, a small pool of a single donor's milk is pasteurized individually
  - This allows the donor milk to be tested for bacillus cereus as well as drugs without dilution from other donations
  - Donor is flagged in Timeless as "Pending testing"
- Once all testing from the initial pool passes, the donor may be pooled with other donors
- 6,061 pools of donor milk have been tested
  - =2,736,901 ounces of donor milk
  - Representing 3,331 milk donors

Mothers' Milk Bank

Colorado Based. Nationwide Reach.

Rocky Mountain Children's Health FOUNDATION