

WORK INSTRUCTIONS

6.1.1 Confirmation of Benzodiazepines and Z drugs in Blood and Urine

REFERENCE MATERIAL

Calibrators and Controls

- Stock Solutions
1 mg/mL single component benzodiazepine-class reference solutions.
- Calibrator Solutions
The calibrator solutions must contain 7-aminoclonazepam, 7-aminoflunitrazepam, zopiclone, zolpidem, chlordiazepoxide, quetiapine, midazolam, flurazepam, nitrazepam, alpha-hydroxyalprazolam, alpha-hydroxytriazolam, oxazepam, nordiazepam, clonazepam, lorazepam, alprazolam, flunitrazepam, temazepam, and diazepam
 - 1.0 µg/mL Target mix in methanol
Add 10µL each 1mg/mL or 25µL 250µg/mL Stock Solution to \cong 6mL MeOH (LCMS grade) in 10mL ball flask. QS with MeOH. Solution is stable for one-year when stored under refrigeration.
 - 10.0 µg/mL Target mix in methanol
Add 250µL each 1mg/mL Stock Solution to \cong 20mL MeOH (LCMS grade) in 25mL ball flask. QS with MeOH. Solution is stable for one-year when stored under refrigeration.
- Positive Control
Negative urine or blood can be spiked with working solutions, but the compounds in that solution **cannot** be the same lot as was used for the calibrators. At minimum, the control must contain two compounds included in the scope of the method and fall between the approximate concentrations of 75 and 400 ng/mL.

Conjugated Controls (Urine samples only)

- Spiked Negative urine (must be the same lot for both positive and negative Glucuronide controls)
- Stock Solution
100µg/mL Oxazepam Glucuronide or Lorazepam Glucuronide
- Direct spiking
Spike negative urine with 30µL of 100µg/mL stock solution or 300µL working solution.
- Working Glucuronide Solution (10ng/µL)
Add 1mL 100µg/mL Stock Solution to 10mL MeOH. *Solution is stable for one year when stored under refrigeration.*

Internal Standard

- Stock Solution
100 µg/mL 7-aminoflunitrazepam-D7, alphahydroxyalprazolam-D5, oxazepam-D5, nordiazepam-D5, clonazepam-D4, temazepam-D5, diazepam-D5
- Working Internal Standard Solution (1.0 µg/mL ISTD mix in methanol)
Add 100µl of 100µg/mL stock solution to \cong 9mL MeOH in 10mL ball flask. QS with MeOH. *Solution is stable for one year when stored under refrigeration.*

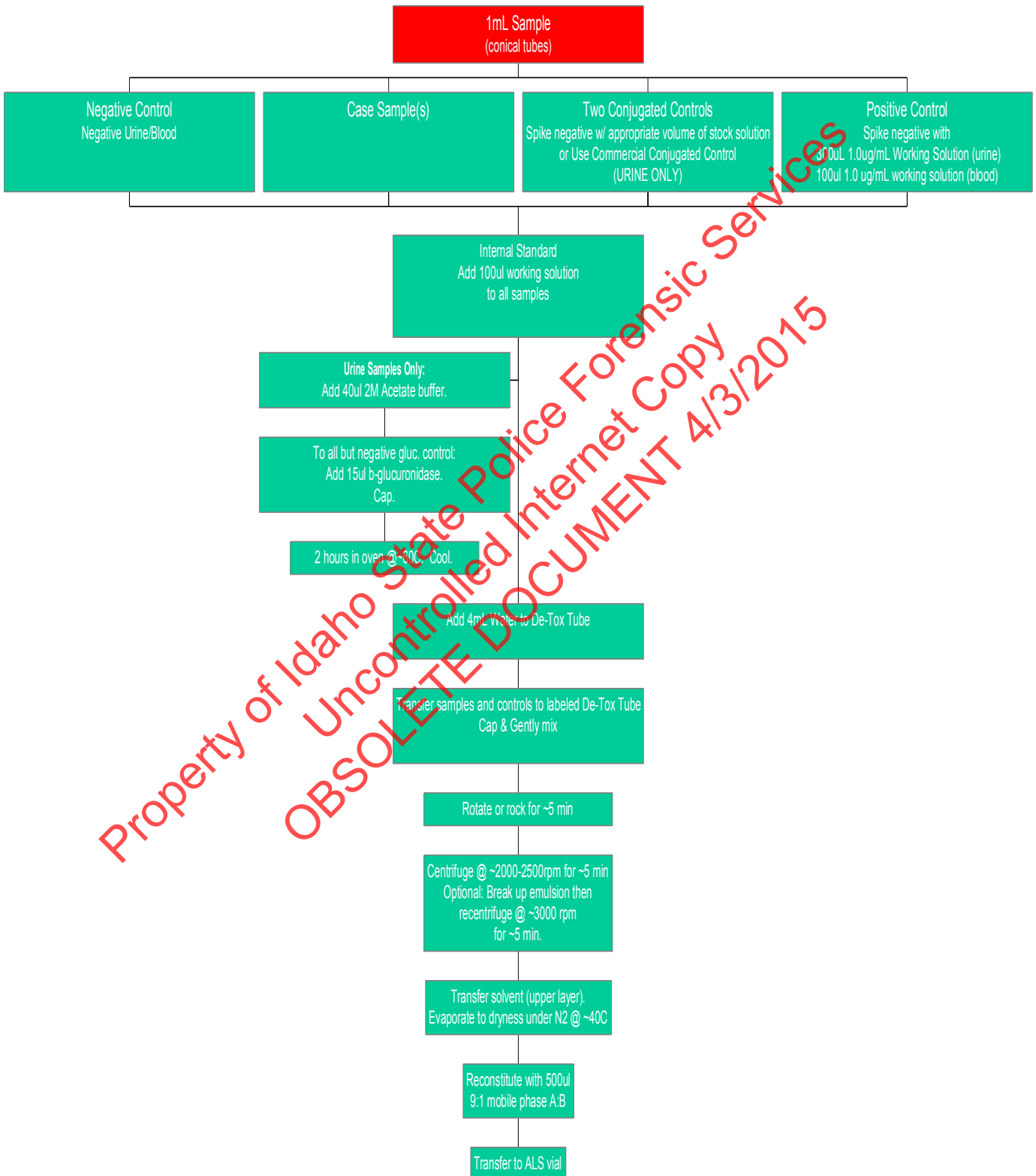
COMMENTS

This method has instructions for the preparation of both urine and blood casework samples.

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SAMPLE PREPARATION:



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Revision History

Revision No.	Issue Date	Revision/Comments
0		Original Issue.
1		
2	1/16/2014	
3	3/9/2014	Formatting changes. Addition of revision history page. Adjustment to positive control section to reflect method requirements. Added option for breaking up emulsions.

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