

## WORK INSTRUCTIONS

### 6.1.1 Confirmation of Benzodiazepines and Z drugs in Blood and Urine

#### QUALITY ASSURANCE MATERIAL

##### Working Reference Material Solutions

Corresponding calibrator and control reference materials must be obtained from different vendors, or be from different lot numbers if suitable second vendors are not available. *Working reference material solutions are stable for 12 months when stored under refrigeration.*

#### Calibrators and Controls

- Stock Solutions

1mg/mL or 250ug/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 LC/MS grade MeOH.

- 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 LC/MS grade MeOH.

##### Calibrator Preparation

Add the volume of working calibrator working solution to appropriate tube as indicated below.

Level	ng/mL	Working Solution (ng/µL)	Volume to add (µL)
Blank	-	-	-
1	25	1	25
2	50	1	50
3	100	1	100
4	500	10	50
5	1000	10	100
6	3000	10	300

- Positive Control

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.

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#### 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 ISID mix in methanol)  
Add 100µl of 100µg/mL stock solution to  $\cong$  9mL MeOH in 10mL ball flask. QS with LCMS grade MeOH. *Solution is stable for one year when stored under refrigeration.*

#### COMMENTS

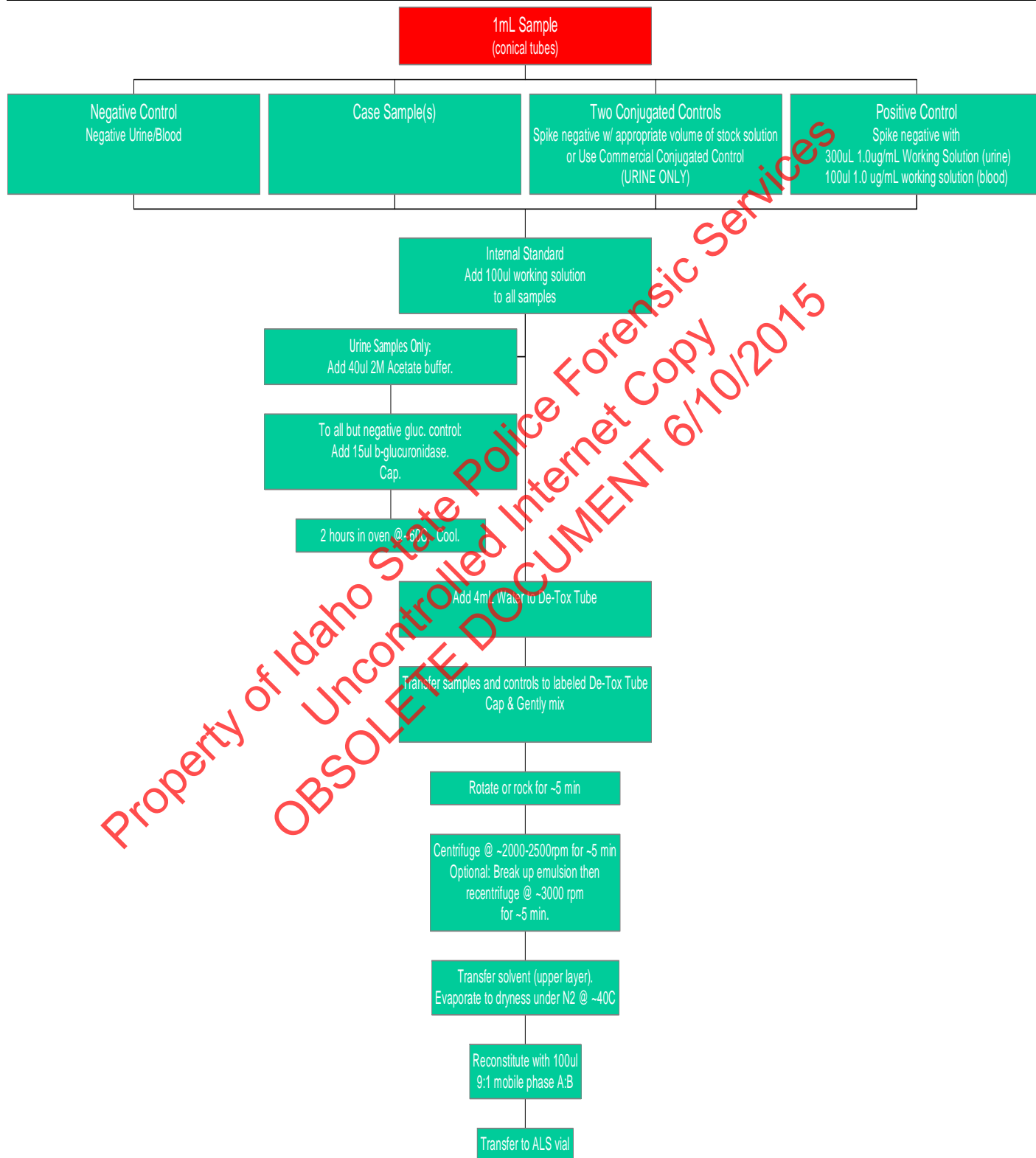
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This method has instructions for the preparation of both urine and blood casework samples.

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#### SAMPLE PREPARATION:



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#### *Revision History*

Revision No.	Issue Date	Revision/Comments
0	04/09/2013	Original Issue.
1	09/06/2013	?
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.
4	04/03/2015	Added spiking charts. Some formatting changes. Changed reconstitution volume to reflect method change.

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