

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	5	1	5
2	10	1	10
3	25	1	25
4	50	1	50
5	100	1	100
6	500	10	50
7	1000	10	100
8	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 (10 µg/mL ISTD mix in methanol)
Add 100µL of 100µg/mL stock solution to ≈ 9mL MeOH in 10mL ball flask. QS with LCMS grade MeOH. *Solution is stable for one year when stored under refrigeration.*

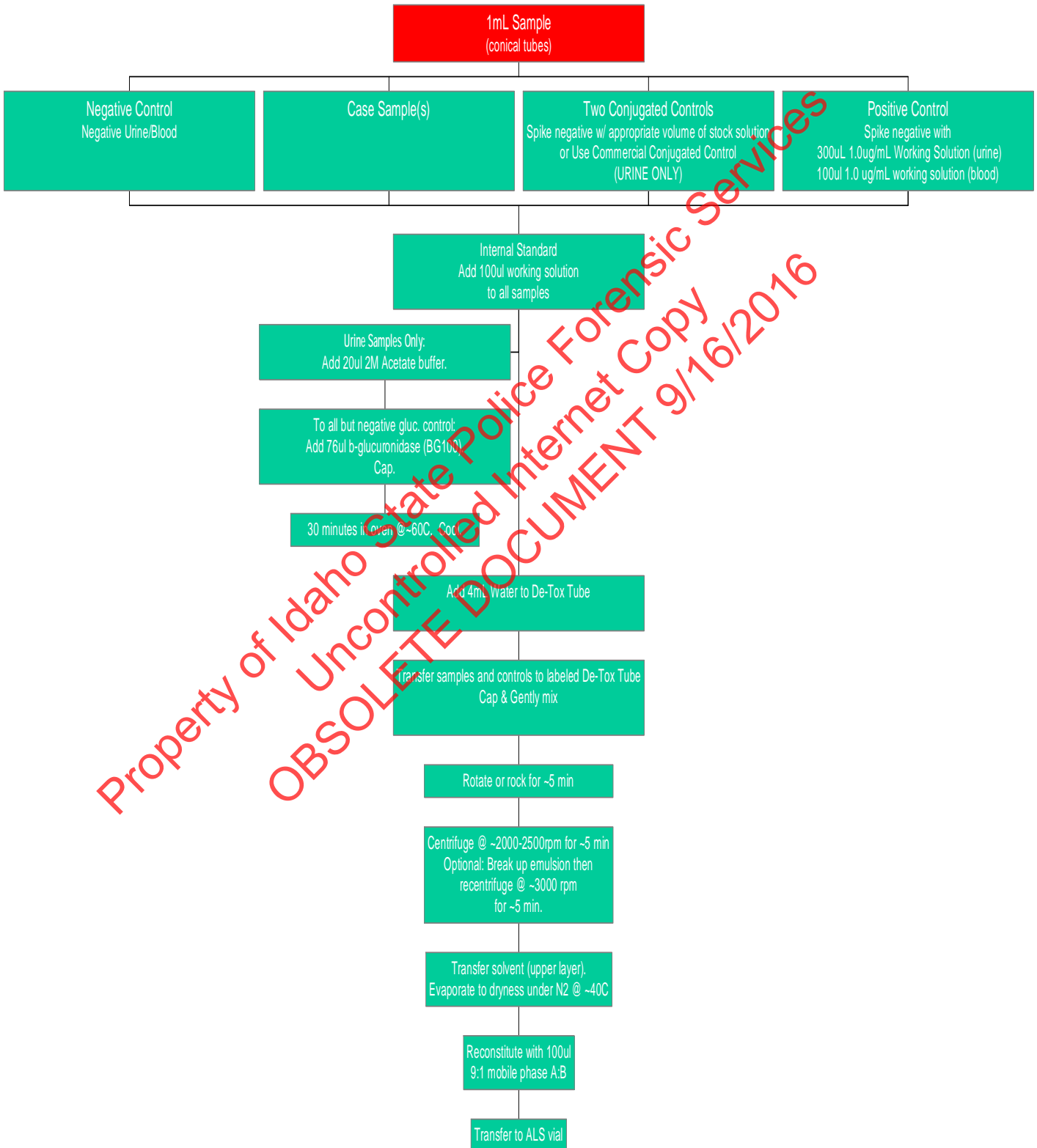
COMMENTS

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	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.
5	06/10/2015	Added 2 calibrators (5 & 10 ng/mL) and updated enzyme hydrolysis procedure to reflect validated changes.