

WORK INSTRUCTIONS

6.1.2 Confirmation of Cannabinoids in Blood and Urine

REFERENCE MATERIAL

Calibrators and Controls

- Stock Solutions

1 mg/mL or 100 µg/mL single component cannabinoid-class reference solutions.

- Calibrator/Control Working Solutions

The calibrator solutions must contain Δ^9 -THC, 11-nor- Δ^9 -THC-9-COOH, 11-hydroxy- Δ^9 -THC, Cannabinol, Cannabidiol

- 1.0 µg/mL Target Mix in methanol

Add 10 µL each (1 mg/mL) or 100 µL (100 µg/mL) Stock Solution to \cong 6 mL MeOH in 10 mL ball flask *EXCEPT* carboxy-THC. QS with MeOH. *Solution is stable for one-year when stored in the freezer.*

- 0.1/0.5 µg/mL Target Mix in methanol

Add 1 mL 1.0 µg/mL Target Mix + 50 µL (100 µg/mL) carboxy-THC stock solution to \cong 8 mL MeOH in 10 mL ball flask. QS with MeOH. *Solution is stable for one-year when stored in the freezer.*

(NOTE: Alternative calibrator/control working solution preparation options are listed in Appendix 1 of the Analytical Method.)

- Calibrator Preparation

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

Sample Type	0.1/0.5 µg/mL Target Mix	1.0 µg/mL Target Mix	100 µg/mL c-THC stock	Or 1.0/5.0 µg/mL Target Mix
1/5 ng/mL Cal 1	10 µL	-	-	-
2/10 ng/mL Cal 2	20 µL	-	-	-
5/25 ng/mL Cal 3	50 µL	-	-	-
10/50 ng/mL Cal 4	100 µL	-	-	-
25/125 ng/mL Cal 5	250 µL	-	-	25 µL
50/250 ng/mL Cal 6	-	50 µL	2.5 µL	50 µL
100/500 ng/mL Cal 7	-	100 µL	5 µL	100 µL

- 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.

- For urine, a single positive control between the approximate concentrations of 5 ng/mL and 25 ng/mL for all compounds except carboxy-THC shall be included in the run. The response for carboxy-THC should be between 25 ng/mL and 125 ng/mL.
- For blood, two positive controls are required (one low, and one mid to high). The low concentration control shall fall between 5 ng/mL and 10 ng/mL for all compounds except carboxy-THC, which shall be between 25 ng/mL and 50 ng/mL. The mid- or high-concentration control shall fall between 25 ng/mL and 100 ng/mL for all compounds except carboxy-THC, which shall be between 125 ng/mL and 500 ng/mL. (Note: for the low control, spike with 5-10 µL of the low control working solution and for the mid to high control, spike with 25-100 µL of the high control working solution).

Conjugated Controls (Urine samples only)

- Spiked Negative urine (must be the same lot for calibrators and controls)
- Stock Solution
100 µg/mL Carboxy-THC Glucuronide
- Working Glucuronide Solution (10ng/µL)
Add 10 µL 100 µg/mL Stock Solution to 990 µL MeOH or Acetonitrile. *Solution is stable for one week when stored under refrigeration.*

Internal Standard

- Stock Solution
100 µg/mL Δ^9 -THC-D3, 11-nor- Δ^9 -THC-9-COOH-D3, 11-hydroxy- Δ^9 -THC -D3, Cannabinol-D3, Cannabidiol-D3
- Working Internal Standard Solution (1.0 µg/mL ISTD mix in methanol)
Add 100 µL of 100 µg/mL stock solution to \cong 9 mL MeOH in 10 mL ball flask. QS with MeOH. *Solution is stable for one year when stored under refrigeration.*

COMMENTS

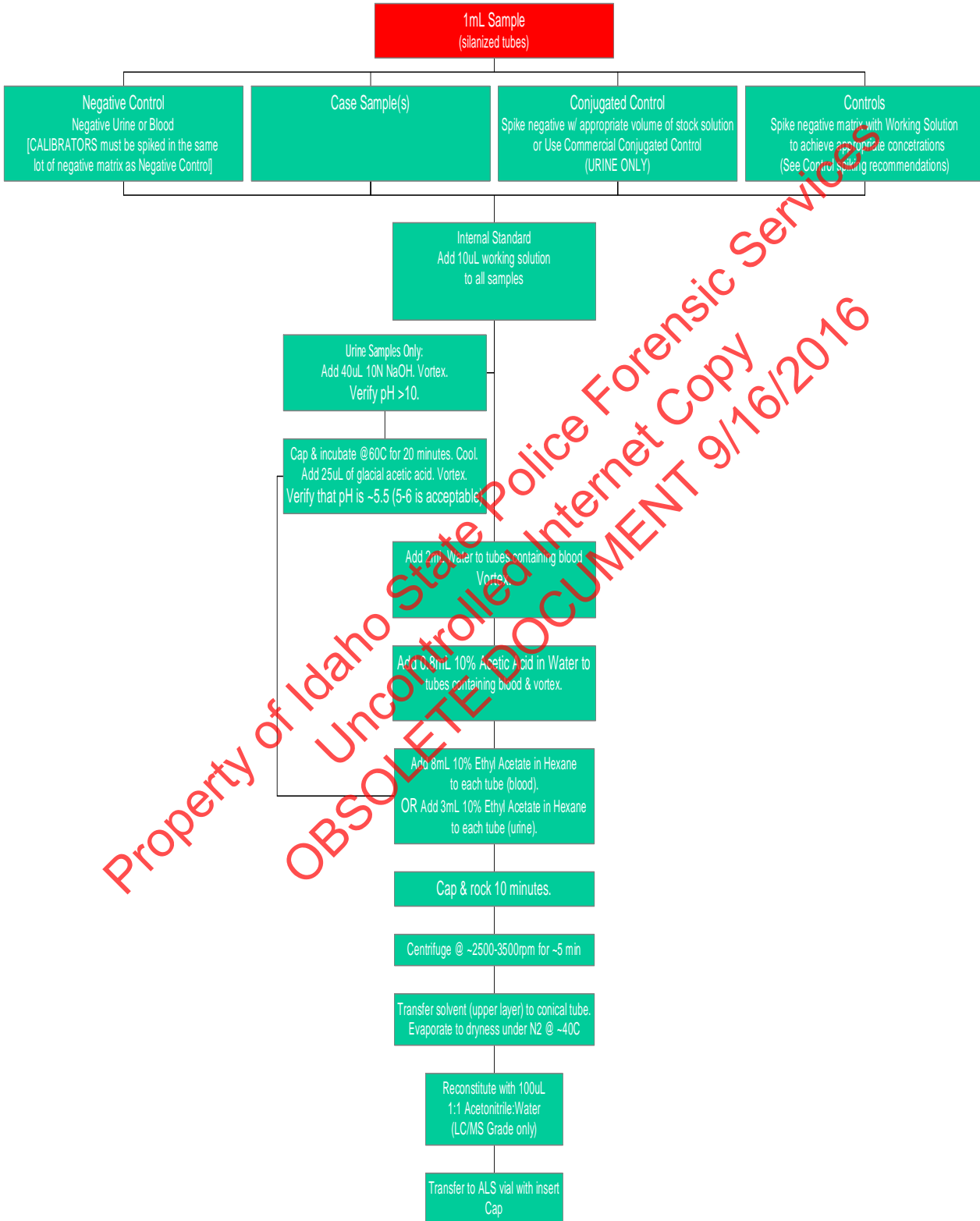
This method has instructions for the preparation of both urine and blood case work samples.

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SAMPLE PREPARATION:



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Revision History

Revision No.	Issue Date	Revision/Comments
0	08/31/2015	Original Issue.
1	07/26/2016	Removed extracted blank requirement, changed concentration requirements for controls

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