

## WORK INSTRUCTIONS

### 3.10.3 Free (Unbound) Codeine and Morphine in Blood UCT 200 mg CLEAN SCREEN® DAU Extraction Column

#### 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 6 months when stored under refrigeration.*

##### **10ng/mL**

Add 100.0 $\mu$ L each 1mg/mL Codeine and Morphine Stock Solution to  $\approx$ 9mL Methanol in a 10mL volumetric class A flask. QS to 10mL.

##### **1ng/mL**

Add 1.0mL 10ng/ $\mu$ L working solution to  $\approx$ 5mL Methanol in a 10mL volumetric class A flask. QS to 10mL.

##### Calibrator Preparation

Add the volume of working Codeine-Morphine mixed RM to appropriate tube as indicated below.

Level	ng/mL	Working Solution (ng/ $\mu$ L)	Volume to add ( $\mu$ L)
1	25	1	25
2	50	1	50
3	100	1	100
4	250	10	25
5	500	10	50
6	1000	10	100

##### Spiked Positive Control Preparation

Add the volume of working Codeine-Morphine mixed RM to appropriate tube as indicated:

ng/mL	Working Solution (ng/ $\mu$ L)	Volume to add ( $\mu$ L)
75	1	75
750	10	75

##### 1.0ng/ $\mu$ L Working Internal Standard Solution

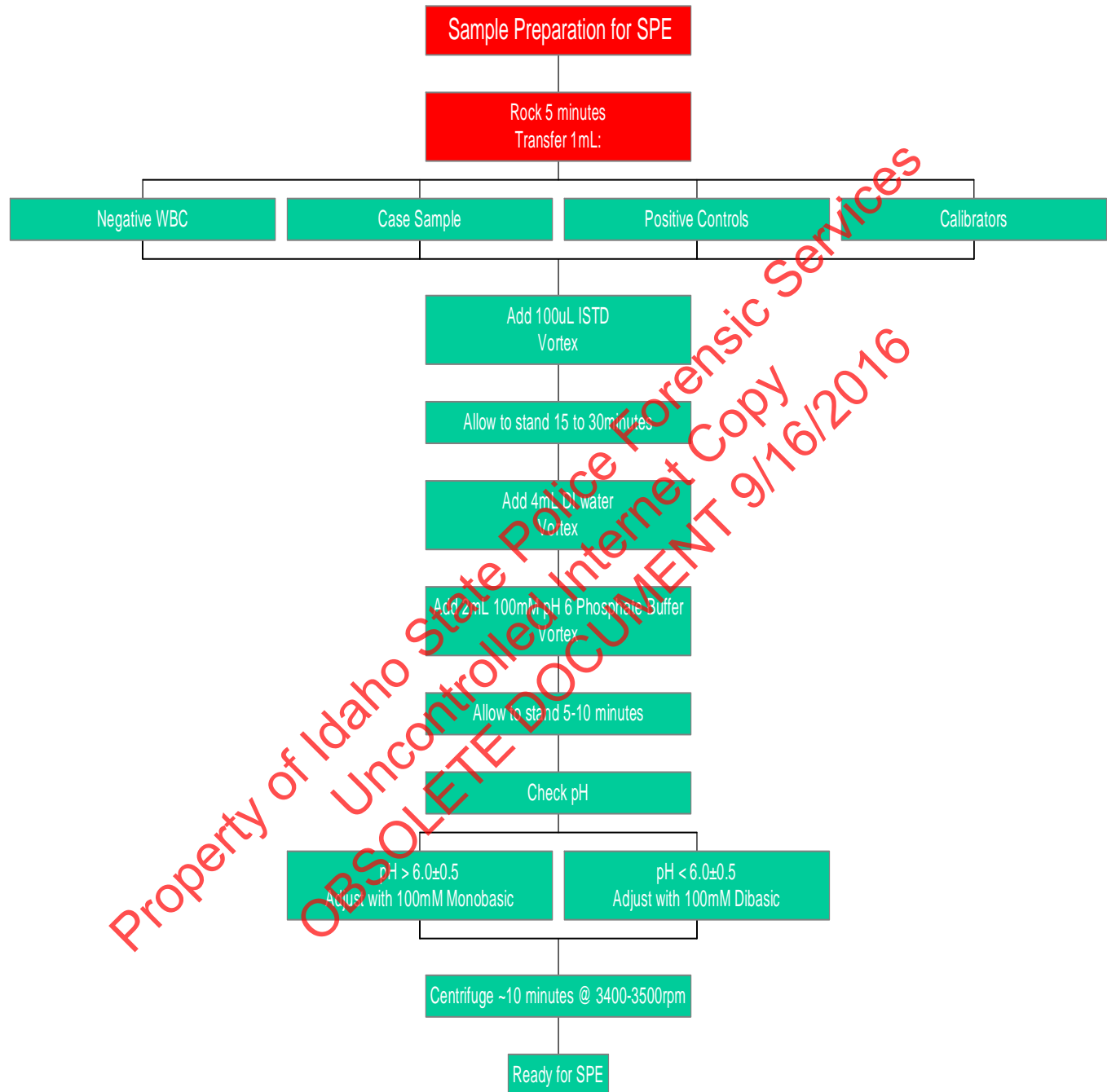
Add 10.0 $\mu$ L each 1mg/mL (1000ng/ $\mu$ L) or 100 $\mu$ L each 100 $\mu$ g/mL (0.1 $\mu$ g/ $\mu$ L) Codeine-D3 or Codeine-D6 and Morphine-D3 or Morphine-D6 Stock Solution to  $\approx$ 9mL Methanol in a 10mL volumetric class A flask. QS to 10mL. *Solution is stable for 6 months when stored under refrigeration.*

##### Optional: Commercial Blood Positive Control

Commercial controls must contain Codeine and Morphine each at a target concentration of 100ng/mL. Refer to package insert for verified value and expected range.

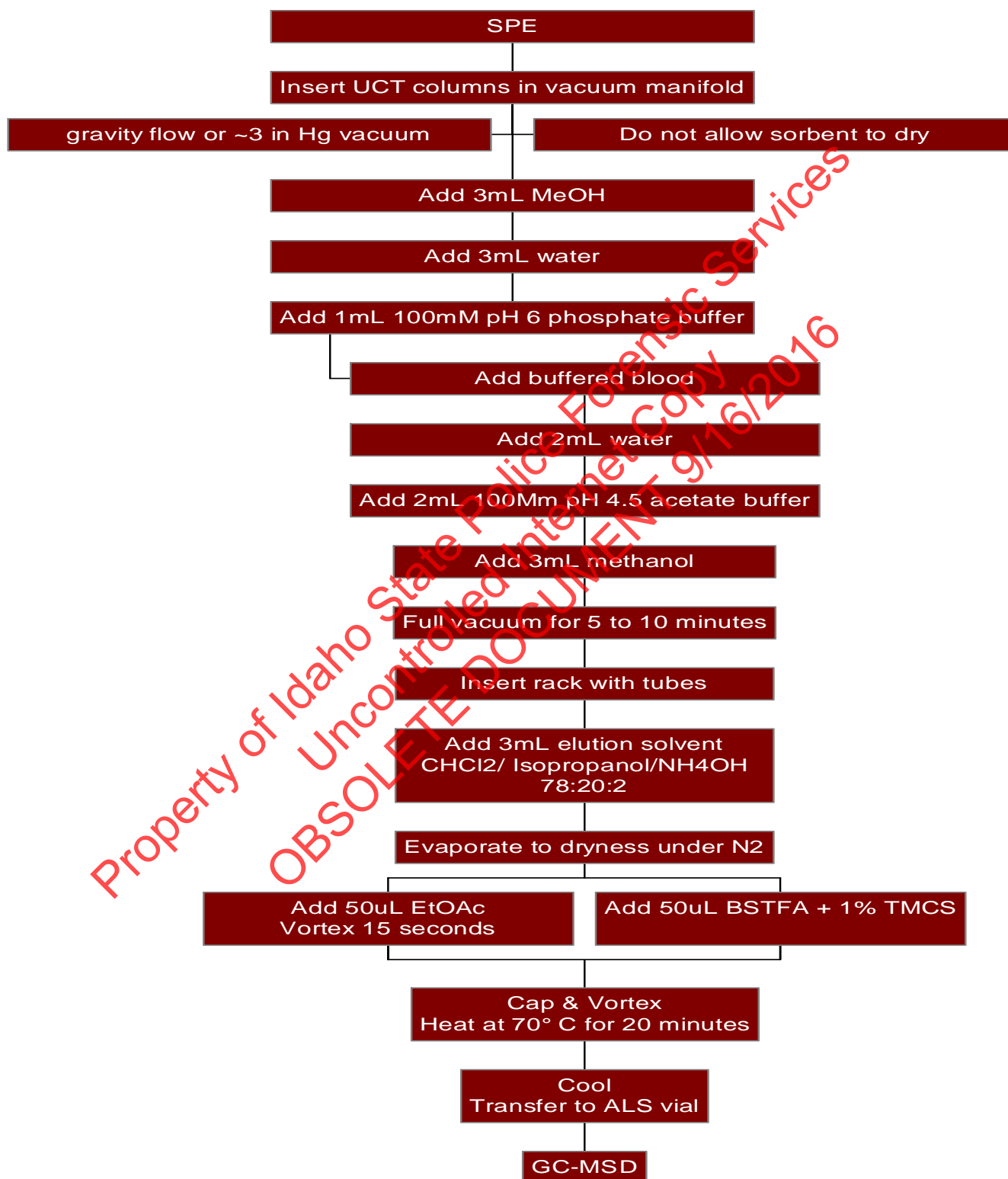
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#### *Revision History*

Revision No.	Issue Date	Revision/Comments
0	06-29-2007	Original Issue
1	03/07/2011	Clarified schemes, updated language
2	01/07/2013	Updated flow chart to reflect changes in method.
3	03/13/2015	Minor changes to reflect method language.

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