



# **TENNESSEE BUREAU OF INVESTIGATION**

## *Forensic Services Division*

### Toxicology Quality Assurance and Procedures Manual

#### 6.15A ELISA Control Preparation

#### **6.15A Enzyme-Linked Immunosorbent Assay (ELISA) Control Preparation and Verification**

##### **6.15A.1 Purpose**

The following procedure will be used for the preparation and verification of positive and negative control material for the ELISA presumptive screening procedure.

##### **6.15A.2 Limitations**

- Matrix matched control materials are not used for the ELISA screening procedure. Validation studies indicated that false positives in serum/plasma or urine case samples are possible when compared to controls prepared in blood. All presumptive positive results shall be confirmed for reporting purposes, except for cases of insufficient sample that will be clearly documented on the report.
- High humidity was shown to increase the variability in B/B0 values, which in turn can impact new control verification. An attempt to control and monitor humidity shall be made, and it is suggested that testing be suspended when humidity levels reach greater than or equal to 70%.

##### **6.15A.3 Specimen Criteria**

Not applicable.

##### **6.15A.4 Safety**

All universal precautions should be followed. The laboratory maintains MSDS information on all chemicals used in the laboratory. If any chemical or reagent carries unusual warnings it would be listed with that item in the Equipment section.

##### **6.15A.5 Equipment**

###### ***Instrumentation***

Tecan EVO Freedom 75

###### ***Supplies***

Various volumetric glassware

Volumetric pipettes and disposable tips

###### ***Chemicals***

Negative blood

100 mM PBS Buffer

Methanol, Optima grade or higher

###### ***Reagents***

- 1:1 Blood:PBS Buffer
  - Combine bank blood or other approved blood product with an equal quantity of PBS buffer. The total volume prepared can vary by the needs of the laboratory.
  - The blood:buffer solution shall be stored in the refrigerator when not in use.



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- Expires one year from the date of preparation, or by the manufacturer's expiration date, whichever comes first.

#### **Reference Materials**

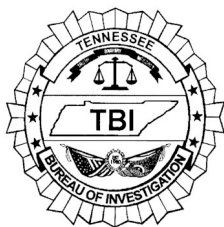
Suggested concentration 1 mg/mL

Morphine, Buprenorphine, Delta 9 THC-COOH, Benzoylecgonine, Secobarbital, Oxazepam, Diphenhydramine, Carisoprodol, Methadone, Tramadol, Ketamine, Zolpidem, (+)-Methamphetamine, (+)-Amphetamine, Fentanyl and Oxycodone

#### **6.15A.6 Preparation Procedure**

##### **ELISA Stock Reference Standard Solutions**

- Store at freezer temperatures
  - All Stock Reference Standard Solutions should be made from new reference materials.
  - Prepared solutions expire one year from date of preparation or manufacturer expiration date, whichever comes first.
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- **Morphine [1000ng/mL]**  
Pipette 50 µL of morphine [1 mg/mL] certified reference material into a 50 mL volumetric flask and dilute to volume with methanol.
  - **Buprenorphine [500 ng/mL]**  
Pipette 25 µL of buprenorphine [1 mg/mL] certified reference material into a 50 mL volumetric flask and dilute to volume with methanol.
  - **THC-COOH [1000 ng/mL]**  
Pipette 50 µL of THC-COOH [1 mg/mL] certified reference material into a 50 mL volumetric flask and dilute to volume methanol.
  - **Benzoylecgonine [1000 ng/mL]**  
Pipette 50 µL of benzoylecgonine [1 mg/mL] certified reference material into a 50 mL volumetric flask and dilute to volume with methanol.
  - **Secobarbital [10,000 ng/mL]**  
Pipette 500 µL of secobarbital [1 mg/mL] certified reference material into a 50 mL volumetric flask and dilute to volume with methanol.
  - **Oxazepam [1000 ng/mL]**  
Pipette 50 µL of oxazepam [1 mg/mL] certified reference material into a 50 mL volumetric flask and dilute to volume with the methanol.
  - **Diphenhydramine [1000 ng/mL]**  
Pipette 50 µL of diphenhydramine [1 mg/mL] certified reference material into a 50 mL volumetric flask and dilute to volume with methanol.
  - **Carisoprodol [100,000 ng/mL]**



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Pipette 1 mL of carisoprodol [1 mg/mL] certified reference material into a 10 mL volumetric flask and dilute to volume with methanol.

- **Methadone [1000 ng/mL]**

Pipette 50 µL of methadone [1 mg/mL] certified reference material into a 50 mL volumetric flask and dilute to volume with methanol.

- **Tramadol [1000 ng/mL]**

Pipette 50 µL of tramadol [1 mg/mL] certified reference material into a 50 mL volumetric flask and dilute to volume with methanol.

- **Ketamine [1000 ng/mL]**

Pipette 50 µL of ketamine [1 mg/mL] certified reference material into a 50 mL volumetric flask and dilute to volume with methanol.

- **Zolpidem [500 ng/mL]**

Pipette 25 µL of zolpidem [1 mg/mL] certified reference material into a 50 mL volumetric flask and dilute to volume with methanol.

- **Methamphetamine [1000 ng/mL]**

Pipette 50 µL of methamphetamine [1 mg/mL] certified reference material into a 50 mL volumetric flask and dilute to volume with methanol.

- **Amphetamine [1000 ng/mL]**

Pipette 50 µL of amphetamine [1 mg/mL] certified reference material into a 50 mL volumetric flask and dilute to volume with methanol.

- **Fentanyl [100 ng/mL]**

Pipette 10 µL of fentanyl [1 mg/mL] certified reference material into a 100 mL volumetric flask and dilute to volume with the methanol.

- **Oxycodone [1000 ng/mL]**

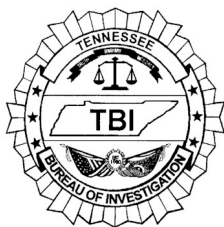
Pipette 50 µL of oxycodone [1 mg/mL] certified reference material into a 50 mL volumetric flask and dilute to volume with methanol.

#### **ELISA Working Reference Control Standards**

- Store at refrigerator temperatures
- Expire two months from the date of preparation, not to exceed the expiration date of the stock solution.

#### **Working Reference Control Standard 1**

To make the Working Reference Control Standard for the 6-drug mixture listed below, add the following amounts of each ELISA Stock Reference Standard Solution to a 10 mL culture tube and dilute to a final volume of 5 mL (4,440 µL) with a 1:1 mixture of negative blood/PBS buffer.



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| DRUG             | STD SOLUTION | AMOUNT USED | FINAL CONCENTRATION |
|------------------|--------------|-------------|---------------------|
| Morphine         | 1000 ng/mL   | 50 µL       | 10 ng/mL            |
| Buprenorphine    | 500 ng/mL    | 10 µL       | 1 ng/mL             |
| THC-COOH         | 1000 ng/mL   | 50 µL       | 10 ng/mL            |
| Benzoyllecgonine | 1000 ng/mL   | 250 µL      | 50 ng/mL            |
| Secobarbital     | 10000 ng/mL  | 125 µL      | 250 ng/mL           |
| Oxazepam         | 1000 ng/mL   | 75 µL       | 15 ng/mL            |

#### Working Reference Control Standard 2

To make the Working Reference Control Standard for the 6-drug mixture listed below, add the following amounts of each ELISA Stock Reference Standard Solution to a 10 mL culture tube and dilute to a final volume of 5 mL (4,050 µL) with a 1:1 mixture of negative blood/PBS buffer.

| DRUG            | STD SOLUTION  | AMOUNT USED | FINAL CONCENTRATION |
|-----------------|---------------|-------------|---------------------|
| Diphenhydramine | 1000 ng/mL    | 250 µL      | 50 ng/mL            |
| Carisoprodol    | 100,000 ng/mL | 50 µL       | 1000 ng/mL          |
| Methadone       | 1000 ng/mL    | 250 µL      | 50 ng/mL            |
| Tramadol        | 1000 ng/mL    | 250 µL      | 50 ng/mL            |
| Ketamine        | 1000 ng/mL    | 100 µL      | 20 ng/mL            |
| Zolpidem        | 500 ng/mL     | 50 µL       | 5 ng/mL             |

#### Working Reference Control Standard 3

To make the Working Reference Control Standard for the 4-drug mixture listed below, add the following amounts of each ELISA Stock Reference Standard Solution to a 10 mL culture tube and dilute to a final volume of 5 mL (4,650 µL) with a 1:1 mixture of negative blood/PBS buffer.

| DRUG            | STD SOLUTION | AMOUNT USED | FINAL CONCENTRATION |
|-----------------|--------------|-------------|---------------------|
| Methamphetamine | 1000 ng/mL   | 125 µL      | 25 ng/mL            |
| Amphetamine     | 1000 ng/mL   | 125 µL      | 25 ng/mL            |
| Oxycodone       | 1000 ng/mL   | 50 µL       | 10 ng/mL            |
| Fentanyl        | 100 ng/mL    | 50 µL       | 1 ng/mL             |

#### Working Reference Negative Control Standard

To make the Working Reference Negative Control Standard, create a 1:1 mixture of negative blood/PBS buffer. It is not necessary for the negative control to be prepared on



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the same day as the positive controls, but the matched positive control must be made from the same lot of negative blood as the matched negative control. Negative blood shall be evaluated and verified as negative prior to its use.

#### **6.15A.7 Performance Characteristics**

Not applicable.

#### **6.15A.8 Analytical Parameters**

Not applicable.

#### **6.15A.9 Data Analysis**

- It is best practice to create and verify a new lot of control material prior to the expiration date of the old material. In this circumstance the new materials shall be analyzed concurrently with the controls previously verified and the difference data means compared.
- The difference data mean for each assay for the new working reference control standard should be within  $\pm 20\%$  of the difference data mean of the corresponding assay for the working reference control standard currently in use. A spreadsheet is available in the document control software to facilitate this calculation.
- If the new working reference control standard is outside of the  $\pm 20\%$  acceptance criteria, or if the previous lot of control material has expired prior to verification of new material, then the scientist/technician shall evaluate the precision of the new lot of material. To do this, the analyst shall make a low positive control (50% below the concentration of the control standard concentration) and a high positive control (100% above the concentration of the control standard concentration.) If the ranges of the low positive control, positive control and high positive control (difference data mean  $\pm 2$  standard deviations for each) do not overlap, then the new working reference control standard will be considered fit for use. A spreadsheet is available in the document control software to facilitate this calculation.

#### **6.15A.10 Corrective Measures**

- If the new lot of working control reference material fails to meet the  $\pm 20\%$  difference data mean criteria on the first analysis, the assay can be repeated.
- If it fails a second time, the precision procedure shall be followed to verify the control material.

#### **6.15A.11 Reporting**

- Documentation of the verification shall be stored, and shall include all appropriate calculations, references to lot numbers.

#### **6.15A.12 References**

See method validation and related policies.