TX 34: Enzyme-Linked Immunosorbent Assay (ELISA)

1. Introduction

Enzyme linked immunosorbent assays (ELISA) have the advantage of rapidly screening small volumes of biological samples for the presence of drugs. The use of an automated system to pipette reagents, wash, and read the plates makes the screening process faster and more reproducible. The testing within this immunoassay procedure is considered presumptive. Any positive results from unknown samples (e.g., evidence) obtained through the use of this procedure must be confirmed by an orthogonal technique (i.e., a second analytical technique based on a different scientific principle) when such results are reported without the stipulation that they are presumptive.

2. Scope

This procedure allows for the screening of blood and urine samples for at least fourteen (14) commonly encountered drugs and drug classes. These include, but may not be limited to: 6-acetylmorphine, amphetamine and analogs, barbiturates, benzodiazepines, buprenorphine, cannabinoids, cocaine metabolite (benzoylecgonine), fentanyl, methadone, methamphetamine and analogs, opiates, oxycodone/oxymorphone, phencyclidine (PCP), and zolpidem. Other classes may be added to the routine screens as deemed necessary, provided the kits have been validated prior to their use.

Generally, for routine post-mortem drug screens involving Office of the Chief Medical Examiner (OCME) evidence, only blood samples will be analyzed using this procedure. For routine antemortem driving under the influence (DUI)-type cases, urine is the most common matrix that is submitted. However, as situations arise, sampling of different matrices for different types of cases may be necessary.

3. Principle

In each assay the micro-well plates come pre-coated with antibodies which are specific for the drug or metabolite being tested. Samples are individually added to analyte-specific wells, along with drug-enzyme conjugate (e.g., horseradish peroxidase). Any analyte (drug) that happens to be in a sample will compete with the corresponding drug-enzyme conjugate for binding sites on the specific antibody within that particular well during a set incubation period. Following the incubation, the drug-specific plate is automatically washed to remove any unbound analyte (drug) or drug-enzyme conjugate. The washing step is the main reason why ELISA is termed a heterogeneous immunoassay technique. Substrate (e.g., tetramethylbenzidine) is then automatically added to each well. Any enzyme (from the drug-enzyme conjugate) that is still bound within the well will cause a color change due to the enzyme having reacted with the added substrate. After another incubation period the

color reaction between the enzyme and the substrate is stopped by the automated addition of acid. The intensity of the resulting solution's color within the wells is automatically measured using an appropriate wavelength by the ELISA instrument. The higher the color intensity within the well's solution, the less drug is present for that particular well's sample. The weaker a color is within a sample's well for a particular drug/drug class, the more drug is present.

Individual tests for a particular class of drug are termed "assays" and are saved as '*.asy' files. Assays can be set up with different cutoff values, as needed. When assays are grouped together and analyzed at the same time, that grouping is called a 'Worklist.' For this procedure, worklists have been designed for samples using a single level calibrator: a cut-off calibrator. When analyzing a Worklist the cut-off calibrator for each particular analyte is analyzed along with case samples, along with a positive control and a negative control for each matrix.
{Note: For this procedure – calibrator, cut-off, and cut-off calibrator are all synonyms}

4. Specimens

This procedure has been validated for blood specimens, but urine sample instructions have been added so that they may be analyzed after appropriate validation. A minimum of 0.5 mL of blood or 1.5 mL of urine is needed to perform all assays. In cases where sample volume is limited it is possible to perform a subset of assays on smaller amounts of sample, but a Lead analyst or higher must be consulted prior to analyzing any small-volume evidence.

5. Equipment/Materials/Reagents

5.1. Neogen Corporation (Lexington, KY) Forensic ELISA Kits:
Each kit should include microwell plates, drug-enzyme conjugate, substrate reagent, and enzyme-immunoassay (EIA) buffer solution. The substrate and buffer are not kit specific and may be interchanged as long as the expiration dates have not passed. The drug enzyme conjugate may need to be diluted with EIA buffer prior to use.

- 6-Acetylmorphine (aka: 6-AM or 6-MAM) (Item #: 134015 or 1304019)
- Amphetamine (Item #: 130815 or 130819)
- Barbiturates (Item #: 130615 or 130619)
- Benzodiazepines (Item #: 130115 or 130119)
- Buprenorphine (Item #: 131915 or 131919)
- Cannabinoids/THC (Item #: 131015 or 131019)
- Cocaine/Benzoylecgonine (Item #: 130315 or 130319)
- Fentanyl (Item #: 131515 or 131519)

- Methamphetamine/MDMA (Item #: 130915 or 130919)
- Methadone (Item #: 131615 or 131619)
- Opiates (Item #: 130415 or 130419)
- Oxycodone/Oxymorphone (Item #: 130715 or 130719)
- Phencyclidine (PCP) (Item #: 130515 or 130519)
- Zolpidem (Item #: 132615 or 132619)

5.2. Dynex DSX Immunoassay Instrument (or equivalent)

5.3. Adjustable volumetric pipettes with appropriate disposable tips

5.4. Vortex mixer

5.5. Routine laboratory supplies

5.6. Calibrator vials (Dynex Item #65940) with caps

5.7. DSX Sample Tips (Dynex Item #65910)

5.8. DSX Reagent Tips (Dynex Item #65920)

5.9. Reagent tubes (Dynex Item #65950)

5.10. Disposable culture tubes with caps (12 x 75 mm or equivalent)

5.11. Specimen diluent (Neogen Corporation (Lexington, KY))

5.12. Methanol (HPLC grade or better)

5.13. Deionized water (Millipore(18+ MΩ grade) or equivalent)

6. Standards and Controls

6.1. Negative Whole Blood Control:
Obtained from Red Cross, OCME, or equivalent – or purchased from a suitable commercial source. Stability and storage can be determined by appropriate source.

6.2. Negative Urine Control:
Obtained in-house, from OCME, or purchased. Store refrigerated or obtain fresh. Stable for at least 2 years when refrigerated.

6.3. 6-Acetylmorphine (6-AM or 6-MAM) Stock Standard (0.1 mg/mL or 1 mg/mL):
Can be purchased from Cerilliant Corp. (or equivalent) or made up fresh from a reference material/standard. Stability and storage determined by manufacturer.

6.4. Amphetamine Stock Standard (1 mg/mL):
Can be purchased from Cerilliant Corp. (or equivalent) or made up fresh from a reference material/standard. Stability and storage determined by manufacturer.

6.5. Barbiturates (Secobarbital) Stock Standard (1 mg/mL):
Can be purchased from Cerilliant Corp. (or equivalent) or made up fresh from a reference material/standard. Stability and storage determined by manufacturer.

6.6. Benzodiazepine (Oxazepam) Stock Standard (1 mg/mL):
Can be purchased from Cerilliant Corp. (or equivalent) or made up fresh from a reference material/standard. Stability and storage determined by manufacturer.

6.7. Buprenorphine Stock Standard (0.1 mg/mL):
Can be purchased from Cerilliant Corp. (or equivalent) or made up fresh from a reference material/standard. Stability and storage determined by manufacturer.

6.8. Cannabinoids (11-nor-9-Carboxy-Δ^9-Tetrahydrocannabinol ; aka: COOH-THC) Stock Standard (1 mg/mL):
Can be purchased from Cerilliant Corp. (or equivalent) or made up fresh from a reference material/standard. Stability and storage determined by manufacturer.

6.9. Benzoylecgonine (BE, BZE) Stock Standard (1 mg/mL):
Can be purchased from Cerilliant Corp. (or equivalent) or made up fresh from a reference material/standard. Stability and storage determined by manufacturer.

6.10. Diphenhydramine Stock Standard (1 mg/mL):
Can be purchased from Cerilliant Corp. (or equivalent) or made up fresh from a reference material/standard. Stability and storage determined by manufacturer.

6.11. Fentanyl Stock Standard (0.1 mg/mL or 1 mg/mL):
Can be purchased from Cerilliant Corp. (or equivalent) or made up fresh from a reference material/standard. Stability and storage determined by manufacturer.

6.12. Methamphetamine Stock Standard (1 mg/mL):
Can be purchased from Cerilliant Corp. (or equivalent) or made up fresh from a reference material/standard. Stability and storage determined by manufacturer.

6.13. Methadone Stock Standard (1 mg/mL):
Can be purchased from Cerilliant Corp. (or equivalent) or made up fresh from a reference material/standard. Stability and storage determined by manufacturer.

6.14. Opiates (Morphine) Stock Standard (1 mg/mL):
Can be purchased from Cerilliant Corp. (or equivalent) or made up fresh from a reference material/standard. Stability and storage determined by manufacturer.

6.15. Oxycodone Stock Standard (1 mg/mL):
Can be purchased from Cerilliant Corp. (or equivalent) or made up fresh from a reference material/standard. Stability and storage determined by manufacturer.

6.16. Phencyclidine (PCP) Stock Standard (0.1 mg/mL or 1 mg/mL):
Can be purchased from Cerilliant Corp. (or equivalent) or made up fresh from a reference material/standard. Stability and storage determined by manufacturer.

6.17. Zolpidem Stock Standard (0.1 mg/mL or 1 mg/mL):
Can be purchased from Cerilliant Corp. (or equivalent) or made up fresh from a reference material/standard. Stability and storage determined by manufacturer.

6.18. Blood Working Drug Solutions (for both Cut-Off and Pos. Ctrl. Blood Solutions):
For the blood working drug solutions (1-4), add the appropriate reference standard solution (see Table 1) via pipette into separate 10 mL volumetric flasks and bring to volume with methanol. Invert to mix and transfer solution into a container (e.g., glass screw cap vial). This will make four (4) working solutions which will be used for preparing the blood cut-off and positive control solutions. Freeze all solutions when not in use.

Table 1: Guide to Preparing Working Drug Solutions for Blood

Name of Solution	Drug	Reference Standard Solution (mg/mL)	Volume of Reference Std. Sol'n (µL)	Final Volume of MeOH (mL)	Working Drug Solution Concentration (µg/mL)
Working Drug Solution #1 (for Blood)	Amphetamine	1	20	10	2
	Secobarbital	1	40	10	4
	Oxazepam	1	10	10	1
	Morphine	1	10	10	1
Working Drug Solution #2 (for Blood)	Buprenorphine	0.1	10	10	0.1
	Oxycodone	1	10	10	1
	Zolpidem	0.1	50	10	0.5
Working Drug Solution #3 (for Blood)	BE	1	20	10	2
	Methadone	1	10	10	1
	Methamphetamine	1	20	10	2
	11-nor-9-carboxy-Δ^9-THC	1	10	10	1
Working Drug Solution #4 (for Blood)	6-AM	0.1	50	10	0.5
	Fentanyl	0.1	10	10	0.1
	Phencyclidine	0.1	50	10	0.5

Table 2: Guide to Preparing Cut-Off Blood Solutions

Name of Solution	Drug	Volume of Working Drug Solution to be added (µL)	Spiked Blood Concentration (ng/mL)
Cut-Off Solution #1 (for Blood)	Amphetamine	10	20
	Secobarbital		40
	Oxazepam		10
	Morphine		10
Cut-Off Solution #2 (for Blood)	Buprenorphine	10	1
	Oxycodone		10
	Zolpidem		5
Cut-Off Solution #3 (for Blood)	BE	10	20
	Methadone		10
	Methamphetamine		20
	11-nor-9-carboxy-Δ^9-THC		10
Cut-Off Solution #4 (for Blood)	6-AM	10	5
	Fentanyl		1
	Phencyclidine		5

Table 3: Guide to Preparing Positive Control Blood Solutions

Name of Solution	Drug	Volume of Working Drug Solution to be added (µL)	Spiked Blood Concentration (ng/mL)
Pos. Ctrl. Solution #1 (for Blood)	Amphetamine	100	200
	Secobarbital		400
	Oxazepam		100
	Morphine		100
Pos. Ctrl. Solution #2 (for Blood)	Buprenorphine	100	10
	Oxycodone		100
	Zolpidem		50
Pos. Ctrl. Solution #3 (for Blood)	BE	100	200
	Methadone		100
	Methamphetamine		200
	11-nor-9-carboxy-Δ^9-THC		100
Pos. Ctrl. Solution #4 (for Blood)	6-AM	100	50
	Fentanyl		10
	Phencyclidine		50

6.19. Blood Cut-Off Solutions (aka: Blood Calibrator Solutions) –
For the blood cut-off [calibrator] solutions (1-4), add the appropriate Working Drug Solutions (see Table 2) into separate appropriate containers (i.e., test tubes) along with 990 µL of blank whole blood. Cap and vortex sufficiently to mix. This will make four (4) cut-off blood solutions.
While appropriate types of blood should be used to match the samples being tested (i.e., ante mortem versus post mortem), ante mortem blank blood is acceptable for all solutions when necessary. Freeze all Working Drug solutions when not in use.

6.20. Blood Positive Control Solutions –
For the blood cut-off [calibrator] solutions (1-4), add the appropriate Working Drug Solutions (see Table 2) into separate containers (e.g., glass screw cap vial) along with 900 µL of blank whole blood. Cap and vortex sufficiently to mix. This will make four (4) positive control blood solutions.
While appropriate types of blood should be used to match the samples being tested (i.e., ante mortem versus post mortem), ante mortem blank blood is acceptable for all solutions when necessary. Freeze all Working Drug solutions when not in use.

6.21. Urine Working Drug Solutions (for both Cut-Off and Pos. Ctrl. Blood Solutions):
For the urine working drug solutions (1-4), add the appropriate reference standard solution (see Table 4) via pipette into separate 2 mL volumetric flasks and bring to volume with methanol. Invert to mix and transfer solution into a container (e.g., glass screw cap vial). This will make four (4) working solutions which will be used for preparing the urine cut-off and positive control solutions. Freeze all solutions when not in use.

6.22. Urine Cut-Off Solutions (aka: Blood Calibrator Solutions) –
For the blood cut-off [calibrator] solutions (1-4), add the appropriate Working Drug Solutions (see Table 5) into separate containers (e.g., glass screw cap vial) along with 990 µL of blank urine. Cap and vortex sufficiently to mix. This will make four (4) cut-off urine solutions. Freeze all solutions when not in use.
While appropriate types of urine should be used to match the samples being tested (i.e., ante mortem versus post mortem), ante mortem blank urine is acceptable for all solutions when necessary.

6.23. Urine Positive Control Solutions –
For the blood cut-off [calibrator] solutions (1-4), add the appropriate Working Drug Solutions (see Table 6) into separate containers (e.g., glass screw cap vial) along with 900 µL of blank urine. Cap and vortex sufficiently to mix. This will make four (4) positive control urine solutions. Freeze all solutions when not in use.
While appropriate types of urine should be used to match the samples being tested

(i.e., ante mortem versus post mortem), ante mortem blank urine is acceptable for all solutions when necessary.

Table 4: Guide to Preparing Working Drug Solutions for Urine

Name of Solution	Drug	Reference Standard Solution (mg/mL)	Volume of Reference Std. Sol'n (µL)	Final Volume of MeOH (mL)	Working Drug Solution Concentration (µg/mL)
Working Drug Solution #1 (for Urine)	Amphetamine	1	100	2	50
	Secobarbital	1	40	2	20
	Oxazepam	1	40	2	20
	Morphine	1	60	2	30
Working Drug Solution #2 (for Urine)	Buprenorphine	0.1	10	2	0.5
	Oxycodone	1	2	2	1
	Zolpidem	1	2	2	1
Working Drug Solution #3 (for Urine)	BE	1	60	2	30
	Methadone	1	60	2	30
	Methamphetamine	1	60	2	30
	11-nor-9-carboxy-Δ^9-THC	1	10	2	5
Working Drug Solution #4 (for Urine)	6-AM	1	2	2	1
	Fentanyl	1	2	2	1
	Phencyclidine	1	5	2	2.5

Table 5: Guide to Preparing Cut-Off Urine Solutions

Name of Solution	Drug	Volume of Working Drug Solution to be added (µL)	Spiked Urine Concentration (ng/mL)
Cut-Off Solution #1 (for Urine)	Amphetamine	10	500
	Secobarbital		200
	Oxazepam		200
	Morphine		300
Cut-Off Solution #2 (for Urine)	Buprenorphine	10	5
	Oxycodone		10
	Zolpidem		10
Cut-Off Solution #3 (for Urine)	BE	10	300
	Methadone		300
	Methamphetamine		300
	11-nor-9-carboxy-Δ^9-THC		50
Cut-Off Solution #4 (for Urine)	6-AM	10	10
	Fentanyl		10
	Phencyclidine		25

Table 6: Guide to Preparing Positive Control Urine Solutions

Name of Solution	Drug	Volume of Working Drug Solution to be added (µL)	Spiked Urine Concentration (ng/mL)
Pos. Ctrl. Solution #1 (for Urine)	Amphetamine	100	5000
	Secobarbital		2000
	Oxazepam		2000
	Morphine		3000
Pos. Ctrl. Solution #2 (for Urine)	Buprenorphine	100	50
	Oxycodone		100
	Zolpidem		100
Pos. Ctrl. Solution #3 (for Urine)	BE	100	3000
	Methadone		3000
	Methamphetamine		3000
	11-nor-9-carboxy-Δ^9-THC		500
Pos. Ctrl. Solution #4 (for Urine)	6-AM	100	100
	Fentanyl		100
	Phencyclidine		250

7. Calibration

This procedure is calibrated each time it is used by analyzing cut-off calibrators and a set of two controls (negative and positive) for each assay. Cut-off calibrators are analyzed in duplicate. This procedure is not designed to be quantitative, but can be used to estimate the amount of drug present for purposes of directing later confirmation and quantitation testing.

The following criteria must be met for the calibration of each assay to be considered in control:

- The optical density values of the cutoff calibrator solutions must be less than the optical density values of the negative control solutions.
- The optical density values of the cutoff calibrator solutions must be greater than the optical density values of the positive control solutions.

8. Sampling

Not applicable.

9. Procedure

9.1. Allow all ELISA kit components to be used to come to room temperature. Note: keep any plate well strips sealed in the zipper lock pouch (with desiccant) at all times prior to use.

9.2. Dilute the negative blood controls, blood cutoff calibrator solutions, positive blood control solutions, and blood samples 1:5 with EIA dilution buffer (e.g., 200 µL of blood along with 800 µL of EIA dilution buffer) into glass culture tubes (i.e., 12 x 75 mm) and vortex to mix. Prepare evidential blood samples in duplicate.

9.3. Turn on the instrument using the power switch on the right side of the instrument. Note: the instrument can be operated with the cover down or with the safety interlock key inserted with the cover up.

9.4. With the computer booted to the Windows screen, double click on the "Revelation DSX" icon.

9.5. Check all solutions and connections, follow software prompts, and start the instrumental analysis.

9.6. When the system is ready to be shut down, the following steps should be taken:

- Empty the waste tip container.

- Empty the liquid waste container, as needed.
- Remove plates from plate trays.
- Refill reagent tips, sample tips, and clean the pipettor.
- Turn off power to instrument.
- Shut down computer.

Table 7: Typical Plate Layout Example (Three-Strip Analysis)

Negative Control	Sample #03 (repeat)	Sample #07 (repeat)
Cut-Off (initial)	Sample #04 (initial)	Sample #08 (initial)
Positive Control	Sample #04 (repeat)	Sample #08 (repeat)
Sample #01 (initial)	Sample #05 (initial)	Sample #09 (initial)
Sample #01 (repeat)	Sample #05 (repeat)	Sample #09 (repeat)
Sample #02 (initial)	Sample #06 (initial)	Sample #10 (initial)
Sample #02 (repeat)	Sample #06 (repeat)	Sample #10 (repeat)
Sample #03 (initial)	Sample #07 (initial)	**Cut-Off (repeat)**

10. Instrumental Parameters

The inserts with each commercial kit are used as guides for the instrumental procedures. Some changes have been made to deal with matrix issues or to improve limits of detection beyond manufacturer specifications. Most instrumental parameters (e.g., workspace coordinates, pipette arm alignment) are set by the manufacturer during service visits and will not be changed by users. Other parameters (e.g., sample positions, additional controls) will be set during sequence preparation and will depend on the number of samples and desired assays to analyze.

11. Decision Criteria

A specimen is considered to give a positive response for a particular drug class/analyte if the optical density reading for that sample is less than or equal to the optical density reading for the cutoff calibrator for that drug class/analyte. Due to possible matrix effects, any specimen that gives an optical density reading close (e.g., within 10%) to the respective cutoff calibrator may be considered a 'positive' result.

In instances wherein optical density values for samples approach those values for cutoff solutions, yet are still below the negative control's values and are outside the 10% window

(see above), such samples can be considered 'possibly positive.' However, all 'possibly positive' results will still be reported as negative (i.e., non-positive) within this procedure.

If further analyses are needed for non-positive results, analysts will seek guidance from a Lead analyst or higher. If a specimen is deemed positive for a particular drug class, a separate analysis utilizing a different analytical principle should be performed in order to confirm the positive immunoassay result(s).

12. Calculations

Not applicable.

13. Uncertainty of Measurement

Not applicable.

14. Limitations and Quality Assurance

14.1. Limit of Detection (LOD): The limits of detection for the following drugs have been shown to be the values that follow. Some of these may have been administratively set and could be lower than the concentration value actually listed within Table 7.

14.2. Specificity : A listing of cross reactivities of various drugs and metabolites can be found within each kit insert.

14.3. Matrix effects are a known limitation with regards to ELISA testing. Calibrators and controls should be matrix matched (i.e., prepared in post mortem blood if analyzing post mortem specimens). Matrix-matching the calibrator and control solutions helps to minimize the impact of matrix effects.

14.4. Reference drug standards may be used which have different concentrations than what are listed within the procedure. When this happens analysts need to change dilutions, as needed, in order for concentration values to correlate with those listed within the tables.

14.5. All kit contents, calibrators, and controls should be kept refrigerated when not in use.

14.6. Do not shake or vortex conjugate. In order to mix the conjugate solutions simply invert, do not shake the solutions.

14.7. Remove any large bubbles from any solutions that have been loaded into the instrument. This needs to be done because bubbles can interfere with the level-sensing capabilities of the instrument. The presence of bubbles can result in what's called a missed pipette with little-to-no sample getting analyzed.

14.8. Expired kit components should not be used. The Neogen K-Blue substrate, EIA dilution buffer, 10x wash concentrate, and sulfuric acid stop solution can all be

shared between kits so long as the expiration dates on the reagent and parent kits have not lapsed.

14.9. All assays have been developed to utilize the Neogen EIA dilution buffer and H_2SO_4 stop solution regardless of assay kit type.

14.10. Only aliquot sufficient reagent volumes for analysis. Do not return excess reagents to stock containers.

14.11. Once diluted, the wash concentrate has a shelf life of five (5) days at room temperature.

Table 8: Blood and Urine Limits of Detection (aka: Cut-off/Calibrator Concentrations)

Drug Analyzed (Assay Class)	Blood Cutoff Concentrations (ng/mL)	Urine Cutoff Concentrations (ng/mL)
6-Acetylmorphine (6-AM)	5	10
Amphetamine (Amphetamines)	20	50
Secobarbital (Barbiturates)	40	200
Oxazepam (Benzodiazepines)	10	200
Buprenorphine	1	5
11-nor-9-carboxy-Δ^9-THC (Cannabinoids)	10	50
Benzoylecgonine (BE ; Cocaine-related)	20	300
Fentanyl	1	10
Methamphetamine (MDMA-related)	20	300
Methadone	10	300
Morphine (Opiates)	10	300
Oxycodone	10	10
Phencyclidine (PCP-related)	5	25
Zolpidem	5	10

15. Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *DSS General Laboratory (GL) SOPs* regarding Laboratory Safety for further guidance.

16. References

Dynex User Manual

Neogen package inserts for each assay (Neogen Corporation, Lexington, KY)

Example of Maintenance Log

ELISA (DSX) Maintenance Log **Month:_____ Year: _____**

Daily Maintenance									
Initial & Date →									
Verify – Self Test Passed ☑									
Fill Wash Bottle-A with Diluted Neogen Wash Buffer (if needed) ☑									
Fill Bottle D with DI Water (if needed) ☑									
Empty Waste Tip Container ☑									
Run Wash Check/Maintenance Protocol ☑									
End Work:									
Wipe Pipettor with 70% Alcohol Sol'n ☑									
Empty Waste Tip Container ☑									
Clean/Disinfect ELISA Instrument Work surface with 70% Alcohol Sol'n ☑									
Empty and Clean the Liquid Waste Container (as needed) ☑									
Weekly Maintenance									
Remove Diluted Wash Buffer and DI Water from Bottles A & D ☑									
Rinse Tanks 3-5 Times with DI Water ☑									
Replace Wash and DI Water in Bottles ☑									
Wipe Pipettor with 70% Alcohol Sol'n ☑									
Refill Bottles with Diluted Wash Buffer in Bottle A ☑									
Refill Bottles with DI Water in Bottle D ☑									
Disinfect Tip Waste and Liquid Waste Container with 70% Alcohol Sol'n (If 10% Bleach Sol'n is Used, Rinse 5x with DI Water) ☑									
Monthly Maintenance:									
Initial & Date →									
Disinfect Tip Waste and Liquid Waste Container with 70% Alcohol Sol'n (if 10% Bleach is used, Rinse 5x with DI Water)☑									

Rev. #	History
01	New document
02	Section 2 - changed 15 to 14 and removed diphenhydramine from entire document. Section 5.11, removed balance from equipment list. Allowed for 0.1 mg/mL concentration to be used for the following reference standard solutions: 6-MAM, fentanyl, PCP, Zolpidem (updated table 1 and table 2 accordingly). Increased secobarbital from 2.5 µg/mL to 4 µg/mL (and volume from 25 µL to 40 µL) within Table 1 and Table 2. Changed spiked blood concentration for secobarbital in Table 3 to 400 ng/mL. Made corrections in 6.22 and 6.23 (blood to urine). Updated section 7 – removed 'and positive controls' and 'average.' Updated section 9.2. Removed specific instructions (9.5 – 9.13). Updated section 9.14 for clarity. Removed section 9.15. Updated section 10. Grammatical change made to sections 14.7 and 14.8.