Section 1.0
Blood and Urine Toxicology

1.2 Enzyme Immunoassay Screening for Drugs-of-Abuse in Blood

1.2.1 BACKGROUND

Refer to Analytical Method 1.1.

1.2.2 SCOPE

This analytical method employs EMIT for the qualitative screening for drugs-of-abuse in blood specimens. EMIT is commonly used for the detection of drugs-of-abuse in urine. EMIT, when applied to forensic whole blood testing, requires an extraction procedure prior to analysis. The EMIT assays will be run on a microprocessor controlled automatic chemistry analyzer, the VIVA-E®. This method has limitations in that the data may have to be evaluated to determine whether to proceed with confirmatory testing rather than relying solely on the assay cut-off as the indicator. The instrument will be operated in accordance with manufacturer's guidelines. There are no specific or unique safety concerns for this method. Universal precautions will be applied while processing blood. Each assay has an established administrative threshold or cut-off, the cut-offs employed are indicated in the following table.

Assay	***Calibrator***	***Blood Cut-off***
Amphetamines	d-Methamphetamine	200ng/mL
Benzodiazepine	Lormetazepam	100ng/mL
Cannabinoid	11-Nor-9-Carboxy-THC	20ng/mL
Cocaine Metabolite/-M	Benzoylecgonine (BECG)	100ng/mL
Methadone	Methadone	100ng/mL
Opiate	Morphine	200ng/mL

1.2.3 EQUIPMENT AND SUPPLIES

1.2.3.1 Viva-E™ Analyzer
1.2.3.2 Air-displacement pipettes and appropriate tips.
1.2.3.3 Disposable 16 x 100mm round bottom glass screw-top tubes
1.2.3.4 Screw Cap for 16mm O.D. tubes
1.2.3.5 Disposable polyethylene pipettes
1.2.3.6 Disposable 1 mL plastic specimen cups
1.2.3.7 Disposable 13X75 polypropylene tubes
1.2.3.8 15mL HDPE Bottle and 30mL HDPE Bottle (EMIT® Reagents)
1.2.3.9 Tube Rocker
1.2.3.10 Vortex Mixer
1.2.3.11 Evaporative concentrator equipped with compressed nitrogen.

1.2.4 REAGENTS

1.2.4.1 DI water
1.2.4.2 Isopropanol (ACS Grade)
1.2.4.3 n-Butylchloride (ACS Grade)
1.2.4.4 Methanol (ACS Grade)
1.2.4.5 Hydrochloric Acid (ACS Grade)
1.2.4.6 Syva EMIT® II Plus Assay Kits
1.2.4.7 Assay Reagents

Manufacturer Provided

- 0.1N Hydrochloric Acid (Cleaning Solution A)
- 0.1N Sodium Hydroxide (Cleaning Solution B)
- System Solution
- Sodium Hypochlorite (Needle Rinse)
- 0.825M Tris-HCl Buffer Concentrate-pH 8.0

For working buffer, place buffer concentrate into a 200mL volumetric flask. QS to volume with DI water. Buffer solution may be used for 12 weeks when stored at room temperature.

1.2.5 REFERENCE MATERIAL

1.2.5.1 Stock Reference Material Solutions

Obtain 1mg/mL (1000ng/µL) Benzoylecgonine, Lormetazepam, Methadone, d-Methamphetamine, Morphine, and 100µg/mL (100ng/µL) 11-nor-9-THC-Δ^9-COOH. Vendor provided ***Certificate of Analysis*** must be stored centrally.

1.2.5.2 Working Solutions

1.2.5.2.1 **Working *Calibrator* Solution**

Add ≅9mL methanol to 10mL volumetric flask. Add the amount of methanolic reference material indicated in the following table. QS to 10mL with methanol.

Compound	Volume (µL)	Final Concentration (ng/µL)
Benzoylecgonine	50	5
Lormetazepam	50	5
Methadone	50	5
d-Methamphetamine	50	5
Morphine	50	5
11-Nor-9-Carboxy-Δ9-THC	100	1

Record lot numbers of stock reference material on

reagent log. Solution is stable for 12 months when stored in freezer.

1.2.5.2.2 **Working High *Control* (Level 5) Solution**

Reference material used to prepare control should be from a different source that used for calibrator reference material whenever possible.

Add ≅9mL methanol to 10mL volumetric flask. Add the amount of methanolic reference material indicated in the following table. QS to 10mL with methanol.

Compound	Volume (µL)	Final Concentration (ng/µL)
Benzoylecgonine	100	10
Lormetazepam	100	10
Methadone	100	10
d-Methamphetamine	250	25
Morphine	200	20
Carboxy-Δ9-THC	200	2

Record lot numbers of stock reference material on reagent log. Solution is stable for 12 months when stored in freezer.

1.2.5.2.3 **Working Additional *Control* Solution**

Add ≅9mL methanol to 10mL volumetric flask. Add the amount of methanolic reference material indicated in the following table. QS to 10mL with methanol.

Compound	Volume (µL)	Final Concentration (ng/µL)
Benzoylecgonine	50	5
Lormetazepam	50	5
Methadone	50	5
d-Methamphetamine	50	5
Morphine	50	5
Carboxy-Δ9-THC	50	0.5

Record lot numbers of stock reference material on reagent log. Solution is stable for 12 months when stored in freezer.

1.2.6 ASSAY SET-UP

1.2.6.1 Blood Cut-off Calibrators

For application of EMIT assays, the whole blood application must parallel the urine assay set-up. The following blood cut-off calibrators are prepared so that the blood calibrators contain the same analytes as the corresponding urine cut-off calibrator (as shown in table below). The same lot of negative blood used to prepare the negative control will be used to prepare calibrators.

Assay	*Calibrator*	*Cal. No.*	*Blood Cut-off (ng/mL)*
Amphetamines	d-Methamphetamine	2	200
Benzodiazepine	Lormetazepam	4	100
Cannabinoid	Carboxy-THC	3	20
Cocaine-M	Benzoylecgonine	3	100
Methadone	Methadone	3	100
Opiate	Morphine	1	200

1.2.6.1.1 Calibrator 1 (200ng/mL)

To 1mL of negative whole blood, add 40µL working reference material.

1.2.6.1.2 Calibrator 2 (200ng/mL)

To 1mL of negative whole blood, add 40µL working reference material.

1.2.6.1.3 Calibrator 3 (20ng/mL or 100ng/mL)

To 1mL of negative whole blood, add 20µL working reference material.

1.2.6.1.4 Calibrator 4 (100ng/mL)

To 1mL of negative whole blood, add 20µL working reference material.

1.2.6.2 Blood Controls

Use the same lot of negative blood used to prepare the negative control to make high control.

1.2.6.2.1 High Control

To 1mL of negative whole blood, add 20µL of high control stock solution. The following table shows resulting concentrations.

Assay Controls for:	*Calibrator*	*Final Conc. (ng/mL)*
Amphetamines	d-Methamphetamine	500
Benzodiazepine	Lormetazepam	200
Cannabinoid	Carboxy-THC	40
Cocaine-M	Benzoylecgonine	200
Methadone	Methadone	200
Opiate	Morphine	400

1.2.6.2.2 Blood Additional Control Preparation
(- 25% and +50% of Assay Cut-off)

Additional Quality Controls Benzodiazepine, Cocaine-M & Methadone Assays 100ng/mL Cut-off		
	25% Below	50% Above
Concentration (ng/mL)	75	150
Working Additional Controls Solution: 5ng/µL		
Add the following volumes to 1mL each of whole blood:	15µL	30µL

Additional Quality Controls Amphetamine & Opiate Assays 200ng/mL Cut-off		
	25% Below	50% Above
Concentration (ng/mL)	150	300
Working Additional Controls Solution: 5ng/µL		

Additional Quality Controls Cannabinoid Assay 20ng/mL Cut-off		
	25% Below	50% Above
Concentration (ng/mL)	15	30
Working Additional Controls Solution: 0.5ng/µL		
Add the following volumes to 1mL each of whole blood:	30µL	60µL

1.2.6.2.3 Negative Control

Add 1mL negative whole blood.

1.2.7 ANALYZER QUALITY CONTROL

1.2.7.1 Calibration

1.2.7.1.1 Prior to each screening run, the Viva-E must be calibrated with calibrators prepared along with controls and case samples.

1.2.7.2 Pre-run Controls

1.2.7.2.1 To confirm that the analyzer is properly calibrated for each assay, controls are analyzed and evaluated. Following calibration a minimum of one Negative and High Positive blood control must be run.

1.2.7.2.2 Appropriate control responses are:

- Level 0/Negative Control indicating negative response.
- Level 5/High Control indicating positive response.

1.2.7.2.3 If controls fail, they may be re-run. If a control continues to fail, the instrument must be recalibrated.

1.2.7.3 In-run Controls

1.2.7.3.1 In each casework analysis run, a minimum of the following controls must be included in rotor sample positions. Preparation is described in section 1.2.6.

1. Negative Control
2. High Positive Control
3. Benzodiazepine, Cocaine-M & Methadone -25% Control
4. Benzodiazepine, Cocaine-M & Methadone +50% Control
5. Amphetamines, Opiate & Cannabinoids -25% Control
6. Amphetamines, Opiate & Cannabinoids +50% Control

1.2.8 BLOOD EXTRACTION PROCEDURE

1.2.8.1 Initial Set-up

Label two extraction tubes for each positive controls, negative controls and case samples.

1.2.8.2 Sample Preparation

1.2.8.2.1 Add 3mL n-Butylchloride:Isopropanol (1:1) to extraction tube.

1.2.8.2.2 While vortexing, slowly add 1mL blood unknown, blood calibrator, positive blood control **or** negative blood control.

1.2.8.2.3 After blood is added, vortex an additional 30 seconds.

1.2.8.2.4 Allow tube to rock for 10 minutes.

1.2.8.2.5 Vortex tube for approximately 10 seconds.

1.2.8.2.6 Centrifuge tube at 3200rpm for 5 minutes.

1.2.8.2.7 Transfer supernatant to an additional pre-labeled tube.

1.2.8.2.8 Add 2-3 drops 1% HCl in Methanol.

1.2.8.2.9 Evaporate to dryness under nitrogen in evaporator.

1.2.8.2.10 Reconstitute residue in 300µL EMIT buffer.

1.2.8.2.11 Vortex tube for approximately 15 seconds.

1.2.8.2.12 Allow tube to sit for 10 minutes.

1.2.8.2.13 Transfer extract to sample cup for analysis.

1.2.8.3 Viva-E™ Instrumental Analysis

Refer to current Viva-E™ Operation Guide and manual.

1.2.9 INTERPRETATION OF ASSAY RESULTS

1.2.9.1 Positive Result

A positive result for a sample is indicated by the rate (dABS/m) that is equal or greater than the cut-off calibrator. At the discretion of the analyst, results at or near the minus 25% of cut-off control may also be sent forth for confirmatory testing.

1.2.9.2 Negative Result

A negative result for a sample will routinely be considered an absorbance rate of less than the cut-off calibrator, however, this is at the discretion of the analyst based upon the criteria in 1.2.9.1.

1.2.10 RUN ACCEPTANCE CRITERIA

1.2.10.1 Assay Controls

1.2.10.1.1 **High Positive Control Response**

High positive controls must indicate a positive result with an absorbance/rate comparable with previous data.

1.2.10.1.2 **Negative Control Response**
Negative controls must indicate a negative result with an absorbance/rate comparable with previous data.

1.2.11 DISTRIBUTION OF ASSAY INFORMATION

1.2.11.1 Original EMIT analysis report must be included in case file.

1.2.11.2 A copy of assay results for calibrators and controls need not be included in individual case files.

1.2.11.3 Original data for calibration and controls for each analysis must be stored centrally in the laboratory where the analysis was performed until archiving, or destruction.

1.2.12 QUALITY ASSURANCE REQUIREMENTS

1.2.12.1 Blood samples are to be stored under refrigeration.

1.2.12.2 Refer to toxicology manual section 5.1 for pipette intermediate check and calibration requirements.

1.2.12.3 Refer to toxicology manual section 5.10 for authentication of reference material requirements.

1.2.13 REFERENCES

1.2.13.1 Thompson, S.G., *Principles for Competitive-Binding Assays. in:* Clinical Chemistry: Theory, Analysis, Correlation, edited by Kaplan, L.A., Pesce, A.J. and Kazmierczak, S.C., pp. 246-260, Mosby, 2003.

1.2.13.2 Hand, C. and Baldwin, D., *Immunoassays in:* Clarke's Analytical Forensic Toxicology, edited by Jickells, S. and Negrusz, A., pp. 375-391, Pharmaceutical Press, 2008.

1.2.13.3 E.M.I.T. Blood Screening Procedure, Montana Department of Justice Forensic Sciences Division, courtesy of Jim Hutchison, April 2008.

1.2.13.4 Enzyme Multiplied Immunoassay (EMIT) Enzymatic Assays for Drug Screening in Urine, Whole Blood Extracts and Other Biological Fluids, Washington State Toxicology Laboratory, Courtesy of Melissa Pemberton, August 2008.

1.2.13.5 Viva-E™ Operator's Manual, Article No.: 6002-380-410-01, Version number: 1.0/08-04

1.2.13.6 Viva-E™ System Operations Guide, T216, 6/4/07, D01320.

Revision History

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1.2 Enzyme Immunoassay Screening for Drugs-of-Abuse in Blood

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