1.0 INTRODUCTION

The Enzyme-Multiplied Immunoassay Technique (EMIT) assay is an immunological screening method. An enzyme that is bound to a drug/analyte is used in the procedure. The enzyme is capable of catalyzing a substrate and indirectly causing a reaction wherein a chemical absorbance change occurs. When an enzyme-labeled drug/analyte is mixed with a sample that already contains the target drug (along with the appropriate substrate and oxidizing agent), the amount of absorbance within the resulting mixture will be proportional to the amount of drug/analyte within the sample. In the detector-part of the instrument, radiation of a fixed intensity is passed through a solution and the transmitted light's intensity is measured and compared. The absorbance of the radiation will be [roughly] proportional to the amount of drug/analyte within the sample. When no drug/analyte is present in a sample, most of the enzyme-labeled drug/analyte is bound to antibodies and the enzyme is rendered inactive. Enzyme-substrate reactions within EMIT cause an indirect absorbance-change event by causing NAD^+ (nicotinamide adenine dinucleotide) to convert to NADH (hydrogenated form). Instrumentation uses monochromatic light to measure the amount of NAD^+ that is converted to its reduced form, NADH. The NADH absorbs ultraviolet light more than NAD^+. The more drug/analyte in the sample, the greater the absorbance of radiation when light is passed through the sample. The technique is considered homogenous since all reagents are mixed together and no rinsing of samples occurs.

Ethyl alcohol can also be analyzed using this instrument. However, the ethanol assay is based on an enzymatic reaction only and not due to an immunological interaction. Alcohol dehydrogenase (ADH) catalyzes the oxidation of ethyl alcohol to acetaldehyde, while NAD^+ is subsequently converted to its NADH reduced form. A change in absorbance (i.e., production of NADH) results when ethanol and ADH react. The increase in absorbance of light is directly proportional to the concentration of ethyl alcohol.

2.0 SCOPE

Enzyme multiplied immunoassay techniques (EMITs) have the advantage of rapidly screening small volumes of biological samples for the presence of drugs. The use of an automated system allows fast and reproducible screening tests. All immunoassay tests are only presumptive and may be subject to false positive or false negative results. For confirmatory purposes, any results obtained through the use of this procedure must be accomplished through the use of a structurally elucidating analytical technique (e.g., gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/mass spectrometry (LC/MS)). Other analytes/classes of drugs may be added to this procedure provided that their reagents/kits have been validated prior to use. This technique is generally geared towards the analysis of urine for the possible presence of drugs and/or ethanol. Blood, serum, or plasma can also be used within this procedure for drug analysis, but additional sample preparation will be needed. The EMIT procedure will not be used for ethanol analysis within blood, serum, or plasma samples.

3.0 PRINCIPLE

In each assay, samples (e.g., urine or blood extract) are mixed with reagents (e.g., Reagent 1 and Reagent 2) in a certain sequence. No washing of solutions is performed (as in ELISA – enzyme-linked immunosorbent analysis) and all the reactions occur within one homogeneous mixture. After a certain amount of time, monochromatic ultraviolet radiation is passed through each solution and intensities are compared in order to determine if any absorbance changes have occurred. Reagent 1 usually contains a substrate, a UV-detection compound, and a drug/antigen-specific antibody. Reagent 2 usually contains an enzyme-labeled drug/antigen.

If a sample is positive (i.e., the solution contains the drug/analyte being tested), antibody/antigen reactions will occur that are specific for the drug/analyte of interest. The majority, if not all, of the antibody sites will be taken up by the sample's drugs/antigens. When Reagent 2 is added to the solution, an enzyme-labeled drug/antigen is introduced. Because all of the antibody sites have been taken, the enzyme-labeled drug/antigen will not bind and will be free-floating in solution. The enzyme-part of the enzyme-labeled drug/antigen will react with free-floating substrate molecules and acts as a catalyst. When an enzyme catalyzes a substrate, the reaction causes NAD^+ to be reduced to NADH. Unlike substrates, enzymes are not consumed so they continue to multiply the reaction by constantly causing NADH to be produced until no more substrate remains. Since NADH absorbs ultraviolet radiation, the instrument will report a relatively high absorption value. The amount of drug in a sample will be proportional to the amount of ultraviolet light that is absorbed by the homogeneous solution.

If a sample is negative (i.e., the solution contains no drug/analytes specific to that particular test), antibody/antigen reactions will not occur when Solution 1 is added to the sample. All of the antibody sites will be free and open. When Reagent 2 is added to the solution, an enzyme-labeled drug/antigen is introduced and will be able to react with free-floating antibodies. As a result the enzyme will not be able to react with the substrate and the light-absorbing agent (NAD^+) will not reduce to NADH. Since NADH absorbs ultraviolet radiation, the amount of ultraviolet light that is absorbed by the homogeneous solution will be minimal and the instrument will report a relatively low absorption value.

Quantitation can occur using EMIT technology when solutions that contain varying known amounts of specific analytes are analyzed contemporaneously with unknown samples. A resulting calibration graph comparing analyte concentration with instrumental absorbance values can used to calculate unknown sample concentrations. Control samples can ensure that quality results are produced from calculations. Within this procedure quantitative data can be obtained from ethanol analyses within urine samples. However, these values are not officially reported and are typically used only for informational purposes when comparing EMIT data to future analytical analyses (i.e., headspace-gas chromatography), if needed.

4.0 SPECIMENS

4.1 Urine: Approximately 650 µL.

4.2 Blood: 1 mL
Within this procedure, serum and plasma will be treated the same as blood with respect to sample preparation/extraction. If limited volumes are encountered, seek guidance from the Lead Examiner and/or the Deputy Director.

4.3 Other matrices: Depends on controls and validation. Examiners should consult the Lead Examiner and/or the Deputy Director prior to analyzing other matrices.

While this procedure has been validated for blood and urine specimens, other matrices may be substituted and may be analyzed. This can be done provided that appropriate controls have been analyzed and, if necessary, appropriate validation has occurred for the particular matrix.

Evidence should be stored refrigerated when not under examination. It is acceptable for an analyst to individually transfer aliquots of samples into temporary containers (e.g., test tubes) even if the EMIT testing will not be performed on the same date. This occurrence should be documented within the case notes and/or on the batch paperwork. Aliquots will be treated in the same manner as the original evidence samples (i.e., prevented from cross-contamination, degradation, and/or sample loss), can remain in the control of the analyst if placed under proper seal, and do not need be sub-itemized. It is acceptable for the analyst, in the process of aliquoting multiple case samples (i.e., creating a batch), to put the boxes of evidence in one convenience container and seal the container for overnight storage in an evidence refrigerator. In such cases the evidence can remain in the analyst's possession according to the chain of custody. In these circumstances all evidence will be considered in the control of the analyst listed within the chain of custody.

Work lists for EMIT can be generated through JusticeTrax (JT). Specimens comprising an analytical batch can be obtained from the JT work list.

Some evidence (e.g., suspected Driving Under the Influence (DUI) cases) is maintained in the Toxicology Unit for a limited time period (e.g., approximately 8 weeks after the last toxicology report for the submission has been released). In the absence of notification from the contributor, or of any legal actions or reason to maintain the samples, such evidence can be discarded in an appropriate medical waste disposal container. Samples from fatality cases (i.e., those cases wherein someone has died as a result of the accident), samples from cases that have pending legal actions, and samples from cases where specific requests have been made for retention of evidence can be held and stored indefinitely, or until directed by appropriate authorities.

5.0 EQUIPMENT / MATERIAL / REAGENTS

5.1 Viva-E EMIT analyzer workstation and related equipment and supplies (e.g., sample cups) (Siemens or equivalent)

5.2 Sorvall ST16 (Thermo Scientific)

5.3 Standard laboratory glassware, plastic ware, and equipment (e.g., test tubes)

5.4 EMIT II Plus Assay Kits: (Siemens or equivalent)

Multidrug Kits	Urine Cut-Off (ng/mL) ; Calibrator/Ctrl Kits
Amphetamine (9C309UL ; 10445420)	1000 (Level L3)
Barbiturates (9D029UL ; 10445422)	200 (Level L3)
Benzodiazepine (9F029UL ; 10445429)	200 (Level L3)
Cocaine (9H029UL ; 10445437)	300 (Level L3)
Methadone (9E029UL ; 10445426)	300 (Level L3)
Opiate (9B309UL ; 10445416)	300 (Level L1)
PCP (9J029UL ; 10445441)	25 (Level L3)
Cannabinoid (9N029UL ; 10445469)	100 (Level L4)
Ethanol (9K309UL ; 10445452)	0.010 – 0.600 g/dL (see 5.7)

5.5 Immunalysis Assay Kits: (Siemens or equivalent)

Kit	Urine Cut-Off (ng/mL) ; Calibrator/Ctrl Kits
Oxycodone (302UR-0100 ; 10991156)	300 (see 5.8)
Buprenorphine (336 UR-0100 ; 10991178)	5 (see 5.8)

5.6 EMIT II Calibrator/Control Kits (Siemens or equivalent)

Level 0: 9A509UL (10445406) Cal 0 Control Kit – Negative Control

Level 1: 9A529UL (10445407) Multidrug Cal 1 Calibrator Kit (used for opiates only)

Level 3: 9A569UL (10445409) Multidrug Cal 3 Calibrator Kit

Level 4: 9A589UL (10445410) Multidrug Cal 4 Calibrator Kit (used for THC only)

Level 5: 9A609UL (10445411) Multidrug Cal 5 Calibrator Kit – Positive Control

5.7 Ethyl Alcohol (Siemens or equivalent)

Negative Calibrator: 9K029UL (10445445)

100mg/dL Calibrator: 9K059UL (10445448)

Low Control (0.036 - 0.044): 9K049UL (10445447)

High Control (0.270 - 0.330): 9K079UL (10445449)

5.8 Immunalysis (Siemens or equivalent)

Calibrator (Oxycodone ; 300): C302UR-10-1-300 (10991160)

Low (Neg.) & High (Pos.) Control (Oxycodone ; 225 & 375): C302UR-10-2-300 (10991161)

Calibrator (Buprenorphine ; 5ng/mL): C336 UR-10-1 (10991180)

Low (Neg.) & High (Pos.) Controls (Buprenorphine ; 3.75 & 6.25): C336 UR-10-2 (10991181)

5.9 Liquichek Urine Toxicology Controls (Bio-Rad or equivalent)

Level S1E: contains target analytes

Level S2E: contains target analytes

Level S3: used for Cannabinoid Only (125ng/mL)

5.10 Reference standard solutions (Cerilliant or equivalent)

5.11 Needle Rinse Solution (Sodium Hypochlorite solution) (ACS Grade or equivalent)

5.12 System Solution (3203-063 ; 10445247) (Siemens or equivalent)
Used to flush EMIT system and avoid bacterial growth
Fill water container with ~10 L deionized water and 25 mL of system solution

5.12 Hydrochloric Acid (0.1N HCl) (ACS Grade or equivalent)

5.13 System Solution (Siemens or equivalent)

5.13 Methanol (MeOH) (ACS Grade or equivalent)

5.14 Acetonitrile (CH_3CN or ACN) (ACS Grade or equivalent)

5.15 Water (Deionized (DIW), Millipore, or equivalent in-house supply)

5.16 Whole blood (Blood bank or equivalent)

5.17 Methanolic hydrochloride (1% MeOH-HCl) – can be prepared by diluting 100 µL of concentrated HCl into 10 mL of methanol. Stable for at least one (1) month at refrigerator temperature

6.0 STANDARDS / CONTROLS

6.1 Working Standard Solution (for blood procedure only)

6.1.1 Mixture of ten (10) analytes in methanol used to make the positive control and calibrator/cut-off blood solutions.

6.1.2 Two (2) sets of the Working Standard Solution should be made:
- One Working Standard Solution should be made and used for preparation of the blood positive control solution.
- The second Working Standard Solution should be made by another Examiner, if available, and should be used for the preparation and the blood calibrator/cut-off solution.

6.1.3 Each Working Standard Solution can be prepared by adding 5mL of MeOH to a volumetric flask (or equivalent), adding the appropriate amounts of drug analytes (see table below), and diluting to volume (10mL) with MeOH.

Table: Summary for preparation of the working standard solutions*

Drug	Reference Standard	Volume of Reference Standard (µL)	Concentration of Working Standard Solution (in 10mL of MeOH) (µg/mL)
Methamphetamine	1.0 mg/mL	50	5
Secobarbital	1.0 mg/mL	5	0.5
Diazepam	1.0 mg/mL	5	0.5
Benzoylecgonine	1.0 mg/mL	10	1
THC	1.0 mg/mL	10	1
Morphine	1.0 mg/mL	20	2
Methadone	1.0 mg/mL	20	1
Phencyclidine (PCP)	1.0 mg/mL	1	0.1
Buprenorphine	100 µg/mL	5	0.05
Oxycodone	1.0 mg/mL	5	0.5

*(Note: Two (2) separate lot preparations of this solution should be made –
One solution used for blood Positive Control and the other used for blood Calibrator/Cut-off)
(Stable when stored in freezer for 1 year (or 1 year from last verification))

6.2 Control Solutions

6.2.1 Negative Control Urine
Level 0: 9A509UL (10445406) Cal 0 Control Kit

6.2.2 Positive Control Urine
Level 5: 9A609UL (10445411) Multidrug Cal 5 Calibrator Kit

6.2.3 System Check Positive Control Urine
These solutions only need to be used when the system is calibrated
Liquichek Urine Toxicology Controls (Bio-Rad):
Level S1E: contains target analytes

Level S2E: contains target analytes
Level S3: used for Cannabinoid Only (125ng/mL)

6.2.4 Negative Control Blood:
Drug-free blood.

6.2.5 Positive Control Blood
Composed of a mixture of ten (10) analytes in blood - prepared from the methanolic Working Standard Solution.
Can be prepared by adding 1mL of negative control blood to a test tube, adding 100µL of the MeOH Working Standard Solution, capping, and mixing well.

Table: Summary for preparation of the blood positive control solution

Drug	**Volume of Working Std. (µL)**	**Concentration of Blood Positive Control (in 1mL of blood) (ng/mL)**
Methamphetamine	100	500
Secobarbital		50
Diazepam		50
Benzoylecgonine		100
THC		100
Morphine		200
Methadone		200
Phencyclidine (PCP)		10
Buprenorphine		5
Oxycodone		50

(Stable when stored in freezer for 1 year (or 1 year from last verification))

6.3 Calibrator/Cut-Off Solutions

6.3.1 Urine Calibrator Solutions
Use EMIT II Calibrator/Control Kits
Level 1: Multidrug Cal 1 Calibrator Kit (used for opiates only)
Level 3: Multidrug Cal 3 Calibrator Kit (everything but opioid and THC)
Level 4: Multidrug Cal 4 Calibrator Kit (used for THC only)

6.3.2 Blood Calibrator Solutions
Composed of a mixture of ten (10) analytes in blood - prepared from the methanolic Working Standard Solution.

Can be prepared by adding 1mL of negative control blood to a test tube, adding 50µL of the MeOH Working Standard solution, capping, and mixing well.

Table: Summary for preparation of the blood calibrator/cut-off Solution

Drug	Volume of Working Std. (µL)	Concentration of Blood Calibrator/Cut-Off (in 1mL of blood) (ng/mL)
Methamphetamine	50	250
Secobarbital		25
Diazepam		25
Benzoylecgonine		50
THC		50
Morphine		100
Methadone		100
Phencyclidine (PCP)		5
Buprenorphine		2.5
Oxycodone		25

(Stable when stored in freezer for 1 year (or 1 year from last verification))

7.0 VALIDATION OF REAGENTS

Validated reagents can be marked with a green dot/sticker which indicates that the reagents used for the specific batch of EMIT samples have been validated. Materials (e.g., controls, calibrators/cut-offs, etc.) are considered validated when all samples produce results as expected.

8.0 PROCEDURE (The following steps can be used for an analysis)

8.1 Instrument Preparation

8.1.1 Perform system refill:

8.1.1.1 Remove the rotor and cuvette covers and open instrument door.

8.1.1.2 Remove all covers from reagents in the reagent rotor.

8.1.1.3 Ensure that needle rinse and HCl bottles are full.

8.1.1.4 Ensure that tubes in positions W and B are filled with water and needle rinse solution appropriately.

8.1.1.5 Inspect wash arms, mixers, and cuvette rotors. Ensure that the system solution containers are filled adequately and that waste containers have enough available space so they don't get full during analyses.

8.1.1.6 Check and ensure that there are adequate amount of reagents present in the reagent bottles prior to initiating instrument operation.

8.1.1.7 Check syringes and tubing for bubbles.
If necessary, tap where needed to remove bubbles.

8.2 Instrument Calibration (Urine)

The EMIT instrument should be calibrated once per week and per manufacturer's instruction. Calibration should be performed after any system change, including reagent refill, solution lot change, or bottle change. Bottles should be changed for new reagent lot numbers. It is important to not overfill bottles. Reagent(s) should be filled only up to the shoulder of the bottles. For urine samples, drugs of abuse calibration utilizes levels 0, 1, 3, and 4 of the Calibrator/Control kits and ethanol calibration utilizes negative and 100mg/dL calibrators.

When calibrator or control solutions do not have enough volume remaining to adequately analyze a batch of samples, it is not recommended for calibrator/control solutions of differing lot numbers to be combined. However, if this needs to happen, the Deputy Director will be consulted and the system should be recalibrated so that the new solution(s) can be validated. Additionally, since these new solutions will no longer be comprised of just one lot number, a new analyst-generated lot number will be created (e.g., MPR03022016). Information listing the lot numbers of each of the newly created solution will be recorded and associated with the analyst-created lot number. All information should be kept either in sample batch documents and/or in the case file(s).

8.2.1 Place labeled sample cups or test tubes in appropriate positions on the sample rotor.

8.2.2 Pipet and load calibrators and controls as follows:

Table: General guide for position placement – alternate positions are permissible.

Position of Sample Cup/Test Tube	Description	Approximate Volume (µL)
C1	Level 0 Calibrator/Control	500
S2	Level 1 Calibrator	300
S4	Level 3 Calibrator	500
S5	Level 4 Calibrator	300
S7	Ethanol Level 0.0 Calibrator	200
S8	Ethanol Level 0.1 Calibrator	200

8.2.4 If needed, clear the previous run positions on the sample rotor.

8.2.5 Load calibrators and controls on the instrument.

8.2.6 Start calibration on EMIT instrument.

8.2.7 Evaluation of Urine Calibrator/Cut-Off Solutions:
At the completion of each calibrator analysis, the instrument will pause until the calibrator solution result is accepted by the operator. One or more of the following should be valid in order for a calibrator solution to be deemed 'acceptable:'

8.2.7.1 An analyte's calibrator/cut-off solution is acceptable if replicate absorbance values are within ± 5% of each other.

8.2.7.2 An analyte's calibrator/cut-off solution is acceptable if the "Reported" absorbance value is within ± 5% of the "Reported" absorbance value for the previously analyzed batch's calibrator/cut-off solution.

8.2.7.3 An analyte's calibrator/cut-off solution can be acceptable if its "Reported" absorbance value is within ± 5% of the mean "Reported" absorbance value from the past ten (10) "Reported" values for that calibrator/cut-off solution.

Note: "Reported" values are the average of the "Replicate" values for a particular calibrator.

If a calibrator is not accepted, the operator should either re-analyze the same calibrator or place a new aliquot of the calibrator in the instrument and re-analyze.

If a calibrator solution is not acceptable based on the above criteria, even after the operator re-analyzes the calibrator solution, then a Lead Examiner (and possibly a service engineer) should be contacted. If justified, the Deputy Director may allow the Examiner to accept the calibrator result for an analyte on a provisional basis. When this occurs, the Deputy Director must document and justify their decision within the affected case file(s) and within the batch documentation. In these situations, all positive and negative controls must work as expected. If any of the subsequent controls don't perform as expected, and the calibrator solutions had problems passing quality control measures, then the entire batch will be considered a failure and remediation measures will need to be implemented (i.e., service engineer notified).

8.3 Instrument Calibration (Blood)

Blood Calibrator/Control Solutions:

Blood calibrator solutions will not be used as official calibrators within the instrument software. They will be analyzed as samples (see Sample Analysis section) and their absorbance values will be used during calibration evaluations.

Evaluation of Blood Calibrator/Cut-Off Solutions:

Evaluation is not done like it is with the evaluation of urine calibrator/cut-off solutions (i.e., prior to sample analysis). See section later in procedure regarding blood calibrator/cut-off solution evaluation.

8.4 Sample Analysis

8.4.1 Urine Samples

8.4.1.1 For drug analyses, only one (1) urine sample per individual should be analyzed. If two (2) or more urine samples were submitted for the same individual, analyze the earliest sample. The other sample(s) should be analyzed only when necessary. If the other samples are to be analyzed, the Examiner should document the reasoning for this in the case file(s). Questions on whether to consume limited-volume samples should be directed to the Lead Examiner or the Deputy Director prior to analysis.

8.4.1.2 For ethanol analyses, both urine samples should be analyzed.

8.4.1.3 Urine samples are usually analyzed without any additional preparation (i.e., neat). Any problems with turbidity or sample degradation should be dealt with on an individual basis. Any sample clean-up or sample preparation steps will be recorded in the case notes and/or case file(s).

8.4.1.4 If needed, centrifuge the urine sample and isolate the supernatant into an EMIT test tube. Alternate clean-up procedures are acceptable, when needed. If utilized, these types of clean-up procedures need to be documented and placed within the case notes.

8.4.1.5 Analyze the urine sample(s) and the urine controls (positive and negative) using the EMIT instrument.

8.4.1.6 The urine Liquichek controls (i.e., S1E, S2E, S3) need only be analyzed during calibration. They are used only as a system check and not for a sample batch control check. Thus, if the urine calibration has been established for the week, then subsequent analyses of the S1E, S2E, and S3 controls do not have to be done.

Table: General guide for volumes of calibrators/controls/samples

Description	Approximate Amounts (µL)
Level 0 Control/Calibrator	500
Level 1 Calibrator	300
Level 3 Calibrator	500
Level 4 Calibrator	300
Oxycodone Low Control	100
Oxycodone High Control	100
Buprenorphine Low Control	100
Buprenorphine High Control	100
Ethanol Level 0.0 (Negative) Cal	200
Ethanol Level 0.10 Calibrator	200
Ethanol Low Control	250
Ethanol High Control	250
S1E Liquichek	500
S2E Liquichek	500
S3 Liquichek	250
Level 5 Control	600
Case samples	600

8.4.2 Blood (Serum/Plasma) Samples

8.4.2.1 Only one (1) sample per individual should be analyzed.

8.4.2.2 If two (2) or more samples were submitted for the same individual, analyze the earliest sample. The other sample(s) should be analyzed only when necessary. If the other samples are to be analyzed, the Examiner should document the reasoning for this in the case file(s). Questions on whether to consume limited-volume samples should be directed to the Lead Examiner or the Deputy Director prior to analysis.

8.4.2.3 Analyze the appropriate controls (positive and negative) and the appropriate blood calibrant/cut-off solutions using the EMIT instrument.

8.4.2.4 The blood calibrant/cut-off solutions should be analyzed twice using the EMIT instrument (similar to the urine calibrators being analyzed in replicate).

8.4.2.5 Blood samples are subjected to a protein precipitation procedure prior to analysis.

8.4.2.5.1 Individually pipette 1.0 mL of each [blood] specimen into an appropriately labeled test tube

8.4.2.5.2 Add 2 mL acetonitrile to each tube and cap

8.4.2.5.3 Vortex-mix each sample for at least 30 seconds

8.4.2.5.4 Centrifuge samples for at least 5 minutes using at least 800 rpm

8.4.2.5.5 Individually decant [top] solvent layer from each specimen and place into appropriately labeled test tubes or cups

8.4.2.5.6 Individually add 25 µL of 1% MeOH-HCl to each tube

8.4.2.5.7 Vortex-mix

8.4.2.5.8 Evaporate to dryness using $N_{2(g)}$ at $\leq$ 40º C

8.4.2.5.9 Reconstitute each sample with 500µL* water

8.4.2.5.10 Vortex-mix for at least 30 seconds

*Note: Alternatively, such as when limited volumes of samples are encountered, 250µL of water can be used for reconstitution. However, a cup needs to be used instead of a test tube during analysis. This volume must be documented within the batch documentation and the case file(s). The Lead Examiner should be consulted and calibrator/cut-off concentrations may need to be adjusted.

8.4.2.6 Blood samples are not tested for ethanol using EMIT.

9.0 EVALUATION

9.1 Urine calibrator and control evaluation

9.1.1 Print a calibration sheet with cut-off values from the main menu.
See the previous section for evaluation of urine calibrator/cut-off solutions.

9.1.2 Check the Level 0 and the S1E (when appropriate) controls and ensure that the EMIT software reported their results as negative.
Samples should not be analyzed until control criteria are met.

9.1.3 Evaluate the sample batch at the completion of the sequence.
Each analyte should be checked to ensure that the EMIT flagged the correct result.
Any problems with certain analyte assays will be addressed individually and on a case-by-case basis.

9.1.4 Control Acceptable Results:
All positive urine control's absorbance values should read above their respective calibrator's/cut-off's absorbance values and should flag as positive, as appropriate, by

the EMIT instrument software.
When analyzed, the S2E control and the S3 [cannabinoid] control should flag positive.
Note: the word 'appropriate' was used due to different positive control solutions being analyzed as controls for various analytes.

All negative urine control's absorbance values should read below their respective calibrator's/cut-off's absorbance values and should flag as negative by the EMIT instrument software.
When analyzed, the S1E control should flag negative.

9.1.5 Samples are usually analyzed as a batch (or group) and utilize common controls and calibrators for quality control purposes. Because substantial paperwork would have to be replicated if controls and calibrator data were included within each sample's case file, the complete paperwork [batch packet] can be kept separately (e.g., within the Toxicology Unit). This batch packet should contain all evaluation (i.e., Quality Control (QC)) documentation. Specific results for each case are filed in their appropriate case file(s). The QC results can be printed from the EMIT instrument. Batch packet data is usually transferred to long-term storage after a year since passed its collection date.

Note: Due to varying analyte concentrations, the above criteria may not always be met for some analytes within batches. Because this is a presumptive screening assay the batch may, depending on the magnitude and nature of the failure, be accepted by the Deputy Director or Technical Lead(s). If the batch is accepted in these circumstances, appropriate documentation must justify the acceptance decision and such documentation will be kept in the case file(s) and/or batch data. For urine samples, if the above criteria are not met and there are positive findings by the EMIT, the replicate urine samples must fully pass QC evaluation set forth in this document in order for a valid result to be reported.

9.2 Blood calibrator and control evaluation:

Calibration/cut-off absorption values within EMIT analyses of blood samples are lower than corresponding EMIT absorption values for urine samples. Because of this, the EMIT instrument's urine calibration/cut-off data can't be used when analyzing blood samples within this procedure. Absorbance values from blood calibrator solutions will be used as cut-off values when evaluating blood samples. Assessment for blood samples will be based on the comparison of a sample's absorbance reading and each calibrator/cut-off solution's absorbance reading for a particular analyte.

9.2.1 Calibrator solutions should be analyzed in replicate – similar to urine calibrators. One or more of the following should be valid in order for a calibrator solution to be deemed 'acceptable:'

9.2.1.1 An analyte's calibrator/cut-off solution is acceptable if replicate absorbance values are within ± 5% of each other.

9.2.1.2 An analyte's calibrator/cut-off solution is acceptable if the average absorbance value is within ± 5% of the average absorbance value for the

previously analyzed batch's calibrator/cut-off solution.

9.2.1.4 An analyte's calibrator/cut-off solution can be acceptable if its average absorbance value is within ± 5% of the mean of average absorbance values from the past ten (10) average values for that calibrator/cut-off solution.

9.2.2 Control Acceptable Results:
All positive blood control's absorbance values should read above their respective calibrator's/cut-off's absorbance values

All negative blood control's absorbance values should read below their respective calibrator's/cut-off's absorbance values and should flag as negative by the EMIT instrument software.

Note: Most blood positive control samples will [erroneously] indicate a negative 'Reportable' result from the EMIT software because the software will be using urine calibration/cut-off data during its evaluation process. These results should be disregarded and manual evaluation by the analyst must occur.

9.2.3 Blood sample batches can be utilized. However, since the calibration data stored within the EMIT instrument is for urine samples, blood calibration/cut-off solutions need to be analyzed contemporaneously with each batch of blood samples. However, when sample-batches are utilized, the complete paperwork [batch packet] can be kept separately (e.g., within the Toxicology Unit). This batch packet should contain all evaluation (i.e., Quality Control (QC)) documentation. Specific results for each case are filed in their appropriate case file(s). For blood analyses, the urine QC results should not be printed from the EMIT instrument. Batch packet data is usually transferred to long-term storage after a year past its collection date.

10.0 INTERPRETATION AND REPORTING OF RESULTS

10.1 Urine Samples (non-Ethanol):

The EMIT instrument will indicate that an analyte is possibly present within a sample based on comparison of absorption values between the sample and the calibration/cut-off solutions.

Positive Results: Occurs when the instrument flags an analyte as being possibly present within a sample. Results from analytes whose absorbance values are greater than, or equal to, the corresponding analyte's calibration/cut-off absorbance values will trigger this response. Urine samples which are EMIT positive may need to be re-sampled and re-analyzed for specific analytes.

Negative Results: Occurs when the instrument flags an analyte as being not detected within a sample. Results from analytes whose absorbance values are lower than the corresponding analyte's calibration/cut-off absorbance values will trigger this response. Any analyte for a drug class having a negative result will not be reported as possibly being present within a

presumptive report (if that type of report is issued).

Elevated Results: Elevated results occur when samples have absorption values significantly greater than negative control absorption values, are close to the cut-off absorption values, but are not equal to (or greater than) the absorption values for the calibration/cut-off concentrations of a particular analyte. While confirmation techniques can be employed to confirm presence/absence of analyte(s) and while elevated values can be noted within case files, samples with elevated results will be treated as 'negative results' (see above). It is at the Examiner's discretion whether confirmatory procedures be used for the sample.

10.2 Blood Samples:

For this procedure, the EMIT instrument is automatically using urine calibration data when evaluating blood samples. The urine calibration/cut-off solutions have absorbance values that are significantly higher than those for corresponding blood samples. When blood samples (i.e., blood extract solutions) are analyzed within this procedure, determination of the possible presence or absence of an analyte will be performed manually by the Examiner and not by the software.

For post mortem cases (or OCME ante mortem cases) an enzyme immunological technique will be performed for Barbituates and Marihuana related compounds.

Instrument-generated Positive Results: Any positive result automatically reported from the EMIT software will indicate that the sample has an analyte concentration greater than, or equal to, that within a corresponding urine calibration/cut-off solution. In these situations, the analyte concentration in the blood sample will be greater than that within the urine calibration/cut-off solution(s). Any positive results for a drug class should be reported in a presumptive test report, if that type of report is issued.

Positive Results: Occurs when a sample's absorbance value for an analyte is greater than, or equal to, the absorbance value for the corresponding analyte's calibration/cut-off solution. Any positive results for a drug class will be used as presumptive information when deciding whether to conduct further examinations on the specimen. If there is an inadequate amount of sample and EMIT results are the only results that are available, and a report needs to be issued, any positive findings will be reported with the necessary caveat that the technique is a presumptive one and is not confirmatory.

Negative Results: Occurs when results from analytes whose absorbance values are lower than the corresponding analyte's calibration/cut-off absorbance values. Any analyte for a drug class having a negative result will not be reported as possibly being present within a presumptive report (if that type of report is issued). Any negative results for a drug class will be used as presumptive information when deciding whether to conduct further examinations on the specimen. If there is an inadequate amount of sample and EMIT results are the only results that are available, and a report needs to be issued, any negative findings will be reported with the necessary caveat that the technique is a presumptive one and is not confirmatory.

Elevated Results: Elevated results are considered Negative Results. They occur when

samples have absorption values significantly greater than negative control absorption values, are close to the cut-off absorption values, but are not equal to (or greater than) the absorption values for the calibration/cut-off concentrations of a particular analyte. While confirmation techniques can be employed to confirm presence/absence of analyte(s) and while elevated values can be noted within case files, samples with elevated results, if reported, will be treated as 'negative results' (see above).

10.3 See case workflows in SOP TX-19 for analytical guidance, based on, sample type and offense.

11.0 LIMITATIONS

The use of EMIT for the screening of forensic samples is considered presumptive and not confirmatory. False positive results can occur due to cross-reactivity. Negative results will occur when analyte concentrations are at levels below calibration/cut-off concentrations. In cases where EMIT data indicates the presence of an analyte, confirmatory procedures will be performed when an identification is required. Quantitative values will not be reported and are typically used for comparison purposes only (e.g., HS-GC data).

12.0 MAINTENANCE

12.1 Weekly Needle Rinse: May be performed after running samples or prior to initiating a new week's usage.

12.1.1 Remove covers from reagent and sample rotors

12.1.2 Remove covers from needle rinse solution and 0.1N HCl bottles

12.1.3 The operator must ensure that the needle rinse solution test tube (Position W on the sample ring) and the needle rinse solution bottle does not get too low. The test tube should be complete filled before starting the needle rinse.

12.2 Bi-annual Preventative Maintenance: Siemens Service personnel

12.3 Rotor Change: Cuvette Rotor can be changed every 10,000 tests. This is normally performed by Siemens Service Personnel. However, if analyst needs to change they should refer to the Operator's manual for directions.

13.0 SAFETY

This procedure is carried out in a laboratory environment and standard safety procedures appropriate for such an environment should be utilized. This includes gloves, safety glasses, and protective clothing (e.g., lab coat), as appropriate. Biological specimens subject to the analytical procedure should be handled using universal precautions. Potentially contaminated items and surfaces should be cleaned and disinfected prior to any further use.

14.0 REFERENCES

Dade Behring; "Viva-E Operators Manual" Dade Behring, Inc. P.O. Box 6101 Glasgow Business Community, Newark Delaware 19714
Blood Immunoassay Screen for Drugs of Abuse using the Olympus AU 400. West Chester County Department of Laboratories and Research: Division of Forensic Toxicology.

Rev. #	History
3	Changed title. Significant format and verbiage changes throughout document. Added blood matrix sample-specific analyses as well as new calibrator/cut-off information. Updated product identifier information. Changed the requirement for the Bio-Rad Liquichek Urine positive controls to be analyzed with every batch to only when the calibrant/cut-off solutions are analyzed. Removed the requirement that all urine samples from the same individual must be analyzed. Removed and updated tables throughout document. Added the use of both cups and test tubes for analyses (sample volume dependent). Removed specific work instructions from document. Updated evaluation and reporting criteria. Added History section to document.
4	Added guidance for the analysis of post mortem blood samples. Removed tables under 10.2, added statement that post mortem cases will be screened for Marihuana and Barbs by an immunological technique, and added 10.3 refering the user to SOP TX-19 for workflows. Updated sections 8.2 (combining of calibrator/control solutions of differing lot numbers), 9.2.2, and 9.2.3, 10.1, 10.2, and 11.0.