1.0 PRINCIPLE: The EMIT assay is an immunoassay screening method based on the "Enzyme-Multiplied Immunoassay Technique." Briefly, an enzyme capable of catalyzing a color-generating reaction is bound to a drug, or drug-group specific antibody. When the antibody-bound enzyme is mixed with sample that may or may not contain the target drug, and the appropriate substrate and cofactors, the amount of color generated will be inversely proportional to the amount of drug. However, the transmittance will be (roughly) proportional to the amount of drug present in the sample. The accuracy of the assay is closest at the calibration concentrations.

2.0 SAFETY: This procedure is carried out in a Laboratory Environment, and standard safety procedures appropriate for such an environment should be utilized, including (minimally) safety glasses and protective clothing (lab coat). 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.

3.0 SAMPLES FOR ANALYSIS: Specimens requiring screening by EMIT are retrieved from the "EMIT Refrigerator" in the Toxicology "Wet Lab" (see section 8.0 Specimen).

4.0 EQUIPMENT:

4.1 Viva-E EMIT analyzer workstation (Seimens, Inc.) and related equipment and supplies (e.g. sample cups)

4.2 Micro-vial centrifuge (Abbott Labs)

4.3 Standard laboratory glassware, plasticware and equipment.

5.0 REAGENTS:

5.1 <u>Reagents available as stock items:</u>

5.1.1.1 EMIT II Plus Assay Kits (Seimens):

Kit	Cut-Off
Amphetamine (9C309UL)	1000 ng/ml
Barbiturates (9D029UL)	200 ng/ml
Benzodiazepine (9F029UL)	200 ng/ml
Cocaine (9H029UL)	300 ng/ml
Methadone (9E029UL)	300 ng/ml
Opiate (9B309UL)	300 ng/ml
PCP (9J029UL)	25 ng/ml
THC (9N029UL)	100 ng/ml

5.1.2 EMIT II Calibrator/Control Kits (Seimens)

Level 0: 9A509UL
Level 1: 9A529UL
Level 3: 9A569UL

Level 4: 9A589UL
Level 5: 9A609UL

5.1.3 Liquichek Urine Toxicology Controls (Bio-Rad)
Level S1: #466; contains target analytes; 20 – 25% below cutoff.
Level S2: #467; contains target analytes; 20 – 25% above cutoff.
Level S3: #463; contains target analytes; ~ 3x above cutoff.

5.1.4 System Solution (Seimens) #3203-063
Reconstituted with dionized water per package directions.

5.1.5 Needle Rinse Solution (Seimens) #7668 (Sodium Hypochlorite solution)

5.1.6 0.1N NaOH; (Seimens) #5636-02

5.1.7 0.1N HCl; (Seimens) #5621-02

5.1.8 N,N-Dimethylformamide (Baker, Reagent Grade or equivalent)

6. Validation of Reagents

Validated reagents are marked with a green dot, indicating the reagent was validated, and the specific EMIT batch on which that process was documented. Newly prepared and/or reconstituted reagents may be evaluated for validity on an EMIT batch, prior to any consideration of sample results. Acceptable performance of all batch control materials and overall batch acceptability (although individual samples may fail) is considered as validation of reagents. Reagents so validated are marked with a green sticker as noted above. Note: The external control solutions are accepted as validated per manufacturer's documentation. As such, they are not subject to in-house validation procedures. They should be evaluated for dates in-use, per manufacturers documentation, and marked with a green sticker as such. Acceptable performance on the External Control Materials is the basis for evaluation of validity of instrument solutions.

7. Instrument Calibration: Based on the Laboratories sample volume, the Viva-E automatic analyzer is calibrated once per week, per manufacturer's instruction. Calibration will also be performed after any system change, including reagent refill, or solution lot change, or bottle change.Bottles must be changed for new reagent lot numbers. Important; Do not overfill bottles – add reagent up to shoulder of bottle only. Calibration utilizes levels 1, 3 and 4 of the Calibrator/Control Kits, as follows:

7.1 Place sample cups in positions S2, S4 and S5 on the sample rotor.
7.2 Add 4 drops Level 1 solution into the S2 sample cup, similarly add 7 drops Level 3 solution into the S4 cup and 4 drops Level 4 solution into the S5 cup.
7.3 Load the rotor on the instrument.
7.4 From the Main Menu Screen, select the tests to be calibrated.(S2 – Opiates;S4 all,EXCEPT Opiate and Cannabinoid;S5 – Cannabinoids).
7.5 Start the calibration process which proceeds automatically
7.6 Evaluation of Calibration: At the completion of each analyte calibration, the instrument will pause until the calibration is accepted or rejected by the operator. The initial acceptance

criteria is that the absorbance value for the level calibrator is within 5% of the previous calibration. If this fails, then the acceptance criteria is +/` 5% of the mean value for that analyte on the previous 10 calibrations. If that fails as well, the instrument manufacturer is contacted, and the Director notified (who may, because this analysis is used as a screen only, choose to accept on a provisional basis, the calibration).

8.0 **SPECIMEN**

Specimens requiring EMIT screening are stored in the "EMIT Refrigerator" prior to analysis. Following aliquot for analysis, such samples are stored in the same Refrigerator. Specimens comprising an analytical batch are listed by lab case number on "EMIT Batch Summary Sheet" (Appendix I).

8.1 All evidence transfers, either between individuals or between an individual and a storage location must be documented on the Chain of Custody for the case, either in the LIMS, or on hard-copy COC document maintained in the Case Jacket.

8.2 When not in the sampling or aliquot process, samples in the toxicology section must be maintained "Under Proper Seal." This is interpreted to mean that the sample, or a container in which the sample is kept is sealed with tamper-evident tape, with the initials and date of person placing the seal on the seal as well.

8.3 Samples must be maintained in such a manner so that they are protected from contamination or deleterious change. Depending on the nature of the sample, this may mean refrigeration or freezing when not in the analytical process.

8.4 Samples are maintained in the Toxicology Section, for 8 weeks, in the absence of notification of any legal action, or reason to maintain the samples. After this period, samples are discarded in the appropriate medical waste disposal container. Samples from homicides, or cases with pending legal action and requests for retention are maintained by the laboratory.

8.5 Preparation of Samples:

8.5.1 Blood samples: Blood are subjected to a protein precipitation procedure prior to analysis; ~800 ul of blood is mixed with an equal volume of N,N-dimethyl formamide, in an appropriately labeled microvial, and the mixture is thoroughly vortexed.The mixture is then centrifuged at ~ 800 rpm, (microfuge) for 5 min. The supernatant is decanted into a test tube for analysis as described below.

8.5.2. Urine samples: Urine samples are normally analyzed without any special preparation. However, if the sample is particularly turbid, the sample may be centrifuged (as noted above) and the supernatant utilized.

9.0 PROCEDURE

Note: The operator should check and ensure that there are adequate amount of reagents present in the reagent bottles prior to initiating instrument operation.

Note: Departure from procedures as specified in this SOP is not anticipated. Should an issue arise that may require such a departure, the issue must be raised with Quality Manager and/or the Director. If the proposed change will not present a change of a magnitude that would require validation, the change may be approved, and the Director will modify and re-issue the SOP accordingly.

Any such procedural changes would be subject to the review process afforded by the quality control measures of the analytical scheme described herein. Hence, any modification or change that produces an unexpected deleterious effect on the analytical procedure would be expected to trigger analysis or batch failure in the QC review stages.

9.1 Daily Start-Up

9.1.1 Perform system refill:

9.1.1.1 Remove rotor and cuvette covers, and open instrument door.

9.1.1.2 Remove all covers from reagents in the reagent rotor.

9.1.1.3 Ensure that needle rinse and HCl bottles are full.

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

9.1.1.5 Inspect wash arms and mixers and cuvette rotors, ensure that system solution containers have an adequate amount and waste containers have adequate available space for the run.

9.1.1.6 Replace rotor and cuvette covers

9.1.1.7 Initiate system refill from the main menu (F5, F1, Rotor/System, Fill/Empty System, Select "Fill system," F1)

9.1.2.8 Check syringes and tubing for bubbles – tap to remove bubbles as necessary.

9.1.2.9 If not already documented, the "EMIT Batch Sheet" is completed by indicating in appropriate spaces, the particular sample/case numbers being analysed. Matrix is also indicated on the form, as well as any pertinent case comments , e.g. DMSA, Fatal.

9.2 Load sample cups and test tubes as appropriate in the sample ring, and add reagent/sample for all samples, calibrators and controls as follows (using a disposable plastic Pasteur pipette for each):

Position	Description	Container	Amount
C1	Level 0 Cal/Ctl (Blank)	sample cup	8 drops
1	S1 Liquichek	test tube	12 drops
2	S2 Liquichek	test tube	12 drops
3	S3 Liquichek	test tube	12 drops
Samples 1-15	Case samples	test tube	12 drops
last	Level 5 Control	test tube	12 drops

Additional samples as needed; note, an additional control is required for 16 or greater samples. If two controls are being run, the analyst should place the first in the middle of the run, and the second control at the end of the run.

9.3 The data from the previous run is cleared from instrument memory;
- 9.3.1 From "main menu", select "Evaluate samples",
- 9.3.2 select "F2 Historic Results",
- 9.3.3 Left click on "Archive Results"
- 9.3.4 From "main menu",select "sample handling",
- 9.3.5 click "F4" (confirm unload)
- 9.3.6 exit screen by clicking "F10".

9.4 Place the sample disk in the instrument, and "request samples" on the instrument operation program, by keying "F8."Under "Request Type",
- 9.4.1 Select "Control"; Under "Tests":
- 9.4.2 Click "Level 0"
- 9.4.3 Click on F8 "New Sample"; Under Sample ID:
- 9.4.4 Type in Case Number; Under "Tests":
- 9.4.5 Click on "Emit"
- 9.4.6 Repeat 9.4.4 – 9.4.5 for all remaining controls and samples.

9.5 Start Instrument:
- 9.5.1 Click F9; "Sample Handling"
- 9.5.2 Click on first sample listed
- 9.5.3 Press enter for rest of samples and controls to the end of the list. (Position indicator circles will turn yellow, as assigned).
- 9.5.4 Print Load List (click F1) and double check correct sample entry vs. manuallyprepared batch list. In the event of an error, return and re-enter as appropriate.
- 9.5.5 Press "F3"to start the instrument. Results for each calibrator, control and sample will print out automatically.in batches of four to a page. To print individual case results click on Main Menu, click on Evaluate samples, Left click on one sample, Left click on F1, Left click on Print. Repeat for all samples.

10.0 Run Write-Up and Evaluation

10.1 Initial evaluation:

10.1.1 Check the level 0 and the S-1 control to ensure the results are negative. If not, the run is considered to have failed and must be repeated. Note; the failure of the batch is noted on the batch summary sheet, and the batch is retained in the EMIT batch file. Depending on the nature of the failure, or in the event of sequential, or a pattern of batch failures, the operator will appraise the Quality Manager and/or the Director of the situation. If action is necessary to address the issue, a Quality Action Request may be initiated to document the response of the laboratory to the issue.

10.1.2 Print a calibration sheet from the main menu, "blank/calib info", print (F1).

10.1.3 The individual results for each calibrator, control, and sample are transcribed on a per-drug basis to the "EMIT BATCH SUMMARY SHEET"
Note : "Negative" results are not so transcribed. (for case samples).

10.2 Quality Control

10.2.1 The batch is evaluated by the analyst at the completion of the run. Each analyte is individually checked. Failure of one analyte does not require failure of another analyte. Failure of the batch for any analyte is clearly documented on the batch summary sheet.

10.2.2 For an analyte to be considered acceptable, the low control solution (S1) must read below the calibrator, and the positive control (S2) must read above the calibrator. The high control (S3) must read above the positive control (S2).

Note: Because of varying analyte concentrations, one of the above criteria may not be met for some analytes in some batches. Because this is a screen assay, (any presumptive positive results are subject to confirmation by GCMS) the batch may, depending on the magnitude and nature of the failure, be accepted by the Director, or Quality Manager, with appropriate documentation and explanation thereof.

10.2.3 EMIT runs are performed as part of screeing batches, containing controls and calibrators. The complete batch packets are in the Toxicology Laboratory. This packet contains all run evaluation documentation. Specific results for each case are filed in the appropriate case file. Results are documented on the "Batch Summary" sticker on each case file. A Batch summary sheet will be produced with each batch. Data on each batch should include fields such as: Sample name, Batch ID (Date of Batch), analysts who generated data, matrix, analyte found (and concentration if applicable), controls run with the batch and results

obtained.

11. Reporting of Results:

11.1 Following an acceptable technical review, the analyst enters the results of the screen as either "pos" or "neg" for each drug/drug group, for each case in the LIMS system.

11.2 The Instrument result sheets are placed in the "EMIT Batches" basket in Tox instrument room, to facilitate GCMS confirmation of screen positive results. Completed batch documentation is stored, by date, in the EMIT Batch binder. If controls do not meet the criteria, the batch can be rejected as a whole or by a case by case basis. The supervisor is notified and proper action is taken to correct any problem. Batches and/or cases shall be repeated as needed.

11.3 Samples generating positive screen results (and in some cases, negative results; see 11.3.1 and 11.3.2, below) for a particular drug or drug class will be considered for confirmatory analysis by GCMS by the analyst. Case numbers from such cases will be added, as appropriate, to the GCMS-Confirmatory batch sheet, maintained in the Toxicology Instrument room.

11.3.1 Urine Samples: Depending on specific case circumstances, cases may be annotated for confirmatory procedures, even in the absence of a positive screen result. (Example; a urine case in which oxycodone is suspected, yet opiate screen procedures are negative may very reasonably be sent for an opiate-specific GCMS confirmatory analysis.)

11.3.2 Blood Samples: The levels of drugs routinely detected in blood may be lower than those detected in urine samples. As such, a blood sample may read "negative" in that the magnitude of the signal for a specific EMIT test is below the cutoff, yet the signal present is adequately elevated above the negative so as to provide an valid indication that the drug (or drug group) may actually be present. Blood EMIT results will be considered by the Analyst from this perspective. Decisions to "override" a "negative" result will be appropriately documented in the case jacket. (Example: A blood case, with an EMIT opiate result of 0.225 (negative = 0.178, low control = 0.234, cutoff calibrator (300 ng/mL morphine) = 0.258); with the result suggestive of the presence of an opiate, the analyst may reasonably choose to list the case for GCMS opiate analysis.) As a general rule, blood EMIT results above the low control (rather than the cutoff calibrator) should be considered for GCMS confirmation.

12. Maintenance:

12.1 Weekly Needle Rinse: May be performed after running samples, or prior to initiating a new weeks 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 While procedure is running, the operator must ensure that the needle rinse solution test tube(Position W on the sample ring) and the needle rinse solution bottle remain full.
12.1.4 From main menu; select F5 "Special Function"
12.1.5 Select F1; Rotor/System; double click "Rotor/Needle Rinse"
12.1.6 Select F3; Needle Rinse

12.2 Bi-annual Preventative Maintenance: Siemens Service personnel

12.3 Rotor Change: Cuvette Rotor can be changed every 10,000 tests. (Normally performed by Seimens Service Personnel.)however if analyst must change see Operator's manual for directions.

12.3.1 To check number of tests on the current cuvette rotor disc:
12.3.1.1 From main menu; select Rotor System
12.3.1.2 Click once on "Change Measurement Disk" ; number of tests will be displayed.
12.3.1.3 Return to main menu

13. References:

13.1 Dade Behring; "Viva-E Operators Manual" Dade Behring, Inc. P.O. Box 6101 Glasgow Business Community, Newark Delaware 19714

A. Sources of Error:

Emit screening of forensic samples is a presumptive technique for the Toxicology section and no positive results are ever reported on the basis of a screen analysis alone. "False positive" results can occur in the screen, in that molecules of similar size, shape and electrical configuration to the target molecule can in some instances, elicit a positive EMIT result. However, because all EMIT screen results are subject to confirmation by GCMS, and because such a similar molecules would not be expected to either chromatograph, or fragment in the MS in the same manner as the target molecule, there is no expectation that a screen false positive would ever result in an erroneous report. "False Negative" results can occur when the target molecule is present at a level below the operative cutoff. In such cases, if the presence of a specific drug is suspected, based on case documents, GCMS confirmatory procedures may be ordered, in the absence of a screen positive result, based on review of case information, as noted in 11.3, above.

Example of controlled Emit batch document TX EMIT-1. Batch documents can vary based on nature of batch.

Pos. #	Description	Amp	Barb	Benzo	Coc	Cann	Opiate	Mdone	PCP	Comment	QC Check
C-1	Level 0										
S-2	Level 1									Opiate Cutoff	
S-4	Level 3									Mix Cutoff Values	
S-5	Level 4									THC Cutoff	
1	"S1E"									Blank	
2	"S2E"									All but THC	
3	"S3"									THC Only	
4											
5											
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Operator Review:
_____ Batch Acceptable?:_____ _____

QC Review By:
_____ Batch Acceptable?:_____ _____