Deviation Request Form (DRF)

Directions: The Initiator will complete Sections A through C. Additional continuation pages can be included if necessary.

Initiator	Stephanie Douthwaite	**Date**	4/4/2019

A. Requested deviation applies to (Technical Procedure – include specific section):

Enzyme Immunoassay Section 5.6
5.6.3 In duplicate, pipette 0.25 ml of each sample to be analyzed into a disposable glass test tube.
5.6.4 Using a pipette or liquid handler diluter, add 2.5 mL of deionized water into each tube.

B. Requested deviation:

Insert allowance for lower prep volumes.

Amend 5.6 to the following

5.6 Sample preparation - For submitted specimens with low volume, 5.6.3 and 5.6.4 may be done at half -volumes.

C. Necessity for the deviation:

Need a way to conserve sample for confirmatory testing when low volumes of specimens are submitted for testing.

D. Technical review and Authorization (to be completed by the Quality Manager and/or Technical Leader)
Comments(to include merits and impacts):

n/a

Approved	☑	Yes	☐	No	Duration	12 months
Signature	F. Wayne Lewallen Digitally signed by F. Wayne Lewallen Date: 2019.04.04 14:04:01 -04'00'				Date	4/4/2019

E. Quality Assurance Authorization (to be completed by the Quality Manager, Forensic Scientist Manager or designee)

Acceptable within general QA guidelines and good laboratory practice?	☑	Yes	☐	No
Significant negative impact to Crime Laboratory Quality System?	☐	Yes	☑	No

Restrictions/limitations:

☑	Authorized	☐	Rejected	Signature	Timothy G. Suggs Digitally signed by Timothy G. Suggs DN: cn=Timothy G. Suggs, o=North Carolina State Crime Laboratory, ou=Quality Manager, email=tsuggs@ncdoj.gov, c=US Date: 2019.04.10 12:13:27 -04'00'	Date	04/10/2019

Enzyme Immunoassay (EIA)

1.0 Purpose - This procedure specifies the required elements for the calibration and use of the Tecan Liquid Handling Workstation for EIA of blood and urine.

2.0 Scope – This procedure applies to Toxicology in the Raleigh, Triad, and Western locations of the State Crime Laboratory.

3.0 Definitions

- **Performance verification –** The initial confirmation of the reliability of a previously or externally validated method or instrument.
- **Quality control (QC) check –** Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
- **ELISA –** Enzyme-Linked Immunosorbent Assay; the EIA technique used in the State Crime Laboratory.

4.0 Equipment, Materials and Reagents

4.1 Equipment

- Tecan Liquid Handling Workstation for ELISA
- Mechanical pipettes
- Liquid handling diluter/pipettor system
- Volumetric flasks, Class A

4.2 Materials

- Stoppers or caps
- Vortex mixer
- Test tubes

4.3 Reagents

- Deionized water
- 1N Hydrochloric Acid

4.4 Primary Reference Materials

- d-Amphetamine
- Benzoylecgonine
- Carisoprodol
- (-)-delta-9-carboxy-11-nor-delta-9-tetrahydrocannabinol (THC-COOH)
- (±)-Methadone
- d-Methamphetamine
- Morphine
- Oxazepam
- Oxycodone
- Phenobarbital
- *cis*-Tramadol HCl
- Zolpidem

4.5 Kits

- Each manufactured kit is assembled with 96 well coated microplates and paired conjugate, substrate, and stop acid reagent. The kits are opened upon receipt, and the components are labeled and separated for proper storage.
- The manufacturer's lot numbers assigned to the plates and conjugates are paired for optimum performance and are not interchangeable. Plates and conjugates with identical lot numbers can be combined.
- Lot numbers of substrate and stop reagent are interchangeable.

4.6 Critical Reagents

- Negative blood/urine
- EIA Calibration Solutions
- EIA Verification Solutions
- Immunalysis 96 well coated microplates for barbiturates, benzodiazepines, carisoprodol, cocaine metabolite, metabolites of delta-9-THC, methamphetamine/MDMA, methadone, opiates, tramadol, zolpidem, amphetamine/MDA, and oxycodone / oxymorphone and each respective Enzyme Conjugate

4.7 Commercial Reagents

- Methanol, ACS and spectrophotometric grade
- Immunalysis TMB Substrate Reagent
- Immunalysis TMBZ Substrate Reagent
- Immunalysis Stop Reagent

4.8 Calibration/Verification Solution Preparation - solutions may be prepared in any amount provided that the component ratios are kept constant. If using 100 µg/ml standards, multiply the standard volumes by 10.

4.8.1 The EIA Verification Solution shall be prepared using standards from manufacturers or lot numbers that differ from the ones used to prepare the calibration solutions.

4.8.2 EIA Calibration Solution 1

4.8.2.1 To a 10 mL volumetric flask, add the following Primary Reference Materials:

- 150 µL of 1 mg/mL phenobarbital
- 25.0 µL of 1 mg/mL oxazepam
- 25.0 µL of 1 mg/mL morphine
- 25.0 µL of 1 mg/mL benzoylecgonine
- 12.5 µL of 1 mg/mL (±)-methadone
- 10.0 µL of 1 mg/mL d-amphetamine

4.8.2.2 Dilute the flask to volume with methanol.

4.8.2.3 Lot number: Eight digit format year/month/day.

4.8.2.3.1 Example: 20101231

4.8.2.4 Expiration: One year.

4.8.2.5 Store in freezer.

4.8.2.6 QC check: Successful QC checks (see **5.4**).

4.8.3 EIA Calibration Solution II

4.8.3.1 To a 10 mL volumetric flask, add the following Primary Reference Materials:

- 12.5 µL of 1 mg/mL (-)-THC-COOH
- 250 µL of 1 mg/mL carisoprodol
- 10.0 µL of 1 mg/mL d-methamphetamine
- 25.0 µL of 1 mg/mL cis-tramadol HCl
- 10.0 µL of 1 mg/mL zolpidem
- 12.5 µL of 1 mg/mL oxycodone

4.8.3.2 Dilute to volume with methanol.

4.8.3.3 Lot number: Eight digit format year/month/day

4.8.3.3.1 Example: 20101231

4.8.3.4 Expiration: One year.

4.8.3.5 Dilute to volume with methanol.

4.8.3.6 Store in freezer.

4.8.3.7 QC check: Successful QC checks (see **5.4**).

4.8.4 EIA Verification Solution I

4.8.4.1 To a 10 mL volumetric flask, add the following primary reference materials:

- 300 µL of 1 mg/mL phenobarbital
- 50.0 µL of 1 mg/mL oxazepam
- 50.0 µL of 1 mg/mL morphine
- 50.0 µL mL of 1 mg/mL benzoylecgonine
- 25.0 µL of 1 mg/mL (±)-methadone
- 20.0 µL of 1 mg/mL d-amphetamine

4.8.4.2 Dilute to volume with methanol.

4.8.4.3 Lot number: Eight digit format year/month/day

4.8.4.3.1 Example: 20101231

4.8.4.4 Expiration: One year.

4.8.4.5 Store in freezer.

4.8.4.6 QC check: Successful QC checks (see **5.4**).

4.8.5 EIA Verification Solution II

4.8.5.1 To a 10 mL volumetric flask, add the following primary reference materials:

- 25.0 µL of 1 mg/mL (-)-THC-COOH
- 500 µL of 1 mg/mL carisoprodol
- 20.0 µL of 1 mg/mL d-methamphetamine
- 50.0 µL of 1 mg/mL cis-tramadol HCl
- 20.0 µL of 1 mg/mL zolpidem
- 25.0 µL of 1 mg/mL oxycodone

4.8.5.2 Dilute to volume with methanol.

4.8.5.3 Lot number: Eight digit format year/month/day

4.8.5.3.1 Example: 20101231

4.8.5.4 Expiration: One year.

4.8.5.5 Store in freezer.

4.8.5.6 QC check: Successful QC checks (see **5.4**).

5.0 Procedure

5.1 Instrument Performance Verification for New Instrumentation

5.1.1 New Tecan work stations shall be installed by a manufacturer representative and shown to meet any manufacturer's requirements.

5.1.2 The Key Operator shall complete a performance verification on new Tecan work stations prior to use for casework.

5.1.3 The performance verification shall include analysis of a minimum of fifteen blood and urine samples with known results. All quality control requirements shall be met.

5.1.3.1 The known blood and urine samples may be prepared or purchased.

5.1.3.2 The results of the known samples shall be substantially comparable to their known results.

5.1.4 The data shall be filed and maintained by Key Operator to document the new instrument set up.

5.1.5 A new entry for the instrument shall be made in the Resource Manager section of FA prior to use in casework. The new entry shall include the following:

- Manufacturer's serial number.
- Unique Section identifier for the new instrument.

- Notation under "Verification Date" to reflect the date the performance verification was completed.

5.2 Maintenance

5.2.1 Record all maintenance in the instrument log at the time it is performed.

5.2.1.1 Maintenance reports provided by an outside vendor shall be stored in the Managed Files of the instrument resource in FA

5.2.2 Notify the Key Operator or designee of instrument problems. The Key Operator or designee shall evaluate the instrument and determine if maintenance or service is needed. If the problem prevents the instrument from properly functioning, the Key Operator or designee shall note in the instrument log that the instrument is "Out of Service." The instrument shall not be used for casework until the problem is corrected and a successful calibration is performed on one assay in triplicate. (See **5.3**) Upon correction of the problem and a successful calibration the Key Operator or designee shall note in the instrument log that the instrument is "In Service."

5.2.3 Maintenance Schedule

5.2.3.1 This is a minimum maintenance schedule. Instrument use may necessitate additional maintenance as determined by the Tecan Key Operator or designee.

5.2.3.2 Daily/With-use –Prior to Analytical Run

5.2.3.2.1 Check syringes for leaks, bubbles or visual contamination. If required, clean the syringes taking care in removing syringes. If the syringes are leaking, replace the caps on the syringe plungers.

5.2.3.2.2 Check the valve and surrounding area for signs of moisture.

5.2.3.2.3 Check the green Teflon coating of the stainless steel tips for damage.

5.2.3.2.4 Check that there are no air bubbles or contamination in the instrument tubing. Tighten the tubing connections or replace the tubing as required.

5.2.3.3 Daily/With-use –After Analytical Run:

5.2.3.3.1 If more than one analytical run is performed on the same day, rinse washer between runs by performing a day rinse prompted directly from the washer.

5.2.3.3.2 Clean the Teflon sample tips by gently wiping it with a lint-free tissue containing isopropyl alcohol.

5.2.3.3.3 Empty the waste container and clean with dilute bleach.

5.2.3.3.4 Perform a night rinse prompted directly from the washer and turn the system off.

5.2.3.3.5 Remove racks from the instrument surface and carefully clean using isopropyl alcohol or a mild detergent.

5.2.3.4 Every Six Months

5.2.3.4.1 Wipe down the washer using isopropyl alcohol. Clean needle heads with appropriate probe.

5.2.3.5 Preventative maintenance shall be performed annually by an outside vendor.

5.3 Calibration and Verification

5.3.1 Calibration and verification samples shall be analyzed with every run.

5.3.2 Calibration and verification samples shall be prepared in a matrix equivalent to the case samples.

5.3.3 Allow all solutions to equilibrate to room temperature prior to use.

5.3.4 Calibration/Verification Sample Preparation

5.3.4.1 Negative Calibration Sample

5.3.4.1.1 Prepare a negative calibration sample as described in **5.6** using the equivalent negative matrix.

5.3.4.2 Prepared EIA Calibration Working Solution - Standards may be prepared in any amount provided that the component ratios are kept constant.

5.3.4.2.1 Add 0.050 mL of the appropriate EIA Calibration Solution to a test tube.

5.3.4.2.2 Add 2.45 mL of the equivalent negative matrix to the test tube.

5.3.4.2.2.1 The final concentration of **EIA Calibration Working Solution I** is 300 ng/mL phenobarbital, 50 ng/mL oxazepam, 50 ng/mL morphine, 50 ng/mL benzoylecgonine, 25 ng/mL methadone, and 20 ng/mL d-amphetamine.

5.3.4.2.2.2 The final concentration of the **EIA Working Calibration Solution II** is 25 ng/mL THC-COOH, 50 ng/mL cis-tramadol HCl, 20 ng/mL d-methamphetamine, 500 ng/mL carisoprodol, 20 ng/mL zolpidem, and 25 ng/mL oxycodone.

5.3.4.2.3 Cap and vortex the test tube.

5.3.4.2.4 Prepare calibration sample as directed in **5.6** on the day of use.

5.3.4.2.5 Dispose of any unused portion according to the State Crime Laboratory Safety Manual.

5.3.4.3 Prepared EIA Verification Working Solution - Standards may be prepared in any amount provided that the component ratios are kept constant.

5.3.4.3.1 Add 0.050 mL of the appropriate EIA Verification Solution to a test tube.

5.3.4.3.2 Add 2.45 mL of the appropriate negative matrix to the test tube.

5.3.4.3.2.1 The final concentration of the **EIA Verification Working Solution I** is 600 ng/mL phenobarbital, 100 ng/mL oxazepam, 100 ng/mL morphine, 100 ng/mL benzoylecgonine, 50 ng/mL methadone, and 40 ng/mL d-amphetamine.

5.3.4.3.2.2 The final concentration of the **EIA Verification Working Solution II** is 50 ng/mL THC-COOH, 100 ng/mL tramadol HCl, 40 ng/mL d-methamphetamine, 1000 ng/mL carisoprodol, 40 ng/mL zolpidem, and 50 ng/mL oxycodone.

5.3.4.3.3 Cap and vortex the test tube.

5.3.4.3.4 Prepare verification sample as directed in **5.6** on the day of use.

5.3.4.3.5 Dispose of any unused portion according to the State Crime Laboratory Safety Manual.

5.4 Critical Reagent QC Check

5.4.1 Prior to use with casework, each new lot of microplates, conjugates, negative blood/urine, calibration solution, and verification solution shall be evaluated by the Tecan Key Operator or designee to establish acceptability.

5.4.2 For each new lot of microplate/conjugate:

5.4.2.1 Pipette and analyze three A/B replicate samples from a Prepared EIA Calibration Working Solution (**5.3.4.2**) and a Prepared EIA Verification Working Solution (**5.3.4.3**) along with a negative calibration sample in accordance with **5.6.**

5.4.3 For each new lot of negative blood/urine:

5.4.3.1 Prepare three A/B replicate samples of the new standard lot being evaluated and analyze in accordance with **5.6.**

5.4.4 For each new lot of calibration solution and/or verification solution:

5.4.4.1 Pipette and analyze one A/B replicate pair from each of three Prepared EIA Calibration Working Solutions (**5.3.4.2**) and/or three Prepared EIA Verification Working Solutions (**5.3.4.3**) along with a negative calibration sample in accordance with **5.6.**

5.4.5 All results shall be analyzed statistically through the use of a Microsoft Excel spreadsheet. This spreadsheet shall determine the mean, standard deviation, and the % Coefficient of Variation (CV).

5.4.6 The verification shall meet the following acceptance criteria prior to approval for use with casework.

5.4.6.1 The % CV of the % b/b_0 shall be less than 20 %.

5.4.6.2 All acceptance criteria listed in sections **5.7.1**-**5.7.4**.

5.4.7 An electronic copy of the spreadsheet shall be placed into the Resource Manager in Forensic Advantage under the appropriate kit or reagent lot. The Toxicology Technical Leader shall approve the verification and document approval in the Resource Manager.

5.5 Instrument Setup

5.5.1 Check system liquid container and fill with deionized water as needed.

5.5.2 Prime the system and washer using the appropriate script(s).

5.5.3 Fill the appropriate reagent troughs with TMB substrate, TMBZ substrate and stop reagent. Ensure that no color develops.

5.5.3.1 Stop solution (1N HCl) may be prepared in-house and used in place of commercially available stop reagent.

5.5.4 Check and fill appropriate conjugate test tubes. Ensure that the enzyme conjugate lot matches the microplate lot being used.

5.5.5 Create an electronic plate layout and position the microplates and the conjugate test tubes to correspond.

5.6 Sample Preparation

5.6.1 Allow all samples to equilibrate to room temperature.

5.6.2 Ensure that all body fluids are homogenous by shaking and/or vortexing.

5.6.2.1 If a homogenous sample cannot be obtained, make a notation on the Immunoassay Sequence Log detailing the condition of the sample and its handling.

5.6.3 In duplicate, pipette 0.25 mL of each sample to be analyzed into a disposable glass test tube.

5.6.4 Using a pipette or liquid handler diluter, add 2.5 mL of deionized water into each tube.

5.6.5 Vortex for approximately 10 seconds. Ensure that there is no foam on the surface of the liquid.

5.6.6 Arrange the duplicate standard and case sample tubes into the appropriate sample racks in the following order:

- Negative Calibration Standard
- Calibration Standard
- Verification Standard
- Case samples
- Calibration II Standard (EVO 150)
- Verification II Standard (EVO 150)

5.6.7 For sequences involving case samples, a second person shall verify the sample placement on the instrument matches the corresponding sequence. Both people shall initial and date the sequence log.

5.6.7.1 This requirement is waived for instruments using a barcode scanner.

5.6.8 Follow steps outlined in the instrument's workstation operating manual.

5.6.9 Record instrument use in the instrument's activity log.

5.7 Acceptance Criteria

5.7.1 An inverse relationship exists between absorbance and concentration.

5.7.2 For each assay, evaluate the % b/b_0 value.

5.7.2.1 For % b/b_0 values that are less than or equal to the b/b_0 of the calibration standard, the result is positive.

5.7.2.2 For % b/b_0 values that are greater than the b/b_0 of the calibration standard, the result is negative.

5.7.3 Absorbance values for the A and B replicates must be within 20% of the mean absorbance.

5.7.4 Quality Control Acceptance Criteria

5.7.4.1 The calibration standard %b/b_0 must be less than 90%.

5.7.4.2 The verification standard % b/b_0 of an assay must be evaluated as positive.

5.7.4.3 For absorbance values of the individual replicates of quality control standards, the following must apply to each of the replicates:

$$Abs_{neg} > Abs_{cal} > Abs_{ver}$$

5.7.4.4 If the quality controls do not meet criteria in **5.7.3** or **5.7.4,** the affected assay will be re-analyzed and no data from the affected assay will be considered for analysis.

5.7.4.4.1 If three or more assays fail in a calibration/verification set, the entire set will be repeated and no data from the set will be considered for analysis.

5.7.5 Data Acceptance Criteria

5.7.5.1 If a case sample does not meet the requirement in **5.7.3**:

5.7.5.1.1 A negative result may be reported if both the A and B replicates of the sample have a higher absorbance than the EIA Calibration Standard mean absorbance.

5.7.5.1.2 A positive result may be reported if both the A and B replicates of the sample have a lower absorbance than the EIA Calibration Standard mean absorbance.

5.7.5.1.3 If **5.7.5.1.1** and **5.7.5.1.2** do not apply, results for the affected assay will not be included in the laboratory report and a note will be recorded in the case notes detailing how the affected assay was evaluated .

5.7.5.1.3.1 The affected assay will be evaluated by use of the appropriate extraction/ instrumental technique and will require additional aliquots for confirmation.

5.7.5.1.3.2 If **5.7.5.1.3** applies to more than one assay, the data will be evaluated by the Toxicology Technical Leader or designee to determine the appropriate action. This action will be documented in the case record.

5.7.6 Quality Control (QC) Data Packet

5.7.6.1 A QC data packet shall be created for all case sample analyses to include the following:

- Summary page with FA workstation reference
- Completed Immunoassay Sequence Log
- Instrument sequence list
- Instrument plate configuration printout
- Assay print outs showing the results of each calibration and verification standard

5.7.6.2 The QC packet will be named with a file name beginning with the name of the procedure (EIA) followed by the eight digit year/month/day format ending with the instrument name. A suffix may be added to differentiate multiple runs.

5.7.6.2.1 Example: EIA20151016YT1-XXX where “Y” will be the regional lab designation such as “R” for Raleigh and “T” is the designation for Tecan.

5.7.6.3 All Quality Control data packs must be administratively and technically reviewed prior to use of the associated case data for reporting. The review and approval will be indicated by signing the summary page.

5.7.6.4 QC packs shall be stored in the Managed Files section of the associated workstation and approved.

5.8 Reporting

5.8.1 The case record shall contain the following:

- Individual case reports showing each assay's % b/b_0

5.8.2 Refer to the technical procedure for Drug Toxicology Reporting.

5.9 Calculations

5.9.1 The % b/b_0 is determined by taking the average absorbance value of the sample duplicates and dividing by the average absorbance value of the negative calibration sample then multiplying by 100.

5.10 Uncertainty of Measurement – N/A

6.0 Limitations

6.1 This is a preliminary drug screen. Refer to the Immunalysis website (see references) for substances known to test positive and negative with this technique.

6.2 Cross reactivity and interference with the enzyme process may cause false positive and false negative results. Refer to *Principles of Forensic Toxicology*, 2nd edition, pages 119-139.

7.0 Safety

7.1 Refer to Laboratory Safety Manual.

7.2 Ensure that the instrument cover is in the down position when the instrument is in use.

8.0 References

Virginia DFS Toxicology Procedure Manual

Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology (2013)

Immunalysis ELISA Kit Inserts, Pomona CA.

Immunalysis Tecan Workstation Operating Manuals

Standard Operating Procedure for NaviTrak-OS, Immunalysis.

References located on the Immunalysis Corporation website.

Levine, Barry, ed. *Principles of Forensic Toxicology.* 2nd edition. AACC Press, 2006, 119-139.

9.0 Records

- EIA calibration data located in Quality Control packets
- FA resource workstation
- Case Record
- Tecan Instrument Activity Log
- Immunoassay Sequence Log

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
02/12/2016	1	Original document
02/22/2019	2	4.1 – Removed volume specification 4.3 – Added 1N HCl 4.4 – Removed concentration specification 4.6 – Removed purchased verification solutions 4.7 – Removed Immunalysis ELISA buffer 4.8 – Renamed and restructured, added multiplier for using 100 µg/ml solutions 4.8.4.1 – Inserted Oxazepam in place of Nordiazepam 5.2.1.1. – New, added instruction to store documents from outside vendors in FA 5.2.3 – Maintenance restructured and broken apart into daily/6 months 5.3 – Removed 5.3.3 – New, (moved from 5.3) 5.3.4 – Reworded for consistency, added allowance to prepare other volumes, changed standard preparation volumes, removed reference to purchased verification solutions 5.4.1 Removed reference to purchased verification solutions 5.4.2 – 5.4.4 Restructured and reworded for consistency.4 – New, separated from 5.4.3 and reworded for consistency 5.4.5 – New, consolidated from 5.4.2 and 5.4.3 5.4.6.2 – Reworded 5.5.2 – Reworded for new instrumentation 5.5.3.1 – New, added in-house stop solution 5.6 – Renamed 5.6.1 – New, (moved from 5.3) 5.6.2 – New, (moved from 5.3) 5.6.4 – Removed Immunalysis ELISA buffer 5.6.6 – Added Cal/Ver II for new instrumentation 5.6.7 – Inserted requirement for sequence verification 5.7.4.4.1 – New 5.7.5.1.2 - Replaced verification with calibration 5.7.5.1.3 – Inserted requirement for note in case notes, (moved from 5.8.7.2), broke apart for single or multiple assay replication evaluation 5.7.6 – Updated for renamed form 5.7.6.4 – New, added instruction to store QC pack in FA workstation 5.8.1 – Removed QC pack inclusion in case record 5.8.2 – Removed 9.0 – Added Immunoassay Sequence Log to records