Name of Procedure:

Toxicology
Use of the Syva® ETS EMIT Analyzer as a Drug Screen

Suggested Uses:

This procedure does not cover every aspect of the instrument used. The operator of the instrument should read the manual for the instrument before using this procedure.

The EMIT analyzer can be used to screen blood samples and other liquids for the presence of the following classes of compounds: cocaine and its metabolites, benzodiazepines, barbiturates, opiates, and metabolites of delta-9-THC.

The EMIT analyzer is designed to analyze urine, but can test blood / serum extracts and other clean liquids.

This is only a screening test. Any positive result must be confirmed using a more specific analysis, such as GC/MS or LC/MS before it can be reported. There are some cases of uncommon substances causing false positives using this test, which must be kept in mind when evaluating the results of this test.

Items Needed to Perform Procedure:

Test tubes,
Stoppers or caps
Vortexer
Centrifuge
Reservoir Filters
Glass boiling beads
Zymark TurboVap LV
Syva ETS EMIT Analyzer
2 mL analyzer cups and caps
Reagent kits for the Syva ETS EMIT, d.a.u. (Cocaine, Barbiturates, Opiates, Benzodiazepines, Cannabinoids (20ng))
EMIT calibrators (Level 0, A Level 1, A Level 2, delta-9 Cannabinoid 20 ng, delta-9 Cannabinoid 50 ng)
Daily Worksheet Syva ETS System (attachment)
Syva ETS System Maintenance Worksheet (attachment)
Automatic pipets, 1ml, 250 uL, 0.010 - 5 ml adjustable

Reagents Used in Procedure:

Phenobarbital, 1 mg/mL
Nordiazepam, 1 mg/mL
Morphine, 1 mg/mL
Benzoylecgonine, 1 mg/mL
Delta-9-carboxy-11-nor-delta-9-tetrahydrocannabinol (THC-COOH), 5 μg/mL
Deionized water
Acetone
Methanol
EMIT Drug Assay Buffer Solution

Stock Calibrator Solution:

1. To a 10 mL volumetric flask, add the following:
   0.5 mL Phenobarbital (1 mg/mL)
   0.025 mL Nordiazepam (1 mg/mL)
   0.025 mL Morphine (1 mg/mL)
   0.025 mL Benzoylecgonine (1 mg/mL)
   2.5 mL Delta-9-carboxy-11-nor-delta-9-tetrahydrocannabinol (5 μg/mL)
   dilute the flask to volume with methanol.

Stock Verification Solution:

Prepare a second solution identical to the working calibrator solution and label this solution the “Working Verification Solution”.

Calibrators:

1. Add 0.100 mL of the stock calibrator solution to the test tube.

2. Add 4.9 mL of a drug free matrix equivalent to the analyte(s) (water, blood, etc.) to the test tube.

3. Cap and vortex the test tube.

4. The concentration of the calibrator is 1000 ng/mL phenobarbital, 50 ng/mL nordiazepam, 50 ng/mL morphine, 50 ng/mL benzoylecgonine, 25 ng/mL THC-COOH.

Verification Sample:

Prepare a verification sample(s) identical to the calibrator solution using the stock verification solution.

Calibrators, Buffers, and Reagents Purchased From an Outside Agency:

1. Prepare Buffers as indicated on the vial, and place in buffer bottle.

2. Prepare the Calibrators and Reagents as indicated on the vial. Mark vials with the appropriate expiration dates.

3. Let the reconstituted reagents remain at room temperature for at least one hour before use.

4. When not in use cap with disposable stoppers and store at recommended temperature.

Instrument Set Up:

1. Warm up the instrument 30 minutes before using.

2. Check fluid levels of the buffer and water bottles in the instrument's cabinet and refill as needed. Ensure that the lines are near the bottoms of the correct bottles. Ensure that the waste bottle is empty.

3. Prime the system when it is in the Standby Mode by pressing the [prime] button and then [1] to prime the buffer and [2] to prime the water.

4. Check the paper by looking for the red stripe along the side of the paper, which indicates that the paper is low. Add paper if needed.

5. Allow the reagents to come to room temperature before using.

6. Seat the reagent rack into the system after removing the stoppers from the reagent vials. (The reagents must be arranged as shown on the calibration setup, from 1 to 5 consecutive: cocaine, barbiturates, benzodiazepines, opiates, cannabinoids (C20)).

7. Check that the cuvette compartment contains a cuvette strip with available wells. Replace during runs as needed.

Calibration of Instrument:

1. Set-up calibration:

    a. Press [CALIB SETUP]
    b. Select the assays to be calibrated. Press [*] to select all the assays displayed, or use the assay keypad to select displayed assays individually.
    c. Select the calibrator levels to be run. (Use #3: neg, cutoff, high.)
    d. Load at least 0.250 mL of each of the calibrating solutions into analyzer cups in a sample tray as listed on the print-out. Load sample tray into system.

2. Calibration:

    a. Press [RUN].
    b. After calibration is complete, for each run, ensure that the two CUT rates are within 14 units of each other, and that the "Cut-Neg Sep" and "Hi-Cut Sep" values are acceptable according to the Reagent Lot information.
    c. Calibration is to be done daily when the instrument is used.

Sample Preparation:

1. Urine samples and clear liquid samples:

    a. Pipet at least 0.250 mL of sample into appropriate sample cup.

2. Whole blood and Serum extraction

    Extract calibrators, blanks, and verification sample(s) with samples.

    a. Pipet 2.5 mL of acetone into each 16 x 125 mm disposable glass test tube.
    b. Pipet 1 mL of whole blood (case, std., or control) into the appropriate tube. Add the blood directly to the acetone. Do not run the blood down the side of the tube.
    c. Cap the tube with a stopper and vortex for approximately 10 seconds.
    d. Allow the tubes to stand for approximately 10 minutes and repeat the vortex step.
    e. Centrifuge the tubes for ten minutes at a minimum of 2500 rpm.

f. Place a 4 mL reservoir containing a frit into a clean 16 x 125 mm glass test tube.
g. Decant the supernatant from step 5 into the reservoir and allow it to completely drain into the test tube.
h. Add 0.5 mL acetone to each reservoir and allow it to drain into the tube.
I. Remove the reservoirs and add a glass boiling bead to each tube.
j. Ensure that water level is adequate in the TurboVap LV. Place the tubes in the TurboVap LV set at 50 ^{o}C and evaporate the liquids until the tubes are completely dry.
k. If the specimens are not going to be analyzed that day they can be sealed and placed in the refrigerator overnight.
l. Immediately prior to analysis, reconstitute the residues with 0.25 mL of a 1:1 methanol:EMIT Drug Assay Buffer Solution.
m. Vortex each tube.
n. Centrifuge the tubes for 10 minutes and transfer the supernatant to an EMIT analyzer cup with a disposable glass Pasteur pipet.
o. Cap the cups until the rack is placed on the analyzer to prevent evaporation of the methanol.

Application of Procedure on Evidence:

1. Sample Programming:

    a. Press [BATCH ENTRY]
    b. Select tray number to be programmed by pressing [SAMP TRAY] and then the sample tray number.
    c. Clear old sample IDs for that tray, if necessary, by pressing [SAMP TRAY] and then [CLEAR].
    d. Enter sample IDs for each cup position used.
    e. Move to the next cup position by pressing [ENTER].
    f. Press [EXIT] when done programming sample IDs.

2. Review Programming:

    a. Select sample tray by pressing [SAMP TRAY] and a number from 1 to 6 to display the desired sample tray.
    b. Check programming:
        1. Ensure that the correct sample IDs have been entered.
        2. Ensure that the correct assays have been selected for each sample.

3. Run Assays:

    a. Select sample tray by pressing [SAMP TRAY] and a number from 1 to 6 to display the desired sample tray.
    b. Press [RUN].

4. Results:

An assay is considered positive if the rate is within 14 units of the calibrator value used to determine the cutoff. The average of two cutoff calibrators values is used to determine the calibrator cutoff value. For every 20 samples a verification sample must be run in the analysis and it must have a rate that is between 90 and 110% of the calibrator. Unless at least one verifier meets the criteria then the entire set must be rerun. At least two blanks must be run during the analysis.

5. Data Record Keeping:

    Use EMIT Worksheet (see attached) or equivalent to record:
    1. Lot numbers of reagents and calibrators.
    2. Expiration dates of reagents and calibrators.
    3. Results of the calibrators, verification samples, blanks, and samples.

Safety Concerns:

Do not block the robotic arm of the system, since the sample needle and liquid sensor are easily broken.

When working with biohazardous samples use protective measures, such as gloves, eye protection, and work with the samples in a biosafety hood.

Maintenance:

The Daily, Weekly, and Monthly sections of the attached maintenance worksheet is performed and logged when the instrument is in use.

Literature References:

Syva ETS Plus System Operator's Manual, ver. 2.0, 1992.

Blood extraction procedure acquired from Georgia Bureau of Investigation.