**Lab 8-1 Name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Toxicology Per\_\_\_\_**

Modified from a Purdue Science Express Lab

You have been granted an internship to work at the FBI Forensics Lab as a toxicologist. Each group of 2 will develop a procedure for testing 3 samples. First you will use an immunoassay as a screening test. If any of the samples test positive then you will use High Performance Liquid Chromatography as a confirmatory test. If any of the samples test positive with the confirmatory test then you will have to determine if the concentration is high enough to be above the confirmatory threshold.

**Part A: Immunoassay**

**Watch this video** [**https://www.youtube.com/watch?v=70TPrfL\_8-M**](https://www.youtube.com/watch?v=70TPrfL_8-M)

**Write out a procedure for this portion of the lab below. You will NOT wash the sample at any time.**

Materials for the Screening Test

Microtiter plate coated with antibody 1

Urine samples

Antibody 2

Colorgenec enzyme substrate

Control 1 – positive

Control 2 – negative

**Part B: High Performance Liquid Chromatography**

Follow the procedure from the slip of paper you were provided based on the drug test you performed in Part A.

\*ALL SAMPLES MUST BE FILTERED PRIOR TO INJECTION\*

Materials for the Confirmatory Test

Drug standard to be used for calibration curve

Urine Sample

HPLC instrument

100L syringe

**Lab 8-1 Procedure**

**Simulated Cannabinoids**

Procedure

Making the THC standards

You will be provided with a 30.0 ng/ml stock solution of 9-carboxytetrahydrocannabinol that will be diluted into the following:

30.0 ng/mL

25.0 ng/mL

20.0 ng/mL

15.0 ng/mL

10.0 ng/mL

5.0 ng/mL

\*ALL SAMPLES MUST BE FILTERED PRIOR TO INJECTION\*

**Lab 8-1 Procedure**

**Simulated Cocaine**

Procedure

Making the Cocaine standards

You will be provided with a 300.0 ng/ml stock solution of benzoylecgonine that will be diluted into the following:

300.0 ng/mL

250.0 ng/mL

200.0 ng/mL

150.0 ng/mL

100.0 ng/mL

50.0 ng/mL

\*ALL SAMPLES MUST BE FILTERED PRIOR TO INJECTION\*

**Lab 8-1 Procedure**

**Simulated Methamphetamines**

Procedure

Making the methamphetamine standards

You will be provided with an 800.0 ng/ml stock solution of d-methamphetamine that will be diluted into the following:

800.0 ng/ml

666.7 ng/ml

533.3 ng/ml

400.0 ng/ml

266.6 ng/ml

133.3 ng/ml

\*ALL SAMPLES MUST BE FILTERED PRIOR TO INJECTION\*

**Lab 8-1 Procedure**

**Simulated Heroin**

Procedure

Making the Heroin standards

You will be provided with a 3000.0 ng/ml stock solution of 6-Acetylmorphine that will be diluted into the following:

3000.0 ng/ml

2500.0 ng/ml

2000.0 ng/ml

1500.0 ng/ml

500.0 ng/ml

\*ALL SAMPLES MUST BE FILTERED PRIOR TO INJECTION\*

**Lab Procedure**

**Simulated PCP**

Procedure

Making the phencyclidine standards

You will be provided with a 40.0 ng/ml stock solution of phencyclidine that will be diluted into the following:

40.0 ng/ml

33.3 ng/ml

26.7 ng/ml

20.0 ng/ml

13.3 ng/ml

6.7 ng/ml

\*ALL SAMPLES MUST BE FILTERED PRIOR TO INJECTION\*

**Lab 8-1 Procedure**

**Simulated Oxycodone**

Procedure

Making the Codeine standards

You will be provided with a 3000.0 ng/ml stock solution of 6-Acetylmorphine that will be diluted into the following:

3000.0 ng/ml

2500.0 ng/ml

2000.0 ng/ml

1500.0 ng/ml

500.0 ng/ml

\*ALL SAMPLES MUST BE FILTERED PRIOR TO INJECTION\*

TEACHER NOTES

**Part A (do NOT use samples from Part A for Part B)**

Have vials or dropper bottles for the samples. Use yellow ammonia for the urine sample that will test positive and match distilled water with yellow food dye (and a tiny bit of green) to match the color of the ammonia.

I tell students that the microtiter plate already has antibody 1 attached to the inside of each well.

Antibody 2 is just distilled water

Control 1 is clear ammonia (positive)

Control 2 is distilled water (negative)

Colorgenec enzyme substrates are just indicators that will change color in basic solution. They are all SLIGHTLY acidified so that they will change color when added to the ammonia. I used BTB, universal indicator, phenolphthalein and methyl red.

**Part B**

**Filter ALL samples before injection.**

**\*Preparation of Stock Solutions\***

I made stock solutions which were double the concentrations immediately below and had students dilute to create a calibration curve.

Caffeine: 0.30 mg/mL

Acetaminophen: 0.126 mg/mL

Aspirin: 0.115 mg/mL

**\*Sample Preparation\***

**Methamphetamine and Cocaine**

Black coffee diluted 1:1 with yellow water (with yellow food dye to make it look like urine)

Stock Standard = 0.015g caffeine in 50 mL volumetric

**Opiates (heroin, oxycodone, hydrocodone, morphine)**

0.0055g aspirin tablet in 50 mL volumetric (with yellow food dye to make it look like urine)

Stock Standard = 0.058 g acetylsalicylic acid in 50 mL volumetric

**THC and PCP**

0.0056g Tylenol tablet in 50 mL volumetric (with yellow food dye to make it look like urine)

Stock Standard = 0.0063g acetaminophen in 50 mL volumetric