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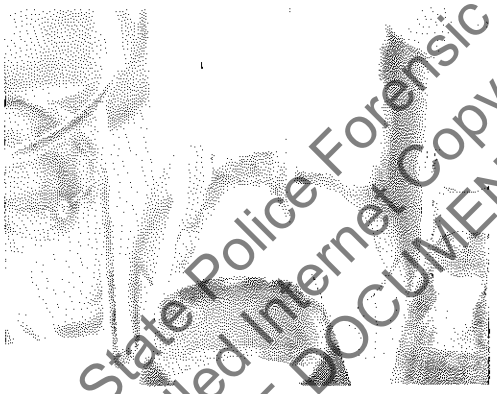

Discipline/Name of Document: Toxicology
2.2.1 Toxi-Lab® Toxi-A Drug Detection System

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Quality Manager

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Date Signed

Section Two
Urine Toxicology

2.2 Thin Layer Chromatography (TLC) Methods
2.2.1 Toxi-Lab® Toxi-A Drug Detection System

2.2.1.1 BACKGROUND

The TOXI-LAB® TOXI-A thin layer chromatography (TLC) drug detection system provides extraction, concentration, inoculation, elution, and visualization steps for the detection of basic and neutral drug compounds in urine speciments.[1] Addition of urine to the Toxi-A tubes results in the urine becoming alkaline. Basic and neutral compounds therefore extract into the tube's organic solvent mixture (1,2-Dichloroethane, Dichloromethane, Heptane and Isopropanol). The solvent is concentrated onto a BLANK TOXI-DISC Blank A. The dried disc is placed onto a TOXI-GRAM A for elution in a developing jar. The resulting position of drugs of interest are visualized by dipping the TOXI-GRAM into a series of solutions. The preliminary identification is based on matching the position of a drug (*Rf*) and visualization color characteristics with that of corresponding reference material. The TOXI-GRAM A includes reference compounds impregnated on preinserted discs. Additional TOXI-LAB discs from the reference collection may be used.

2.2.1.2 SCOPE

This is a summary method for the TOXI-LAB® TOXI-A thin layer chromatography (TLC) drug detection system. The system is used to screen for the presence of a wide variety of basic and neutral drug compounds in urine. The TOXI-A system provides a preliminary result that must be confirmed by GC-MSD.

2.2.1.3 EQUIPMENT AND SUPPLIES

- 2.2.1.3.1 Tube rocker
- 2.2.1.3.2 Laboratory centrifuge
- 2.2.1.3.3 Solvent concentrator with appropriate concentration cups or tubes
- 2.2.1.3.4 Electric (plate) warmer
- 2.2.1.3.5 Fixed and adjustable volume single channel air displacement pipetters, and appropriate tips, capable of accurate and precise dispensing of volumes indicated.
- 2.2.1.3.6 Chromatography jar with cap
- 2.2.1.3.7 Ultraviolet Light Viewer capable of 365nm
- 2.2.1.3.8 Forceps
- 2.2.1.3.9 Disc handling pins
- 2.2.1.3.10 Index cards for use as disc press cards

2.2.1.3.11 TOXI-GRAMS A
2.2.1.3.12 TOXI-DISCS Blank A
2.2.1.3.13 TOXI-LAB A Worksheets
2.2.1.3.14 TOXI-DIP A-1 Stand-off Jar
2.2.1.3.15 TOXI-DIP A-2 Dipping Jar
2.2.1.3.16 TOXI-DIP A-3 Dipping Jar
2.2.1.3.17 TOXI-DIP A-3 Stock Bottle
2.2.1.3.18 TOXI-LAB A Elution Solvent Bottle

2.2.1.4 REAGENTS

2.2.1.4.1 TOXI-TUBES A
2.2.1.4.2 Distilled/Deionized water
2.2.1.4.3 Ethyl acetate (TOXI-LAB Grade)
2.2.1.4.4 Ammonium Hydroxide (ACS Certified Grade)
2.2.1.4.5 Sulfuric Acid (ACS Certified Grade)
2.2.1.4.6 Methanol (ACS Certified Grade)
2.2.1.4.7 Glacial Acetic Acid (ACS Grade)
2.2.1.4.8 Formaldehyde (≅37%) (ACS Grade)
2.2.1.4.9 TOXI-DIP A Reagents
Store at room temperature.

2.2.1.4.9.1 **TOXI-DIP A-1 Formaldehyde Vapors**
Pipet approximately 25mL formaldehyde solution through an opening in the stand-off bottom of A-1 jar. Remove any liquid that ends up on the top surface with paper towel. Cap tightly. Replace solution weekly.

2.2.1.4.9.2 **TOXI-DIP A-2 Concentrated Sulfuric Acid**
Fill A-2 jar with sulfuric acid. Replace with fresh acid when contamination is apparent.

2.2.1.4.9.3 **TOXI-DIP H_20**
Fill H_20 jar with DI water. Water should be changed daily or after every 5 to 10 GRAMs.

2.2.1.4.9.4 **TOXI-DIP A-3 Modified Dragendorff's**
Empty contents of A-3 vial into A-3 jar or stock bottle. Add 10mL acetic acid. While stirring, add DI water to approximately ¼ inch from top. Cap tightly and mix. As reagent is used, replenish from stock.

2.2.1.4.8 Stock Elution Solvent

In TOXI-A Elution Solvent Bottle, mix 87mL ethyl acetate, 3mL methanol and 1.5mL DI water. Cap tightly and mix. Store at room temperature.

2.2.1.5 QUALITATIVE CONTROLS

2.2.1.5.1 Toxi-Control No. 19 and No. 2

2.2.1.5.2 Negative Urine

2.2.1.6 REFERENCE MATERIAL

2.4.1.6.1 TOXI-DISCS Libraries

2.4.1.6.2 TOXI-LAB Drug Compendium

2.2.1.7 METHOD

2.2.1.7.1 Extraction

2.2.1.7.1.1 Label TOXI-TUBES A for negative control, positive control and appropriate laboratory numbers.

2.2.1.7.1.2 Transfer 5mL of casework, negative and positive urine to appropriate TOXI-TUBE A.

2.2.1.7.1.3 Rock TOXI-TUBE A for ≥2 minutes.

2.2.1.7.1.4 Centrifuge tube at ≅2500 rpm for ≥2 minutes.

2.2.1.7.2 Concentration of Extract onto TOXI-DISC

2.2.1.7.2.1 Transfer solvent to heated evaporation cup or tube containing a TOXI-DISC Blank A.

2.2.1.7.2.2 Evaporate solvent to dryness.

2.2.1.7.3 Inoculation

2.2.1.7.3.1 Use disc handling pin to transfer disc to appropriate location on TOXI-GRAM A. Rub the inserted disc gently with clean press card.

2.2.1.7.3.2 Place TOXI-GRAM A on electric warmer with the disc end slightly off the edge. Heat for 30 to 60 seconds prior to elution.

2.2.1.7.4 Elution

2.2.1.7.4.1 Transfer 3mL elution solvent to chromatography jar. Add volume of ammonium

hydroxide indicted on TOXI-GRAMS A jar. Cap and swirl vigorously for a few seconds.

2.2.1.7.4.2 Place TOXI-GRAM A into chromatograph jar and cover. Make sure to not allow the side edges of the GRAM to touch the walls of the jar.

2.2.1.7.4.3 Allow solvent to migrate until the dye spots reach ≅9.5 cm. Remove the GRAM and place face down on electric warmer for 30 to 60 seconds until the fumes have evaporated.

2.2.1.7.5 Visualization

2.2.1.7.5.1 Place GRAM into TOXI-DIP A-1 jar for 5 to 30 minutes.

2.2.1.7.5.2 Remove GRAM and place the lower two-thirds on the warmer for no more than 5 seconds to remove some of the formaldehyde fumes.

2.2.1.7.5.3 Dip GRAM slowly in and out of TOXI-DIP A-2 jar. Hold GRAM over jar for 15 to 60 seconds until the green center of pseudoephedrine develops. Note color characteristics and position of specimen spot(s).

2.2.1.7.5.4 Dip GRAM in and out of water. Hold GRAM over jar for 3 to 5 seconds. Dip quickly once again. Allow blue color of imipramine to fully develop.

2.2.1.7.5.5 Continue dipping in and out of water noting the changing color characteristics of spots, until the morphine and codeine spots turn tan.

2.2.1.7.5.6 Lightly blot GRAM with paper towel to remove excess reagent. Observe GRAM under UV light (365nm). Compare fluorescence of specimen spot(s) with reference drug spots. Note observations.

2.2.1.7.5.7 Dip GRAM into TOXI-DIP A-3 jar for at least 10 seconds. Remove GRAM and note color characteristics and position of specimen spot(s).

2.2.1.7.5.8 Place GRAM into sheet protector and copy with laboratory photocopier.

2.2.1.7.6 Detection

2.2.1.7.6.1 Use location and color characteristics of reference material on gram and Drug Compendium to find corresponding data.

2.2.1.7.6.2 Based on the evaluation of data, additional GRAMs may be run with additional reference material discs.

2.2.1.7.7 Identification Criteria

2.2.1.7.7.1 The position *(Rf)* and color characteristics at each state of visualization of a spot noted for a specimen must correspond to that of reference material.

2.2.1.8 REFERENCES AND RECOMMENDED READING

2.2.1.8.1 Toxi-Lab® A Drug Detection System Instruction Manual, ©1989.

Revision History

Section Two
Urine Toxicology

2.2 Thin Layer Chromatography (TLC) Methods
2.2.1 Toxi-Lab® Toxi-A Drug Detection System

Revision #	Issue Date	Revision
0	10-18-2002	Included with SOPs with only reference to proprietary method.
1	05-07-2007	Full analytical method created.