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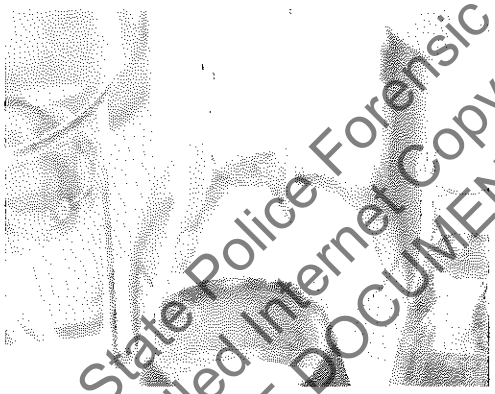

Discipline/Name of Document: Toxicology
2.2.2 Toxi-Lab® Toxi-B Drug Detection System

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Quality Manager

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Date Signed

Section Two
Urine Toxicology

2.2 Thin Layer Chromatography (TLC) Methods
2.2.2 Toxi-Lab® Toxi-B Drug Detection System

2.2.2.1 BACKGROUND

The TOXI-LAB® TOXI-B thin layer chromatography (TLC) drug detection system provides for extraction, concentration, inoculation, elution, and visualization steps for the detection of acidic and neutral drug compounds in urine specimens.[1] The addition of urine to the TOXI-B extraction tube results in acidic and neutral drug compounds being isolated from an acidified solution (pH=4.5) into an extraction solvent (methylene chloride and heptane with zinc chloride to facilitate the extraction process). The solvent is concentrated onto a TOXI-DISC Blank B. The dried disc is placed onto a TOXI-GRAM B 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.

2.2.2.2 SCOPE

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

2.2.2.3 EQUIPMENT AND SUPPLIES

2.2.2.3.1 Tube rocker
2.2.2.3.2 Laboratory centrifuge
2.2.2.3.3 Solvent concentrator with appropriate concentration cups or tubes
2.2.2.3.4 Electric (plate) warmer
2.2.2.3.5 Adjustable volume single channel air displacement pipetter capable of up to 50µL
2.2.2.3.6 Single channel air displacement pipetter capable of dispensing 3mL
2.2.2.3.7 Chromatography jar with cap
2.2.2.3.8 Ultraviolet Light Viewer capable of 365nm
2.2.2.3.9 Forceps
2.2.2.3.10 Disc handling pins
2.2.2.3.11 Index cards for use as disc press cards
2.2.2.3.12 TOXI-GRAMS B

2.2.2.3.13 TOXI-DISCS Blank B
2.2.2.3.14 TOXI-LAB B Worksheets
2.2.2.3.15 TOXI-DIP B-1 Dipping Jar
2.2.2.3.16 TOXI-DIP B-1 Stock Bottle
2.2.2.3.17 TOXI-DIP B-2 Dipping Jar
2.2.2.3.18 TOXI-DIP B-2 Stock Bottle
2.2.2.3.19 TOXI-DIP B-3 Dipping Jar
2.2.2.3.20 TOXI-DIP B-3 Stock Bottle
2.2.2.3.21 TOXI-LAB B Elution Solvent Bottle

2.2.2.4 REAGENTS

2.2.2.4.1 TOXI-TUBES B
2.2.2.4.2 Distilled/Deionized water
2.2.2.4.3 Ethyl acetate (TOXI-LAB Grade)
2.2.2.4.4 Ammonium Hydroxide (ACS Certified Grade)
2.2.2.4.5 Sulfuric Acid (ACS Certified Grade)
2.2.2.4.6 Dichloromethane (ACS Certified Grade)
2.2.2.4.7 TOXI-DIP B Reagents

Prepare dipping jar and stock bottle solutions for each. As reagent is used, replenish from stock. Store at room temperature.

2.2.2.4.7.1 **TOXI-DIP B-1 Diphenylcarbazone Solution**
Completely empty the contents of one B-1 chemical vial into the B-1 Dipping jar or stock bottle. Fill with dichloromethane to approximately ¼ inch from top. Cap tightly and mix.

2.2.2.4.7.2 **TOXI-DIP B-2 Silver Nitrate Solution**
Empty contents of B-2 solution into B-2 dipping jar or stock bottle. Fill with DI water to approximately ¼ inch from top. Cap tightly and mix.

2.2.2.4.7.3 **TOXI-DIP B-3 Mercuric Sulfate Solution**
Completely empty contents of B-3 vial into B-3 jar or stock bottle. Fill approximately ¾ full with DI water. While stirring add 10mL concentrated sulfuric acid. Add DI water to approximately ¼ inch from top. Cap tightly and mix.

2.2.2.4.8 Stock Elution Solvent
Mix 60mL dichloromethane and 40mL Ethyl Acetate in TOXI-B Elution Solvent Bottle.

2.2.2.5 QUALITATIVE CONTROLS

2.2.2.5.1 Toxi-Control No. 19 and No. 3

2.2.2.5.2 Negative Urine

2.2.2.6 REFERENCE MATERIAL

2.2.2.6.1 TOXI-DISCS B-1, B-2, B-3, and B-4.

2.2.2.6.2 TOXI-LAB Drug Compendium

2.2.2.7 METHOD

2.2.2.7.1 Extraction

2.2.2.7.1.1 Label TOXI-TUBES B for negative control, positive control and appropriate laboratory numbers.

2.2.2.7.1.2 Transfer 4.5 mL of casework, negative and positive urine to appropriate TOXI-TUBE B.

2.2.2.7.1.3 Rock TOXI-TUBE B for ≥2 minutes.

2.2.2.7.1.4 Centrifuge tube at 2500 rpm for ≥2 minutes.

2.2.2.7.2 Concentration of Extract onto TOXI-DISC

2.2.2.7.2.1 Transfer solvent to heated evaporation cup or tube containing a TOXI-DISC Blank B.

2.2.2.7.2.2 Evaporate solvent to dryness.

2.2.2.7.3 Inoculation

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

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

2.2.2.7.4 Elution

2.2.2.7.4.1 Transfer 3mL elution solvent to chromatography jar. Add volume of ammonium hydroxide indicted on TOXI-GRAMS B jar. Cap and swirl vigorously for a few seconds.

2.2.2.7.4.2 Place TOXI-GRAM B into chromatograph jar and cover. Make sure to not allow the side

edges of the GRAM to touch the walls of the jar.

2.2.2.7.4.3 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.

2.2.2.7.5 Visualization

2.2.2.7.5.1 Dip the GRAM in and immediately out of TOXI-DIP B-1. Allow GRAM to sit until all of the dichloromethane has evaporated.

2.2.2.7.5.2 Dip GRAM in and out of TOXI-DIP B-2 jar. Note color and position of reactions for specimen spot(s).

2.2.2.7.5.3 After a golden-brown background develops, place GRAM into TOXI-DIP B-3 jar and agitate until the background clears. Note color and position of reactions for specimen spot(s). Place GRAM into sheet protector and copy with laboratory photocopier.

2.2.2.7.5.4 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.

2.2.2.7.6 Detection

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

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

2.2.2.7.7 Identification Criteria

2.2.2.7.7.1 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.

2.2.2.8 REFERENCES AND RECOMMENDED READING

2.2.2.8.1 Toxi-Lab® B Drug Detection System Instruction Manual, ©1989.

Revision History

Section Two
Urine Toxicology

2.2 Thin Layer Chromatography (TLC) Methods
2.2.2 Toxi-Lab® Toxi-B Drug Detection System

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