Section Two
Urine Toxicology

## 2.2 ANSYS® Thin Layer Chromatography (TLC) Methods

### 2.2.4 Toxi-Lab® THC II-PLUS 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (Δ9-THC-COOH) Detection System

#### 2.2.4.1 BACKGROUND

The TOXI-LAB® THC thin layer chromatography (TLC) drug detection system provides for extraction, concentration, inoculation, elution, and visualization steps for the detection of Δ9-THC-COOH in urine specimens.[1] The preliminary identification is based on matching the position of a drug (*Rf*) and visualization color characteristics with that of corresponding reference material.

#### 2.2.4.2 SCOPE

This method is an option to screen for the presence of Δ9-THC-COOH in urine. The results serve to support the results of the enzyme immunoassay (EIA) screen or used in lieu of EIA screen. The TOXI-LAB THC II system provides a preliminary result that must be confirmed by GC-MSD.

#### 2.2.4.3 EQUIPMENT AND SUPPLIES

2.2.4.3.1 Tube rocker
2.2.4.3.2 Laboratory centrifuge
2.2.4.3.3 Solvent concentrator with appropriate concentration cups
2.2.4.3.4 Electric (plate) warmer
2.2.4.3.5 Fixed and adjustable volume single channel air displacement pipetters, and appropriate tips, capable of accurate and precise dispensing of volumes indicated.
2.2.4.3.6 Forceps
2.2.4.3.7 Disc handling pins
2.2.4.3.8 Index cards for use as disc press cards
2.2.4.3.9 TOXI-GRAMS Blank THC-II-PLUS
2.2.4.3.10 TOXI-GRAMS Blank THC-II
2.2.4.3.11 TOXI-DISCS THC
2.2.4.3.12 SPEC·C18·1 Extraction Cartridges
2.2.4.3.13 THC II Wash Reagent 1 Bottle
2.2.4.3.14 THC II Wash Reagent 2 Bottle
2.2.4.3.15 TOXI-DIP THC-1 Reagent Tank with Lid
2.2.4.3.16 TOXI-DIP THC-2 Reagent Tank with Lid
2.2.4.3.17 HCl Reagent Tank with Lid
2.2.4.3.18 Chromatography Tank with Lid (THC-II-PLUS)
2.2.4.3.19 Chromatography Jar with Lid (THC-II)
2.2.4.3.20 TOXI-LAB THC Elution Solvent Bottle

## 2.2.4.4 REAGENTS

***Refer to manual section 5.12 for solution preparation instructions not listed below.***

2.2.4.4.1 11.8N KOH

2.2.4.4.2 Methanol (ACS Grade)

2.2.4.4.3 Wash Reagent 1

**20% Acetic Acid**

2.2.4.4.4 Wash Reagent 2

20% **Methylene Chloride (ACS Grade)** in **n-Heptane (ACS Grade).**

2.2.4.4.5 TOXI-DIP THC-1 Fast Blue BB

Prepared with approximately 1g **Fast Blue BB Salt (Purified Grade).** Add Fast Blue BB to reagent tank for TOXI-DIP THC-1. Add approximately 700mL **Methylene Chloride (ACS Grade).** Solution should be pale yellow in color. Mix well.

Store at room temperature. Solution stable for 2 - 3 months.

2.2.4.4.6 TOXI-DIP THC-2 Diethylamine Fuming

Pipet 40mL **Diethylamine (DEA) (ACS Grade)** through an opening in the standoff to the bottom of the tank. Remove any DEA on standoff surface.

Store at room temperature. Replace DEA weekly.

2.2.4.4.7 Hydrochloric Acid Fuming

Pipet 40mL **Concentrated Hydrochloric Acid (ACS Grade)** through an opening in the standoff to the bottom of the tank. Remove any HCl on standoff surface.

Store at room temperature. Replace HCl weekly.

2.2.4.4.8 THC II Stock Elution Solvent

In THC II Elution Solvent Bottle, mix 50mL **n-Heptane (ACS Grade),** 50mL **Acetone (ACS Grade)** and 1mL **Glacial Acetic Acid (ACS Grade)**. Cap tightly and mix.

Store at room temperature.

## 2.2.4.5 REFERENCE MATERIAL

2.2.4.5.1 Positive Control

Positive Control can be prepared by adding specified amount of Working Control Solution to negative urine and/or obtained commercially. Use the same lot of negative urine to prepare positive control as used to prepare negative control.

2.2.4.5.1.1 **Stock Reference Solution**

100µg/mL (+) 11-nor-9-carboxy-$\Delta^9$-THC

2.2.4.5.1.2 **Working Reference Solution (1800ng/mL)**

Add 900µL Stock Solution to 49.1mL Methanol. Solution is stable for six months when stored at 4°C.

2.2.4.5.2 Negative Control
Negative Urine
May be either in-house verified or commercially obtained.

**2.2.4.6 PROCEDURE**

2.2.4.6.1 Initial set-up
Label extraction tubes and extraction cartridges for the negative control, positive control, and appropriate laboratory numbers.

2.2.4.6.2 60ng/mL Carboxy-THC Positive Control

2.2.4.6.2.1 Transfer 6mL of negative urine to extraction tube.

- Use the same lot of urine used for negative control.

2.2.4.6.2.2 200µL of working reference solution. Vortex.

2.2.4.6.3 Negative Control
Transfer 6mL of negative urine to extraction tube.

2.2.4.6.4 Casework Sample Preparation
Transfer 6 mL of casework urine specimen to extraction tube.

2.2.4.6.5 Sample Hydrolysis

2.2.4.6.5.1 To 6mL of urine, add 12 drops 11.8N KOH. Vortex.

2.2.4.6.5.2 Allow to hydrolyze for 10 minutes.

2.2.4.6.5.3 Add 1.5mL glacial acetic acid. Vortex.

2.2.4.6.6 Extraction

2.2.4.6.6.1 Condition cartridge with 1mL methanol. Aspirate at approximately 5 in. Hg. ***Do not allow the disc to dry.***

2.2.4.6.6.2 Add acidified samples to cartridge reservoirs. Aspirate such that the sample passes through the column no faster than 2mL/min.

2.2.4.6.6.3 Once the sample is completely through the reservoir, remove filter.

2.2.4.6.6.4 Add 1mL 20% acetic acid. Aspirate ≥2 minutes at 10-12 in. Hg.

2.2.4.6.6.5 Add 500µL wash reagent. Aspirate at 10-12 in. Hg.

2.2.4.6.6.6 After solvent has past through, allow to aspirate ≥2 minutes.

2.2.4.6.6.7 Remove disc from cartridge and place into a pre-heated concentrated cup to remove all residual moisture.

2.2.4.6.7 TLC

2.2.4.6.7.1 Place disc into labeled three or 10-channel TOXI-GRAM for THC-II.

2.2.4.6.7.2 Add THC-DISC THC disc.

2.2.4.6.7.3 Note: If not all channels are used, remove excess with razor blade or scissors.

2.2.4.6.7.4 Heat the GRAM, with the disc end slightly off the warmer edge, for 30-60 seconds.

2.2.4.6.7.5 Add 12.5mL of developing solution to chromatography tank (10-channel) or 3mL solution to chromatography jar (3-channel).

2.2.4.6.7.6 Place gram into chromatography tank or jar. Allow dye marker to migrate to ≅ 4 cm. [***This only takes 2-3 minutes***]

2.2.4.6.7.7 Remove GRAM from tank/jar and place face down on warmer for 1-2 minutes.

2.2.4.6.7.8 Dip GRAM into TOXI-DIP THC-1, hold to dry until GRAM becomes speckled.

2.2.4.6.7.9 Place GRAM into TOXI-DIP THC-2 until scarlet spots develop.

2.2.4.6.7.10 Place GRAM in hood so that the Diethylamine (DEA) can evaporate. If any DEA is present when the HCl is added, fuming will occur.

2.2.4.6.7.11 For HCl fuming, either of the following options may be pursued. Option 2 will produce a more intense color which photocopies better.

Option 1: Place GRAM into HCl Fuming Tank until a purple spot develops. Place GRAM into page protector, label and photocopy.

Option 2: Place GRAM on to a page protector. With bulb pipet, add concentrated HCl to just cover GRAM. Note desired color change to deep purple. Label and photocopy GRAM.

2.2.4.6.7.12 Place a copy of GRAM into each associated casefile.

**2.2.4.7 DETECTION AND IDENTIFICATION CRITERIA**

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

**2.2.4.8 REFERENCES AND RECOMMENDED READING**

2.2.4.8.1 Toxi-Lab® THC II 11-nor-Δ9-Tetrahydrocannabinol-9-COOH Detection System Instruction Manual, ©1998.

2.2.4.8.2 Toxi-Lab® THC II-PLUS 11-nor-Δ9-Tetrahydrocannabinol-9-COOH Detection System Instruction Manual, ©1998.

# *Revision History*

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| Revision No. | Issue Date | Comments |
|---|---|---|
| 0 | 10/1991 | Original Issue |
| 1 | 11-27-2001 | Introduction into Reformatted SOP Manual |
| 2 | 04-25-2002 | THC-II Method Summary Added |
| 3 | 10-18-2002 | Refinements, other TLC methods added to alternative method binder |
| 4 | 05-07-2007 | Reformat, c-THC TLC only |
| 5 | 07-28-2008 | Clarified that negative urine used to prepare positive control must be the same lot as used for negative control. |