

Idaho State Police

Forensic Services

Approval for Quality System Controlled Documents



Discipline/Name of Document: Toxicology

2.2.4 Toxi-Lab® THC II-PLUS 11-nor- Δ^9 -tetrahydrocannabinol-9-COOH
acid (Δ^9 -THC-COOH) Detection System

Revision Number: 4

Issue Date: 5/07/2007

APPROVED BY:

Courina C. Purdy
Quality Manager

5/7/07
Date Signed

Section Two

Urine Toxicology

2.2 ANSYS[®] Thin Layer Chromatography (TLC) Methods

2.2.4 Toxi-Lab[®] THC II-PLUS 11-nor- Δ 9-tetrahydrocannabinol-9-COOH acid (Δ 9-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 11-nor- Δ 9-tetrahydrocannabinol-9-COOH (Δ 9-THC-COOH) in urine specimens.¹ The preliminary identification is based on matching the position of a drug (*R_f*) and visualization color characteristics with that of corresponding reference material.

2.2.4.2 SCOPE

The method is an option to screen for the presence of 11-nor- Δ 9-tetrahydrocannabinol-9-COOH in urine. The results serve to support the results of the enzyme immunoassay (EIA) screen or used in lieu of a 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 or tubes
- 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 prepared with **Glacial Acetic Acid (ACS Grade)** and **Distilled/Deionized water**
- 2.2.4.4.4 Wash Reagent 2
Prepared with **n-Heptane (ACS Grade)** and **Methylene Chloride (ACS Grade)** (80:20).
- 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 is stable for 2 - 3 months. Replenish from stock.
- 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.

2.2.4.5.1.1 Stock Reference Solution

100µg/mL (+) 11-nor-9-carboxy- Δ^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

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 Positive Control

For a 60ng/mL Positive Control, add 200µL of working reference solution to 6mL of negative urine in an extraction tube. 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 500mL 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 $\cong 4$ 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 (*R_f*) 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- Δ^9 -Tetrahydrocannabinol-9-COOH Detection System Instruction Manual, © 1998.

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

Property of Idaho State Police Forensic Services
Uncontrolled Internet Copy
OBSOLETE DOCUMENT

Revision History

Section Two

Urine Toxicology

2.2 ANSYS® Thin Layer Chromatography (TLC) Methods

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

Revision #	Issue Date	Revision
0	10/91	Original Issue
1	11-27-01	Introduction into Reformatted SOP Manual
2	04-25-02	THC-II Method Summary Added
3	10-18-02	Refinements, additional methods Added to alternative method binder
4	05-07-2007	Reformat, c-THC TLC only.

Property of Idaho State Police Forensic Services
Uncontrolled Internet Copy
OBSOLETE DOCUMENT