

Idaho State Police  
 Forensic Services  
 Toxicology Section

Section Two  
 Urine Toxicology

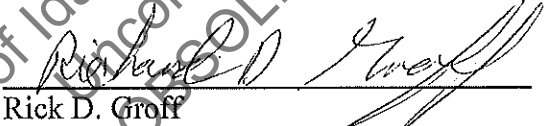
2.2 ANSYS® Thin Layer Chromatography (TLC) Methods

Revision #	Issue Date	History
0	10/91	Original Issue
1	11-27-01	Introduction into Reformatted SOP Manual
2	04-25-02	THC-II Method Summary Added

Approval

Technical Leader:  Date: 04/25/02  
 S C Williamson

Issuance

QC Manager:  Date: 4-26-02  
 Rick D. Groff

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

**2.2.4 Toxi-Lab® THC II-PLUS 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid ( $\Delta^9$ -THC-COOH) Detection System**

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**2.2.4.2 SUMMARY PROCEDURE**

**2.4.4.2.1 STANDARDS**

2.4.4.2.1.1 Stock Standard Solution  
100 $\mu$ g/mL (+) 11-nor-9-carboxy- $\Delta^9$ -THC  
(Radian T-006 or equivalent).

2.4.4.2.1.2 Working Standard Solution (1800ng/mL)  
Add 900 $\mu$ L Stock Solution to 49.1mL  
Methanol. Solution is stable for six months  
when stored at 4°C.

**2.4.4.2.2 CONTROLS**

2.4.4.2.2.1 60ng/mL Carboxy-THC Positive Control Urine

Add 200 $\mu$ L of working standard solution to  
6mL of negative urine and mix.

2.4.4.2.2.2 Negative Control Urine (FS Personnel).

**2.4.4.2.3 PROCEDURE**

2.4.4.2.3.1 Initial set-up

Label sample tubes and extraction cartridges  
for the negative control (NC), positive  
control (PC), and appropriate laboratory  
numbers without prefix.

2.4.4.2.3.2 Sample Preparation

Transfer 6 mL of urine specimen, negative  
control urine, and positive control urine to  
test tube.

2.4.4.2.3.3 Sample Hydrolysis

- To 6mL of urine, add 12 drops 11.8N KOH.
- Vortex
- Allow to hydrolyze for 10 minutes.
- Add 1.5mL glacial acid.
- Vortex

2.4.4.2.3.4 Extraction

- Condition cartridge with 1mL methanol. Aspirate at approximately 5 in. Hg. *Do not allow the disc to dry.*
- Add acidified samples to cartridge reservoirs. Aspirate such that the sample passes through the column no faster than 2mL/min.
- Once the sample is completely through the reservoir, remove filter.
- Add 1mL 20% acetic acid.
- Aspirate  $\geq 2$  minutes at 10-12 in. Hg.
- Add 500mL wash reagent.
- Aspirate at 10-12 in. Hg.
- After solvent has past through, allow to aspirate  $\geq 2$  minutes.
- Remove disc from cartridge and place into a pre-heated concentrated cup to remove all residual moisture.

## 2.4.4.2.3.5

TLC

- Place disc into labeled three or 10-channel TOXI-GRAM for THC-II.
- Add THC-COOH standard disc.
- Heat the GRAM, with the disc end slightly off the warmer edge, for 30-60 seconds.
- Add 12.5mL of developing solution to chromatography tank (10-channel) or 3mL solution to chromatography jar (3-channel).
- Place gram into chromatography tank or jar. Allow dye marker to migrate to  $\cong 4$  cm. [*This only takes 2-3 minutes*]
- Remove GRAM from tank/jar and place face down on warmer for 1-2 minutes.

- Dip GRAM into TOXI-DIP 1, hold to dry until GRAM becomes speckled.
- Place GRAM into TOXI-DIP THC-2 until scarlet spots develop.
- Place GRAM in hood so that the diethylamine (DEA) can evaporate. If any DEA is present when the HCl is added, fuming will occur.
- Place GRAM on to a page protector. With bulb pipet, add concentrated HCl to just cover GRAM. Note desired color change to deep purple.
- Close page protector and label samples and controls.
- Photocopy GRAM, with header information, for each casefile.

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