Weeding Through the Information: Interpreting Laboratory Tests to Determine Second-hand, Recreational, or Medical Use of Marijuana

Paul J. Jannetto, Ph.D., DABCC, FACB, MT(ASCP)
Mayo Clinic
Director, Toxicology & Drug Monitoring Laboratory
Director, Metals Laboratory

Disclosures

• Relevant Financial Relationships:
  • None

• Off Label Usage:
  • None

Objectives

• After this session, the participant will be able to:

  1. Summarize the challenges and limitations of traditional immunoassays and confirmatory tests for synthetic and natural cannabinoids.
  2. Discuss the medical usefulness of cannabinoids and its impact on laboratory testing.
  3. Describe how laboratory testing can be used to identify recent cannabinoid use.
Case #1: A Joint in the Joint

- 25 yo White male
- Arrested for use/sale of marijuana
- Incarcerated 3 months
- Routine DAU testing was performed
  - Amphetamine: Negative (Cutoff = 500 ng/mL)
  - Cannabinoids: Positive (Cutoff = 50 ng/mL)
  - Cocaine: Negative (Cutoff = 300 ng/mL)
  - Opiates: Negative (Cutoff = 300 ng/mL)
  - PCP: Negative (Cutoff = 25 ng/mL)

Case #1 continued

- Is the prisoner still using marijuana?
  1. Yes, the results definitely show the prisoner is still using THC since it is present after 3 months of incarceration.
  2. Since the inmate is obese, it is likely only residual marijuana and doesn’t represent new usage.
  3. No, it is likely due to second-hand smoke from the other inmates/guards.
  4. Unable to determine, need to do additional laboratory testing.
Marijuana: It's Not Just For Brownies Anymore
Medicinal vs. Recreational
Natural vs. Synthetic

History of Marijuana

1500 BC: Earliest Written Reference to Medical Marijuana in Chinese Pharmacopeia
1850: Marijuana added to US Pharmacopeia
1911: Massachusetts 1st state to outlaw cannabis
1942: Marijuana removed from US Pharmacopeia
1970: Controlled Substance Act classifies marijuana as schedule I with "no acceptable medical use"
1976: Netherlands decriminalizes marijuana
1985: Marinol® (Schedule III) approved by the FDA
1996: California first state to legalize medical marijuana
2012: Colorado & Washington legalize marijuana

Medical Marijuana-California

• Proposition 215 passes Nov. 5, 1996 (56% majority)
  • Removes state-level criminal penalties on the use, possession and cultivation of marijuana by patients who possess a "written or oral recommendation" from their physician that he or she "would benefit from medical marijuana."
• Approved Conditions:
  • AIDS
  • Anorexia
  • Arthritis
  • Cachexia
  • Cancer
  • Chronic pain
  • Glaucoma
  • Migrane
  • Persistent muscle spasms, including MS
  • Seizures
  • Severe nausea
  • Other chronic or persistent medical symptoms
State Medical Marijuana Laws

<table>
<thead>
<tr>
<th>State</th>
<th>Year Passed</th>
<th>Possession Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alaska</td>
<td>1996</td>
<td>1 oz, edible 6 plants (3 mature, 3 immature)</td>
</tr>
<tr>
<td>Arizona</td>
<td>2010</td>
<td>2.5 oz, edible 5-12 plants</td>
</tr>
<tr>
<td>California</td>
<td>1996</td>
<td>2 oz, edible 6 plants (3 mature, 3 immature)</td>
</tr>
<tr>
<td>Colorado</td>
<td>2000</td>
<td>2 oz, edible 6 plants (3 mature, 3 immature)</td>
</tr>
<tr>
<td>Connecticut</td>
<td>2012</td>
<td>1 oz, edible 10-30 plants</td>
</tr>
<tr>
<td>Delaware</td>
<td>2011</td>
<td>6 oz, edible 7 plants (3 mature, 3 immature)</td>
</tr>
<tr>
<td>District of Columbia</td>
<td>2012</td>
<td>6 oz, edible 7 plants (3 mature, 3 immature)</td>
</tr>
<tr>
<td>Hawaii</td>
<td>2000</td>
<td>5 oz, edible, 7 plants (3 mature, 3 immature)</td>
</tr>
<tr>
<td>Maine</td>
<td>1999</td>
<td>2 oz, edible 6 plants</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>2012</td>
<td>10,000 oz, edible 10-30 plants</td>
</tr>
<tr>
<td>Michigan</td>
<td>2000</td>
<td>2 oz, edible, 4 plants (4 mature, 4 immature)</td>
</tr>
<tr>
<td>Montana</td>
<td>2009</td>
<td>2 oz, edible, 4 plants (4 mature, 4 immature)</td>
</tr>
<tr>
<td>Nevada</td>
<td>2000</td>
<td>2 oz, edible, 4 plants (4 mature, 4 immature)</td>
</tr>
<tr>
<td>New Jersey</td>
<td>2010</td>
<td>2 oz, edible</td>
</tr>
<tr>
<td>New Mexico</td>
<td>2007</td>
<td>12 oz, edible</td>
</tr>
<tr>
<td>New York</td>
<td>2007</td>
<td>12 oz, edible</td>
</tr>
<tr>
<td>Ohio</td>
<td>2006</td>
<td>25 oz, edible</td>
</tr>
<tr>
<td>Rhode Island</td>
<td>2004</td>
<td>6 oz, edible 5 plants (3 mature, 3 immature)</td>
</tr>
<tr>
<td>Vermont</td>
<td>2009</td>
<td>2 oz, edible 5 plants (3 mature, 3 immature)</td>
</tr>
<tr>
<td>Washington</td>
<td>1998</td>
<td>2 oz, edible 5 plants</td>
</tr>
</tbody>
</table>

With the passage of state medical marijuana laws, the patient or caregiver may cultivate up to 6 marijuana plants in an indoor, indoor setting.

Synthetic Medical Formulations

<table>
<thead>
<tr>
<th>Compound</th>
<th>FDA Approved</th>
<th>Indications</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dronabinol</td>
<td>Yes</td>
<td>• Prophylaxis of chemotherapy-induced nausea &amp; vomiting</td>
<td></td>
</tr>
<tr>
<td>(Marinol®)</td>
<td></td>
<td>• AIDS-related loss of appetite</td>
<td>Oral capsules</td>
</tr>
<tr>
<td>Nabiximols</td>
<td>Yes</td>
<td>• Second-line treatment of chemotherapy-induced nausea &amp; vomiting</td>
<td></td>
</tr>
<tr>
<td>(Sativex®)</td>
<td></td>
<td>• Second-line treatment of spasticity in adults w/ multiple sclerosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>• Cancer pain</td>
<td>Cannabis derived liquid extract as an oromucosal spray</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Neuropathic pain in MS patients</td>
<td></td>
</tr>
</tbody>
</table>

Marijuana Facts

- Prevalence: Most commonly used illicit drug in the United States
- Source: Dried flowers, stems, & leaves of Cannabis sativa (hemp plant)
- Street Names: Blunt, dope, grass, herb, joint, Mary Jane, pot, reefer, skunk, weed
- Effects attractive to abusers: Euphoria, intensified sensual and aesthetic perceptions
- Main Active Ingredient: Delta-9-tetrahydrocannabinol (THC)
- Route of Administration: Ingested (Oral), Smoked
- Bioavailability: Smoked 10-25%, Oral 5-20%
- Peak Concentration: Smoked Within minutes, Oral 1-3 hours
- Elimination t1/2: Smoked 30 hours, Oral 25 hours

Pharmacotherapy 2013;33(2):195-09
Metabolism of Marijuana

Pharmacodynamics

- Delta-9-THC (most psychoactive)
  - ↑ Heart rate
  - ↑ Euphoria
  - ↓ Alertness
  - ↓ Motor instability

- Adverse effects:
  
<table>
<thead>
<tr>
<th>Short-term Use</th>
<th>Long-term or Heavy Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Impaired short-term memory</td>
<td>- Addiction</td>
</tr>
<tr>
<td>- Impaired motor coordination</td>
<td>- Mental health impairment</td>
</tr>
<tr>
<td>- Altered judgment</td>
<td>- Effects on education</td>
</tr>
<tr>
<td>- Psychosis</td>
<td>- Cognitive impairment</td>
</tr>
<tr>
<td>- Pain modulator</td>
<td>- Decreased libido</td>
</tr>
</tbody>
</table>

- Addictive:
  - 1 in 11 become dependent
  - In 2011, 4.2 million met diagnostic criteria for dependence or abuse

Pharmacodynamics Continued

- CB1 receptors
  - Basal ganglia (motor activity)
  - Cerebellum (motor coordination)
  - Hippocampus (short-term memory)
  - Neocortex (thinking)
  - Hypothalamus, Limbic cortex (appetite and sedation)
  - Periaqueductal gray (pain modulator)
  - Immune cells

- CB2 receptors
  - Immune cells (Leukocytes)
  - Tissues
  - Brain on microglia (Alzheimer’s Disease)
Laboratory Testing for Cannabinoids

- Traditional Urine Immunoassay Screen:
  - Target: 11-nor-delta9-THC-9-carboxylic acid (THC-COOH)
  - Cutoffs: 50 ng/mL, 20 ng/mL
- Detection Time (Urine):
  - 2-30+ days (depends on usage duration/frequency, potency/dosage, rate of metabolism, detection limit/sensitivity, etc...)
- False Positives (Urine Immunoassays):
  - Ibuprofen (old immunoassays)
  - Efavirenz (EFV): specifically EFV 8-glucuronide
- Passive Exposure and Urine Immunoassays:
  - Amount depends on size/ventilation of room
  - Amount of Cannabis smoked
  - May result in positive immunoassay (20 ng/mL cutoff)

Elsohly MA. Drug testing in the workplace: Could a positive test for one of the mandated drugs be for reasons other than illicit use of the drug? J. Anal Toxicol. 1995;19:450-58.

Dronabinol (Marinol®) vs. Marijuana

- Common markers/metabolites:
  - Delta9-tetrahydrocannabinol (THC)
  - 11-nor-delta9-THC-9-carboxylic acid (THC-COOH)
- Unique markers/metabolites:
  - Delta9-tetrahydrocannabinol (THCV) – C3 homologue of THC
  - Natural component of cannabis, doesn’t exist in Marinol
  - THCV metabolizes to 11-nor-delta9-tetrahydrocannabinol-9-carboxylic acid (THCV-COOH)


New vs. Residual Marijuana Use

- Consequences of multiple positive urine cannabinoid tests:
  - Discharge from drug treatment program
  - Loss of employment
  - Loss of child custody
  - Incarceration (higher penalties)
  - Martial punishment (military) more severe if multiple usage is established
- Predictive Models:
  1. Less than daily usage:
     - Urine creatinine normalized THC-COOH concentrations, U2/U1 ratios and time intervals between specimen collections
  2. Chronic, daily usage:
     - Urine creatinine normalized THC-COOH concentrations and time intervals between specimen collections

New vs. Residual Marijuana Use
Less Than Daily Users

• Original Model:
  • Normalize to urine creatinine (accounts for varying hydration)
  • (THC-COOH concentration (ng/mL)/creatinine (mg/dL))*100
  • Use ratio of second sample (U2) collected >24 hr to first sample (U1) with a detection limit of 15 ng/mL THC-COOH
  • U2/U1 ratio ≥ 1.5 = new usage
    • ~95% accuracy, 5.5% false positive, 7.4% false negative
  • U2/U1 ratio ≥ 2.5 = new usage
    • ~74% accuracy, 0.1% false positive rate, 24% false negative rate

• Updated Model with time intervals between collections:
  • Normalized ratios compared to time between paired collections
    • 0-23.9 hrs, 24-47.9 hrs, 48-71.9 hrs, 72-95.9 hrs, 96-119.9 hrs, 120-143.9 hrs, and 144-167.9 hrs.


Case #1 continued

• THC-COOH & Cr concentrations in 3-urine samples were obtained (detection limit 15 ng/mL THC-COOH)

<table>
<thead>
<tr>
<th>Specimen</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection Date &amp;Time</td>
<td>11-6-07, 08:00</td>
<td>11-7-07, 09:30</td>
<td>11-11-07, 08:30</td>
</tr>
<tr>
<td>THC-COOH (ng/mL)</td>
<td>250</td>
<td>300</td>
<td>180</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>52</td>
<td>100</td>
<td>35</td>
</tr>
<tr>
<td>THC-COOH/Cr (ng/mg)</td>
<td>481</td>
<td>300</td>
<td>514</td>
</tr>
<tr>
<td>U2/U1 Ratio</td>
<td>NA</td>
<td>0.62</td>
<td>1.71</td>
</tr>
</tbody>
</table>

New vs. Residual Marijuana Use
Chronic, Daily Users

• Model used initial urine THC-COOH normalized to Creatinine (ng/mg)
• U2/U1 ratios collected 2-30 days apart


New vs. Residual Marijuana Use
Chronic, Daily Users

Rule 1: If cannabis reuse is predicted by first/second specimens, it is possible that the last cannabis use was recent and peak urine THC-COOH may have not yet occurred, so use another specimen ≥48 hrs later

Rule 2: If urinary CN-THC-COOH is ≥800 ng/mg in specimen 1 and still ≥200 ng/mg on day 5, collect urine 15 days from day 1 if new usage is predicted


Synthetic Cannabinoids
Synthetic cannabinoids (SCB)

- Originally synthesized as research tools to explore endocannabinoid system and as potential therapeutics
- Sold as "potpourri" or "legal highs"
- Street slang: K2, Spice
- Chemicals designed to have CB1/CB2 binding properties

<table>
<thead>
<tr>
<th>CB1 Effects</th>
<th>CB2 Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced nausea</td>
<td>Reduced inflammation</td>
</tr>
<tr>
<td>Increased appetite</td>
<td>Decreased pain perception</td>
</tr>
<tr>
<td>Improved mood, euphoria</td>
<td></td>
</tr>
</tbody>
</table>

- Potency: 2-100 X more potent than delta9-THC
- >50 SCB identified in US
- Not detectable with standard THC screening tests

Castaneto MS et al. Drug and Alcohol Dependence 2014;144:12-41
Su M et al. Metabolism of classical cannabinoids and the synthetic cannabinoid JWH-018 Clin Pharmacol Ther 2015;

Laboratory Challenges With Synthetic Cannabinoids

- Methodology (Laboratory Developed Tests):
  - GC/MS
  - LC-MS/MS
- Rapidly changing Targets:
  - JWH018
  - XLR 11
  - UR144
  - Etc...
- Interpretation of a "Negative" Result:
  - No recent synthetic THC usage
  - Used another synthetic THC not tested for

Summary

• Marijuana most widely used illicit drug:

• Laboratory Challenges:
  • Passive vs Active Exposure: Depends on cutoff (20 ng/mL vs 50 ng/mL)
  • Medical (Marinol®) vs. Recreational Use:
    • Delta9-tetrahydrocannabinol (THCV) – C3 homologue of THC
    • 11-nor-delta9-tetrahydrocannabinol-9-carboxylic acid (THCV-COOH)
  • Natural vs Synthetic:
    • >50 synthetic cannabinoids, constantly changing structures/targets
    • Traditional immunosassays don’t pickup synthetic THC
    • Lack of commercially available reference standards for LDTs
  • New vs Residual:
    • Creatinine normalized THC-COOH (ng THC-COOH/mg Cr)
    • U2/U1 ratios


Questions & Discussion
Self-Assessment Question #1

A 35 year old male in a drug treatment program for marijuana has two urine specimens collected ~24 hours apart after being in the program for 1 month. The provider wants to know if the patient continues to be abstinent or is still actively using marijuana.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection Date &amp; Time</td>
<td>4-6-15, 08:00</td>
<td>4-7-15, 08:30</td>
</tr>
<tr>
<td>THC-COOH (ng/mL)</td>
<td>650</td>
<td>810</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>95</td>
<td>120</td>
</tr>
<tr>
<td>THC-COOH/Cr (ng/mg)</td>
<td>684</td>
<td>675</td>
</tr>
<tr>
<td>U2/U1 Ratio</td>
<td>NA</td>
<td>0.99</td>
</tr>
</tbody>
</table>

* LC-MS/MS assay detection limit for THC-COOH is 6 ng/mL.

Self-Assessment Question #1 Continued

A. Results suggest no new marijuana usage
B. Results suggest new marijuana usage
C. Need to repeat the test again at 48 hours
D. Unable to determine, patient’s creatinine is too low

Self-Assessment Question #2

A 48 year-old woman living in California with chronic pain is prescribed Marinol®. The physician wants to determine if she is also using other sources of marijuana and orders a cannabinoid screen and confirmation assay. How would you interpret the following results?

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Result</th>
<th>Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine THC-COOH screen</td>
<td>Positive (Cutoff &gt;50 ng/mL)</td>
<td>Negative</td>
</tr>
<tr>
<td>Urine THC-COOH confirmation (LC-MS/MS)</td>
<td>&gt;6400 ng/mL, THC-COOH</td>
<td>&lt;46 ng/mL</td>
</tr>
</tbody>
</table>

A. Patient is compliant and only taking Marinol®
B. Patient is definitely not compliant and using other sources of marijuana
C. Patient is using other sources of marijuana and Marinol®
D. Unable to determine from the test results
Self-Assessment Question #3

A 16 year-old male insists that his positive drug test for marijuana is due to second-hand (passive) exposure from his best friend who drives him to school daily. How should the physician/parents interpret the following test results?

- A. The results are consistent with passive exposure to marijuana
- B. The results are not consistent with passive exposure to marijuana
- C. The results likely represent a false-positive
- D. Collect a second sample >24 hours later and repeat testing

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Result</th>
<th>Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine THC-COOH screen</td>
<td>Positive (Cutoff 50 ng/mL)</td>
<td></td>
</tr>
<tr>
<td>Urine THC-COOH confirmed</td>
<td>425 ng/mL THC-COOH</td>
<td>&lt;6 ng/mL</td>
</tr>
</tbody>
</table>

A. The results are consistent with passive exposure to marijuana
B. The results are not consistent with passive exposure to marijuana
C. The results likely represent a false-positive
D. Collect a second sample >24 hours later and repeat testing