Drug Testing: Answers to Frequently Asked Questions and New Drug Trends

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TASC, Inc
Topics of Discussion

- Sample Media Comparison / Detection
- Urine Dilution
- Ethyl Glucuronide
- Opiate Interpretation
- Benzodiazepine Interpretation
- THC Level Interpretation
- New Drug Trends
What type of testing should my client do?
“Fingleworth, your drug tests show you to be drug free, but also to be 3 months pregnant.”
Sample Media

Various sample media types available to implement a drug abuse monitoring program...

- Urine, Oral Fluid, and Hair most common
- Blood and Sweat - uncommon

Each media type has its advantages/disadvantages
Hair Follicle

- **Pros**
  - Effective baseline test – Unknown drug use history
  - Adulteration difficult / debatable

- **Cons**
  - Not useful for routine monitoring
  - Usage period broad / cannot be pin-pointed
  - Head hair may not be available
  - Limited Test menu
  - Potential issue with treated hair / debatable
  - Expensive
Oral Fluid

- **Pros**
  - Effective field collection
  - Same gender collection not necessary
  - Difficult to adulterate

- **Cons**
  - Short detection period
  - Ineffective for THC Detection
  - Limited test menu
  - Moderate cost
Urine

- Pros
  - Good detection period
  - Large sample size
  - Industry standard
  - Long history of legal acceptance
  - Inexpensive

- Cons
  - Requires visually observed collection to avoid adulteration
  - Potential for specimen dilution
Media Strategies

- Hair
  - Use to establish a “baseline” at beginning of program
  - Use if client misses over 30 continuous days of testing

- Urine
  - Random Schedule: Variable from 1x/month to 2x/week
  - Fixed Schedule (2x/week): Every Mon/Thu or Tue/Fri
  - Intensive: Every Mon/Wed/Fri

- Oral Fluid
  - Medical reasons (Dialysis / Catheter)
  - Testing 2x/week recommended
<table>
<thead>
<tr>
<th>Media</th>
<th>Approximate Detection Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>24-72 hours</td>
</tr>
<tr>
<td>Oral Fluid</td>
<td>12-36 hours / 6-8 hours THC</td>
</tr>
<tr>
<td>Hair</td>
<td>Head hair: 14-90 days prior</td>
</tr>
<tr>
<td></td>
<td>Body hair: 30-365 days prior</td>
</tr>
<tr>
<td>Blood</td>
<td>8-36 hours</td>
</tr>
<tr>
<td>Sweat</td>
<td>1-4 weeks (period patch is worn)</td>
</tr>
</tbody>
</table>
# Urine Drug Detection Periods

**Stimulants:**
- Amphetamines, Cocaine, Ecstasy, Bath Salts
  - 24-72 hrs

**Narcotics / Narcotic Analgesics:**
- Methadone, Opiates, Propoxyphene
  - 24-72 hrs

**Sedative Hypnotics**
- Barbiturates, Benzodiazepines
  - 24-72 hrs / 2-6 wks*

**Hallucinogens:**
- Marijuana
  - 24-72 hrs / 2-6 wks*
- PCP, LSD
  - 2-5 days

**Depressants:**
- Alcohol
  - 1-12 hours
- Ethyl Glucuronide (EtG)
  - 8-72 hours

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*Continuous daily usage history  
Averages only - Will vary depending upon population
My client’s sample was dilute, what does that mean?
Urine Dilution

In vivo dilution is the most common method employed to circumvent a drug test.

**Principle:**

The ingestion of copious amounts of fluids prior to providing a urine sample in order to induce polyuria and ultimately lower the concentration of drugs in the bladder below the detectable limit.
Effects of Water Loading

- Urine Production Rate After Water Loading
- Time (min)
- Urine Production (mL/min)
- 1 Liter
- 2 Liters

L. Kadehjian 2005, Baldes and Smirk, 1934 Macallum and Benson, 1909
Temperature Effect?

**Annual Range:** 4.2% - 5.3%  /  **Annual Average:** 4.7%  /  >2,300,000 samples analyzed

TASC, Inc. 2011
Dilution Interpretation

Intentional or Incidental Dilution?

- Is there a history of diluted samples?

- Are there occurrences of missed scheduled or random testing dates?

- Results of recent prior and subsequent samples “Positive” for drug(s)?

- Is the donor under medical supervision that dictates use of diuretics and/or high fluid intake?
Acting on Diluted Samples

- Establish a procedure for handling diluted samples
  - Define non-compliance
  - Determine ramifications
- Document donors understanding of compliance
- Consider *Negative Dilute* samples to be *Non-Compliant*
I want to test my client for alcohol, what test should I choose?
Urine Alcohol

- Alcohol can be detected in the urine for approximately 12 hours.

- Potential for a false positive due to sugars in urine.
  - Diabetic individuals who are not being treated.
  - Bacteria in urine ferments sugars into alcohol.
  - Glucose test should be performed on a positive sample.
Ethyl Glucuronide (EtG)

Ethyl Glucuronide (EtG) is a unique biological metabolite that is formed in the body after the consumption of ethanol, typically from drinking alcoholic beverages.

- Reported to be detectable in urine typically from 8-80 hours after ingestion, and 2-36 hours in blood
- EtG is detectable over a period roughly 5-6 times longer than traditional urine ethanol testing
- Detectable in oral fluid only a few hours longer than ethanol*
- EtG has also been isolated in hair follicles

*G. Heiseth, B. Yttredal, et al.; JATox: July 2010
Urine EtG

- EtG is realistically detectable for approximately 6-72 hours at the industry norm 500 ng/mL cutoff limit
- Peak urine detection time is approximately 8 hours after ingestion event
- Normal urinary EtG levels in abstainers are <10-80 ng/mL
- Urinary metabolite Ethyl Sulfate (EtS) is also detectable as an additional biomarker
- No direct correlation can be made between urine EtG and BAC (blood alcohol concentration)
- Impairment or intoxication cannot be determined
How much alcohol did my client drink?

- It is **not** possible to determine the amount of EtG that will be produced from a measure of Ethanol (or vice versa) - Retrograde extrapolation **cannot** be performed

- Metabolism of Ethanol and EtG and EtS is genetically determined - Variability between individuals could be a 200-fold difference!

- Age, gender, race, physical health, diet, metabolism, and time of sample collection are but a few significant variables that can affect EtG detected.
Why Test EtS?

- EtG can possibly disappear (or be degraded) in urine due to certain bacterial contamination of the sample.
- EtS is not degraded by common bacterial contaminants.
- EtG can be synthesized by bacteria (such as *E. coli*) in vitro in the presence of alcohol (!)*
- Presence of both EtG and EtS is a strong indicator of alcohol consumption.
- Presence of EtS alone may indicate alcohol consumption in conditions where the sample is contaminated (UTI infection).

*A. Helander, et.al.; ClinChem: August 2007*
Example EtG Observations

- Two non-alcoholic beers
  - EtG concentration after 12 hours: 93 ng/mL
    - Negative

- A teaspoon of communion wine
  - EtG concentration after 12 hours: 77 ng/mL
    - Negative

- Three 1 oz doses of Nyquil over 24 hours
  - EtG concentration after 12 hours: 246 ng/mL
    - Negative
Example EtG Observations

- Single Beer (4.5% Alcohol)
  - Positive EtG above the 500 ng/mL cutoff level for 16 hours
  - Concentration peaking at 4,000 ng/mL after 4 hours

- Three glasses of wine (12% Alcohol) consumed over 3 hours
  - Positive EtG above the 500 ng/mL cutoff level for 32 hours
  - Concentration peaking at 68,000 ng/mL after 14 hours

- Six shots of vodka over 3 hours
  - ETG in the range of 10,000 ng/mL – 100,000 ng/mL
  - Peaked at 16 hours and detectable for 54 hours

Compiled from various sources
Hygiene Products

- Hand sanitizer applied every 15 minutes for 8 hours
  - Maximum EtG of approx 50 ng/mL – **Negative**

- Gargling mouthwash 3 times a day for 5 days
  - Maximum EtG concentration of 117 ng/mL – **Negative**

- Gargling mouthwash 4 times a day for 78 hours
  - Maximum EtG level: 173 ng/mL - **Negative**
Summary

- If usage is denied, confirmation is *Highly Recommended*
  - LC-MS/MS Quantification of EtG and EtS

- Avoid significant sanctions when:
  - EtG is confirmed below 500 ng/mL
  - No detectable EtS (<100 ng/mL) is found

- Consider Medical Conditions
  - Diabetics
  - Clients with Urinary Tract Infections

- Implement a Client Agreement to avoid incidental exposure
My client tested positive for opiates, but I am not sure what they are taking?
Opiates

- 6-MAM (6-monoacetylmorphine)
  - Heroin
- Codeine
  - Tylenol #3/#4, cough syrups with codeine
  - Trace codeine may be present from heroin use
- Morphine
  - MS Contin, Roxanol, Heroin
Opiates

- Hydrocodone
  - Vicodin, Vicoprofen, Tussionex, H-C Tussive Syrup
- Hydromorphone
  - Dilaudid, Vicodin, Vicoprofen, Tussionex, H-C Tussive Syrup
- Oxycodone
  - Oxycontin, Percodan, Percoset, Roxicet
- Oxymorphone
  - Opana, Oxycontin, Percodan, Percoset, Roxicet
Opiate Metabolism

MAJOR AND MINOR METABOLIC PATHWAYS FOR OPIATES AND OPIOIDS

Poppy Seeds and Morphine Drugs

- Codeine → Hydrocodone
  - minor metabolism (high-dose codeine)
- Morphine
  - minor metabolism (high-dose morphine)
- 6-monoacetylmorphine → Heroin

Oxycodone → Oxymorphone

Figure 2
My client is taking Valium, what should they test positive for?
## Benzodiazepines

<table>
<thead>
<tr>
<th>Prescription</th>
<th>Benzodiazepine</th>
<th>Parent Drug/Metabolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Versed</td>
<td>Midazolam</td>
<td>Midazolam, Hydroxymidazolam</td>
</tr>
<tr>
<td>Prosom</td>
<td>Estazolam</td>
<td>Estazolam, Hydroxyestazolam</td>
</tr>
<tr>
<td>Restoril</td>
<td>Temazepam</td>
<td><strong>Temazepam, Oxazepam</strong></td>
</tr>
<tr>
<td>Rohypnol</td>
<td>Flunitrazepam</td>
<td>Flunitrazepam, Desalkylflunitrazepam, 7-Aminoflunitrazepam</td>
</tr>
<tr>
<td>Serax</td>
<td>Oxazepam</td>
<td>Oxazepam</td>
</tr>
<tr>
<td>Valium</td>
<td>Diazepam</td>
<td>Diazepam, Nordiazepam, Temazepam, Oxazepam</td>
</tr>
<tr>
<td>Xanax</td>
<td>Alprazolam</td>
<td>Alprazolam, Hydroxyalprazolam</td>
</tr>
</tbody>
</table>
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<tbody>
<tr>
<td>Ativan</td>
<td>Lorazepam</td>
<td>Lorazepam</td>
</tr>
<tr>
<td>Centrax</td>
<td>Prazepam</td>
<td>Prazepam</td>
</tr>
<tr>
<td>Dalmene</td>
<td>Flurazepam</td>
<td>Flurazepam, Hydroxyethylflurazepam</td>
</tr>
<tr>
<td>Halcion</td>
<td>Triazolam</td>
<td>Triazolam</td>
</tr>
<tr>
<td>Klonopin</td>
<td>Clonazepam</td>
<td>Clonazepam, 7-Aminoclonazepam</td>
</tr>
<tr>
<td>Librium</td>
<td>Chlordiazepoxide</td>
<td>Chlordiazepoxide, Temazepam</td>
</tr>
</tbody>
</table>
My client has been testing for several weeks and is positive for marijuana, are they still using?
THC Detection Period

THC metabolites are fat-soluble, and may be retained in fatty tissue depending upon dosage and recent usage history.

- May take time to produce consistent negative urine samples
  - Casual users: 2-5 days
  - Chronic users: 3-6 weeks
THC:Creatinine (THC:CRE) ratios are commonly used to normalize sample dilution effects.

The ratios can be used directly to monitor THC abstention and elimination, or to determine the probability of a new usage event.

Most effective when interpreting GCMS analyses.
THC:Creatinine Ratio

Ratio is calculated as:

\[
\text{THC (ng/mL)} \times 100 = \text{THC:Cre (mg/mg)}
\]

Creatinine (mg/dL)
THC Elimination

THC Half-Life

Urinary THC excretion half-life is 1-10 days depending on usage history (mean half-life is 3.0 ± 2.3 days)†

1 day for infrequent/casual users
10 days for heavy/chronic users

THC Clearance

THC Clearance Data

99% of Population Negative by 6 weeks
Median: 18 days

D. Kramer; TASC (2009)
Determining a Usage Event

Medical-Legal Method
Manno, et. al. (1984)‡

If THC:Creatinine ratio between samples increases $\geq 50\%$, new usage on or between these dates is suspected

False Positive/Interpretation Rate: 0.1%
False Negative/Interpretation Rate: 24%

THC Concentration vs. THC:Creatinine Ratio

Dehydrated Example

<table>
<thead>
<tr>
<th>Time</th>
<th>THC (EIA)</th>
<th>Creatinine</th>
<th>THC:Cre</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>150</td>
<td>105</td>
<td>143</td>
</tr>
<tr>
<td>3</td>
<td>128</td>
<td>130</td>
<td>98</td>
</tr>
<tr>
<td>7</td>
<td>170</td>
<td>190</td>
<td>89</td>
</tr>
<tr>
<td>10</td>
<td>75</td>
<td>120</td>
<td>63</td>
</tr>
<tr>
<td>15</td>
<td>40</td>
<td>120</td>
<td>33</td>
</tr>
<tr>
<td>22</td>
<td>30</td>
<td>99</td>
<td>30</td>
</tr>
</tbody>
</table>

Diagram showing THC Concentration vs. THC:Creatinine Ratio with data points indicating dehydration.
Summary

- Most clients are testing negative by 1-3 weeks
- 99% of clients will test negative by 6 weeks
- THC:CRE ratios should decrease at least 50% every 10 days
- An increase in THC:CRE ratio of 50% suggests new usage
Synthetic Cannabinoids

Spice/K2
Cannabinoid Receptors

- Synthetic cannabinoids are referred to as substances with structural features which allow binding to one of the known cannabinoid receptors, i.e. CB₁ or CB₂, present in human cells.
- The CB₁ receptor is located mainly in the brain and spinal cord and is responsible for the typical physiological and particularly the psychotropic effects of cannabis.
- The CB₂ receptor is located mainly in the spleen and cells of the immune system.
Synthetic Cannabinoids

- **Classical cannabinoids** (THC, other constituents of cannabis; and their structurally related synthetic analogues e. g. HU-210, AM-906, AM-411, O-1184)
- **Nonclassical cannabinoids** (cyclohexylphenols or 3-arylcyclohexanols such as CP-47,497-C8, CP-55,940, CP-55,244)
- **Hybrid cannabinoids** (combinations of structural features of classical and non-classical cannabinoids, e. g. AM-4030)
- **Aminoalkylindoles** (AAIs), which can be further divided into naphtoylindoles (e. g. JWH-018, JWH-073, JWH-398, JWH-015, JWH-122, JWH-210, JWH-081, JWH-200, WIN-55,212); phenylacetylindoles (e. g. JWH-250, JWH-251); naphthylmethylindoles and benzoylindoles (e. g. pravadoline, AM-694, RSC-4).
- **Eicosanoids** (endocannabinoids such as anandamide, and their synthetic analogs e. g. methanandamide)
- **Others**, diarylpyrazoles (selective CB1 antagonist Rimonabant®), naphtoylpyrroles (JWH-307), naphthylmethyldindolene or derivatives of naphthalene-1-yl-(4-pentyloxy)naphthalene-1-yl)methanone (CRA-13).
Synthetic Cannabinoids

- Most common products:
  - Spice and K2
- Sold in head shops and online
- Many varieties/scents
- Labeled and sold as incense: “Not for Human Consumption”
- Herbs and botanicals treated with synthetic cannabinoids
Physiological Effects

- Elevated Blood Pressure and Heart Rate
- Elevated Body Temperature
- Bloodshot eyes
- Swaying
- Slurred speech
- Tremors – possible Seizures and Convulsions
Psychological Effects

- Euphoria
- Time dilation
- Short-term memory loss
- Anxiety and Agitation
- Paranoia and Hallucinations
- Psychological (and Physiological) dependence documented
Spice vs. Marijuana

- Often psychoactive effects similar to marijuana
- “High” can vary depending on the synthetic cannabinoid
- No standard Spice\K2 blend on market
Detection

- Most compounds are **not** detected by standard drug screening tests (Negative on standard THC screen)

- Detection period estimated to be 24-72 hours in urine
  - Primarily detect metabolites in urine
  - Typically JWH metabolites analyzed due to availability of standards
  - Newer Synthetic Cannabinoid ingredients may differ in metabolism

- Shorter detection period in blood and oral fluid
  - Parent drug detected

- Testing methodology utilized
  - ELISA currently designed to detect aminoalkylindoles
  - Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS)
  - Expensive
Detection Issues

- No standards set for Spice/K2 detection
  - Legal cutoff not established
  - Relatively new in Forensic Drug Testing industry

- Metabolism and retention not well-defined
  - Purified standards continue to be synthesized
  - Limited published research on retention

- Sample Media Variability
  - Urine: Only metabolites observed – not well defined
  - Oral Fluid: Parent drug found – possible shorter detection period

- Testing methodologies still in development
  - Many methods for isolating compounds from sample matrix
  - Varying sensitivity and specificity of instrumentation
Future Challenges

- Hundreds of potential compounds can be used in the manufacturing process of Spice products
- Moving target – Spice industry responds to legislation, Laboratories must respond to latest trend
- Lack of complete understanding of metabolism for all known synthetic cannabinoids
- Development of affordable screening tests that react with a wider range of synthetic cannabinoids
- Lack of certified metabolite standards (getting better) for LC/MS method development
Synthetic Stimulants

Bath Salts
Bath Salts

- Stimulant like Amphetamines

- Substituted cathinones -- Methylenedioxypyrovalerone (MDPV) mephedrone, and methylone are the chemicals most often found in “bath salts”

- Cathinone is a chemical derived from the Khat plant

- Consumed orally or nasally
Where/how is it sold?

- Sold in head shops, convenient stores and online
- Packaging
  - Bath Salts
  - Plant Food – White Snow
  - Insect Repellent – White Lightning
  - Stain Remover – Thunda Cat
  - “NOT FOR HUMAN CONSUMPTION”
Effects

- Severe side effects
  - Suicidal thoughts
  - Agitation
  - Combative/Violent behavior
  - Confusion
  - Hallucinations/psychosis
  - Increased heart rate
  - Hypertension
  - Chest Pain
  - Death or serious injury

- The speed of onset is 15 minutes, while the length of the high from these drugs is four to six hours.
Increasing Problem

- TODAY  |  January 04, 2013

**Navy’s anti-drug ad aims to scare sailors**

Navy officials say a new ad aimed at a designer drug called bath salts was produced after an alarming spike in its use by sailors in 2012, but some are calling the video over the top. NBC’s Jim Miklaszewski reports.

- http://www.today.com/video/today/50362252
Thank you for your time.