Upon arrival, please use the Chat box to provide your first and last name and your phone number (if you called in using your phone).

Please provide first and last names for those with you on the call.

Please include your practice or organization name.

Please remember to mute the phone line by dialing *6 (#6 to unmute) and/or mute the speakers on your computer.
Fundamentals of Urine Drug Testing

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Disclosures

• Clayton Wilburn: I have no relevant financial relationships to disclose or conflicts of interest to resolve

• Jill Warrington: I am on the Blue Cross Blue Shield Community Advisory Council in which I receive reimbursement for time/travel. I am employed solely by the University of Vermont Medical Center and the Larner College of Medicine.
For appropriate use of testing...

Know:

- THE USE
- THE PATIENT
- THE TEST
What is substance use testing?

**It IS**
- An objective measure of substance use
- A tool used by physicians to support patient care
- A mechanism to support treatment adherence

**It is NOT**
- A substitute for active therapy
- A strategy to punish or harm patients on their pathway to recovery

*It does, however, require understanding the test to use this technique effectively.*
Sources of samples for testing

- Hair
- Oral Fluids
- Blood
- Urine

Most common body fluid used: Urine

**Pro’s**
- Easy access
- Long window of detection
- Broad test menus

**Con’s**
- Difficult to establish time since last dose
- Tampering issues arise
Two types of testing

**Screening**
- Designed to provide fast, early diagnostic information
- Compare to other screening tests (e.g.: mammography)
  - Moderate sensitivity
  - Poor specificity
- Method used: Immunoassay

**Definitive or confirmatory**
- Designed to provide more diagnostic certain information
  - Excellent sensitivity
  - Excellent specificity
- Method used: Chromatography-Mass spectrometry
Screening

Point-of-Care

Lab-Based Immunoassays

Both rely on the ability of an antibody to detect a drug
Screening: Point of Care Testing

Point-of-Care

- Also known as POCT (Point-of-Care Testing)
- Used in the office
- Can be in the form of a cup or test strip
- Look often for the absence of a line to call a drug positive
- It’s a yes or no answer
- Often 7-10 drugs per cup
Screening: Lab-Based Immunoassays

Laboratory – based immunoassays

- It’s sent to the lab
- It’s performed on lab instruments
- Report may be qualitative (yes or no) or quantitative
- Can detect ~10-25 drugs
- Usually 1-6 hour turnaround time
Screening immunoassays: How they work
A simplified representation

Step 1: Pour urine into test tube

Step 2: Add antibodies, sample placed in analyzer

Step 3: If drug present, antibody binds and emits light

Step 4: Detector measures amount of light

The Lab-Based Immunoassay is shown here; similar process on a Point-of-Care Testing cup.
Confirmation (or Definitive) testing

Liquid-Chromatography Tandem Mass Spectrometry (LC-MS/MS)

- This is not a screening test
- This is used to definitively identify a drug
- The sample is sent to the laboratory
- The turnaround time can be 2-4 days
- It is run on an instrument known as a liquid chromatography tandem mass spectrometer
- It is a composite of two separate methods:
  - Liquid chromatography – separates the drugs
  - Mass spectrometry – fragments drugs with identification of drug by its fragments
LC-MS/MS: How they work
A simplified representation

**Liquid Chromatography (LC)**
Chemical separation

Urine containing many drugs

Urine is run through a column to separate drug

**Tandem Mass spectrometry (MS-MS)**
Fragments & Identifies drug by its fragments

Drug is fragmented

Drug is fragmented again

Fragments identified at detector
How accurate is the test?

POCT

Lab immunoassays

Confirmation testing
  LC-MS-MS

Diagnostic Certainty (or Result Reliability)

Low → High
Diagnostic Certainty

**Sensitivity**
- Ability to detect drug taken
- How low can you detect
- How well you detect specific drug in a given class
  i.e. Lorazepam in Benzodiazepines

**Specificity**
- Ability to detect just the drug(s) of interest
- Low specificity = more unwanted cross reactions
  i.e. Bupropion in Amphetamines

*In screening assays there is always a balance between the two*
### Possible Results

<table>
<thead>
<tr>
<th>Possible Results</th>
<th>Drug Taken</th>
<th>Screen Positive</th>
<th>Confirmation Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>True Positive (TP)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>True Negative (TN)</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>False Positive (FP)</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>False Negative (FN)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Putting It All Together

• A Test’s Sensitivity and Specificity Drive its Reliability

• For Screening Immunoassays:

  ![Result Reliability Diagram]

- Low
  - Amphetamines
  - Benzodiazepines

- High
  - Fentanyl
  - PCP
  - Opioids
  - Barbiturates
  - Methadone
  - THC
  - Cocaine
  - Buprenorphine
Comparison of strengths and weaknesses

- **POCT**
  - Speed: Fast
  - Cost: Cheap
  - Accuracy: Least

- **Lab immunoassays**
  - Speed: Slow
  - Cost: Pricey
  - Accuracy: Most

- **Confirmation testing**
## Comparison of assays

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>POCT</th>
<th>Lab EIA</th>
<th>Confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed</td>
<td>🟢</td>
<td>🟠</td>
<td>🟥</td>
</tr>
<tr>
<td>Cost</td>
<td>🟢</td>
<td>🟠</td>
<td>🟥</td>
</tr>
<tr>
<td>Accessibility</td>
<td>🟢</td>
<td>🟠</td>
<td>🟥</td>
</tr>
<tr>
<td>Test menu</td>
<td>🟥</td>
<td>🟠</td>
<td>🟢</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>🟥</td>
<td>🟠</td>
<td>🟢</td>
</tr>
<tr>
<td>1. How low can you go?</td>
<td>🟢</td>
<td>🟠</td>
<td>🟢</td>
</tr>
<tr>
<td>Specificity</td>
<td>🟥</td>
<td>🟠</td>
<td>🟢</td>
</tr>
<tr>
<td>1. Can you tell me the drug?</td>
<td>🟥</td>
<td>🟠</td>
<td>🟢</td>
</tr>
<tr>
<td>2. Are you sure it’s that drug?</td>
<td>🟥</td>
<td>🟠</td>
<td>🟢</td>
</tr>
</tbody>
</table>
Complexities of testing

Complicated by:

Tests
- False positives and negatives
- Highly technical language
- Complex metabolic pathways
- Denied use or nonadherence
- Tampering
- Variable cutoffs and methods

Patients
- Lack of standardization
Complexities of testing

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- Variable cutoffs and methods
Common causes of false positives

- **Cross-reactivity of immunoassay (most common)**
  - Antibody
  - Drug of interest
  - Illicit drugs
  - Over the Counter drugs
  - Some foods/drinks
  - Bacterial production (!)
  - Antibodies can (incorrectly) pick up on some/any of these

- **Interfering substance in confirmation testing (very rare)**
  - Unlike in immunoassay, this will be identifiable and called out on the patient report
Common causes of false negatives

1) Too little drug to detect, due to:
   • When drug consumed
   • Dilutions
   • Degradation (rare)

2) Can’t detect, due to:
   • Cutoff of the assay (below level of detection)
   • Drug is masked by other materials in urine (e.g. adulterants).
   • The incorrect test was ordered.
   • Tampering with the specimen cup.
   • Low cross reactivity in drug class

Specimen validities help work out whether dilution or adulteration has occurred.

*Recognize some could be characterized as true positives depending upon clinical context*
Complexities of testing

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Lack of standardization
- Variable cutoffs and methods
<table>
<thead>
<tr>
<th>Parent Drug</th>
<th>Metabolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heroin</td>
<td>6-acetylmorphine or 6-AM</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>Norbuprenorphine</td>
</tr>
<tr>
<td>Methadone</td>
<td>2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Ethyl Glucuronide (EtG)/Ethyl Sulfate (EtS)</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Benzoylecgonine (BCE)</td>
</tr>
<tr>
<td>Methylphenidate</td>
<td>Ritalinic acid</td>
</tr>
</tbody>
</table>
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- Lack of standardization
Simplified metabolic pathway: Opioids

HEROIN → 6-AM → CODEINE → HYDRO-CODONE

MORPHINE → HYDRO-MORPHONE
### Opioids: A more comprehensive metabolite list

<table>
<thead>
<tr>
<th>Opioids</th>
<th>Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>Norbuprenorphine&lt;br&gt;Norbuprenorphine-3-glucuronide&lt;br&gt;Buprenorphine 3-glucuronide</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>Hydroxybutorphanol&lt;br&gt;Nobutorphanol</td>
</tr>
<tr>
<td>Codeine</td>
<td>Hydrocodone (minor)&lt;br&gt;Norcodeine&lt;br&gt;Morphine</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>Norfentanyl</td>
</tr>
<tr>
<td>Heroin</td>
<td>Morphine&lt;br&gt;Codeine (contaminant)&lt;br&gt;6-monoacetylmorphine (6-AM)</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>Hydromorphone&lt;br&gt;Dihydrocodeine&lt;br&gt;Normorphine&lt;br&gt;Norhydrocodone&lt;br&gt;Hydrocodol&lt;br&gt;Hydromorphol</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Opioids</th>
<th>Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydromorphone</td>
<td>Dihydromorphone&lt;br&gt;Hydromorphone-3-glucuronide</td>
</tr>
<tr>
<td>Methadone</td>
<td>2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)&lt;br&gt;2-ethyl-5-methyl-3,3-diphenylpyrrolidine</td>
</tr>
<tr>
<td>Morphine</td>
<td>Hydromorphone (minor)&lt;br&gt;Morphine-3-glucuronide&lt;br&gt;Morphine-6-glucuronide&lt;br&gt;Normorphine</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>Oxymorphone&lt;br&gt;Noroxycodone&lt;br&gt;Oxycodols and their oxides</td>
</tr>
<tr>
<td>Oxymorphone</td>
<td>Oxymorphone-3-glucuronide&lt;br&gt;Oxymorphol</td>
</tr>
<tr>
<td>Propoxyphene</td>
<td>Norpropoxyphene</td>
</tr>
<tr>
<td>Tramadol</td>
<td>O-desmethyl-tramadol&lt;br&gt;Nortramadol</td>
</tr>
</tbody>
</table>
Common metabolic pathways: Benzodiazepines

- Oxazepam
- Temazepam
- Nordiazepam
- Chlordiazepoxide
- Chlorazepate
- Diazepam
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Patients

Lack of standardization
A word about the patient’s journey

“ They are willing to give up *everything they care for* in order to take a drug—it is not enough to say that addiction is a chronic brain disease. What we mean by that is something very specific and profound...and that healthy people take for granted, *free will.*”

- Nora Volkow, Director of National Institute on Drug Abuse (NIDA), 2015

https://www.drugabuse.gov/about-nida/noras-blog/2015/06/addiction-disease-free-will
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Patients
- Lack of standardization

Lack of standardization
Types of Specimen Tampering

**SUBSTITUTION**
Replacing one’s urine with other urine or substances

**DILUTION**
Intentionally “watering down” urine through hydration or adding liquid to sample

**ADULTERATION**
Adding products to mask appearance of drugs
## Ways to identify/limit tampering

<table>
<thead>
<tr>
<th>Types of Tampering</th>
<th>Counter-response (at collection or in laboratory)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substitution</td>
<td>Collections: Observed and/or Randomization Laboratory: Innovative detection of synthetic urine</td>
</tr>
<tr>
<td>Dilution</td>
<td>Collections: Observed collections and/or Randomization Laboratory: Creatinine, specific gravity</td>
</tr>
<tr>
<td>Adulteration</td>
<td>Collections: Observed collections and/or Randomization Laboratory: pH, General oxidants</td>
</tr>
</tbody>
</table>
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- **Patients**
  - Denied use or nonadherence
  - Tampering

- **Lack of standardization**
  - Variable cutoffs and methods
A brief word about differences between labs

- Laboratories are heavily regulated but lack standardization

- Laboratories use different methods
  - Different antibodies in immunoassays
  - Prepare specimens differently for confirmation testing
  - Use different equipment
  - Multiple methods can be in one hospital system

- Reports vary and require orientation to use most effectively
A 34 year old pregnant woman is 18 weeks into her pregnancy and comes to your office for an ultrasound. She has a history of a Stimulant Use Disorder and you periodically are monitoring her with Urine Drug Testing. At intake you perform a urine drug Point-of-Care test (POCT) and you see that her urine is positive for cocaine. Prior urine drug testing has been negative.

What do you do?
Case 1

After discussing this finding with the patient, she admits to recent use of cocaine.

What do you do?
Is any additional testing required?
Case 2

A 22 year old woman with a history of an Opioid Use Disorder is 32 weeks pregnant. You have been monitoring her with urine drug testing. To date, her urine drug testing has been negative. You have an in-house laboratory that you send the urine sample to and it comes back positive for fentanyl.

What do you do?
Case 2

Given the rate of false positives with fentanyl testing and she has been adherent to your treatment plan to date (negative prior urine drug testing), you suspect this is a false positive but send the laboratory to perform a confirmation test for fentanyl. The confirmation test is negative for norfentanyl and fentanyl.

What do you do?

Is any additional testing required?
Summary

• Intent was to provide a broad level overview

• We reviewed the three types of urine drug testing options including:
  • Point-of-Care testing – an in-office drug testing option, provides rapid, accessible answers, but least reliable test result
  • Lab-Based Immunoassay testing – a testing option that is sent to a laboratory, provides reasonable turnaround times, a more extensive selection of drugs but produces a moderate number of false positives and false negatives
  • Confirmation testing – a testing option that is sent to a laboratory, is slower and expensive, but provides the most accurate answer

• Discussed the complexities that influence result interpretation

*Any test result must be considered in the context of the patient to provide the best care*
Questions?

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• Jill Warrington: 802-863-4105 or jwarrington@aspenti.com