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**Objectives**
Provide an overview of how clinical drug testing is performed and what is detected/not detected
Provide references and resources to help with questions about specific drugs and/or situations
Raise the audience's comfort level with performing drug tests and responding to questions from clinicians

**What do you expect when you order a hamburger?**

**Wide variance of testing and results in a “drug screen”**
Different options for different purposes and/or patient situations
Variance with which drugs detected, confirmed or quantified → wide range of costs
Technical limitations of methods and protocols
Limitations of specimen collection and types of specimens
Variance in drug dose, route of administration, subject metabolic capacity, etc.

**Purpose/Situation**
Forensic or Full Clinical Toxicology screen
identify “everything”
screening and confirmatory methods
positive/negative may not be enough - measuring blood levels for toxicity could be required
Expensive, laborious
Employment / drug abuse programs
Identify only drugs of abuse/illicit drugs-no OTC or prescription meds
Expectations/assumptions
lots of negatives in low risk populations like pre-employment
preferred drug(s) of abuse known
“Big 5" from federal government: THC metabolites, cocaine metabolites, opiate metabolites, PCP and amphetamines
Simple, cheap screening protocols
Hybrid protocols
More involved testing than just the “Big 5” but heavy reliance on rapid, cheap methods of screening
Driven by available instrumentation, staff expertise, availability of rapid turnaround from reference lab etc.
Heavily influenced by “what can we provide?” rather than “what do we need to provide?”

**Concept of Drugs/ Toxic Compounds**
Drug taken for particular effect
Can have other effects (“side effects”)
Body recognizes as foreign → Tries to excrete, Tries to metabolize and excrete
What is in the body? What is excreted?
“Parent” drug in body, some in excreta
“Metabolites” in body, more in excreta
“Parent” drug- source compound
Metabolites
Results from body’s changes of parent compound
May be inactive or have their own effects
Structurally similar to parent compound- may be measured along with parent
Presence is usually predictable- Can be used to identify drug use, sometimes better than parent

Specimen types
Ability to detect drugs and/or metabolites varies widely with specimen type, when it is collected, what drug(s) were taken and the route(s) of administration.
Drug testing information skewed toward urine specimens as this is the most common.

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Advantages</th>
<th>Disadvantages</th>
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</thead>
<tbody>
<tr>
<td>Urine</td>
<td>Non-invasive</td>
<td>Can measure urine levels, but they are affected by urine volume so usually not meaningful</td>
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<tr>
<td></td>
<td>Plentiful and stable specimen</td>
<td>Can’t correlate concentrations to behavior- drug excretion ≠ impairment</td>
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<td></td>
<td>Highest concentration parent drugs and metabolites</td>
<td>Cross-reactivity of structurally similar metabolites</td>
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<td></td>
<td>Depending on drug, positive for a long time</td>
<td>Easier to substitute or adulterate than blood</td>
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<td></td>
<td>Easy to analyze, little debris and fewer interferences</td>
<td>Positive primarily with recent intake</td>
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<td></td>
<td></td>
<td>Difficult to collect w/o contamination in neonates</td>
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<tr>
<td>Blood</td>
<td>Can measure meaningful levels</td>
<td>Lower concentrations and fewer metabolites - assays more difficult</td>
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<tr>
<td></td>
<td>Levels correlate better with behavior or impairment, so more often used for legal purposes</td>
<td>Specimen has more interferences</td>
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<tr>
<td></td>
<td></td>
<td>Invasive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Positive primarily with recent intake</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lots of debris in specimen</td>
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<tr>
<td>Gastric</td>
<td>High amounts of drug recovered</td>
<td>Invasive</td>
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<tr>
<td></td>
<td>Parent drugs only- no metabolites</td>
<td>Cannot tell how much got into body</td>
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<td></td>
<td></td>
<td>Only positive with VERY recent intake</td>
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<tr>
<td></td>
<td></td>
<td>Lots of debris in specimen</td>
</tr>
<tr>
<td>Breath</td>
<td>Little to no debris</td>
<td>Only detects volatile substances</td>
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<tr>
<td></td>
<td>Non-invasive</td>
<td>Ethanol only routine analyte</td>
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<tr>
<td></td>
<td>Correlates with blood</td>
<td>Low concentration exhaled</td>
</tr>
<tr>
<td>Meconium and umbilical cord tissue</td>
<td>Detects maternal drug abuse in last 2 trimesters</td>
<td>Limited window to collect specimen</td>
</tr>
<tr>
<td></td>
<td>Meconium superior for exposure but cord faster to obtain</td>
<td>Lots of debris in specimen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specialized testing techniques</td>
</tr>
<tr>
<td>Hair</td>
<td>Non-invasive, stable for months</td>
<td>Assays/results not fully reliable yet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hair type variance for drug absorption (black hair absorbs the most drug)</td>
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<tr>
<td></td>
<td></td>
<td>Drug concentrations low</td>
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<tr>
<td>Sweat</td>
<td>Non-invasive</td>
<td>Limited specimen volume</td>
</tr>
<tr>
<td></td>
<td>Little debris</td>
<td>Drug concentrations low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Assays/results not fully reliable yet</td>
</tr>
<tr>
<td>Oral fluid</td>
<td>Non-invasive, easy to collect</td>
<td>Limited specimen volume</td>
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<tr>
<td></td>
<td>Correlates with blood levels so quantified results can be meaningful</td>
<td>Drug concentrations low</td>
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<tr>
<td></td>
<td>Sample adulteration difficult</td>
<td>Assays/results not fully reliable yet</td>
</tr>
</tbody>
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Drug Testing 101

Start with “screening” method
- In general, sensitivity > specificity
- Cheap and easy
- Many screening methods automated – immunoassay
  - Antibodies often cross react with parent drug and metabolites = “class analysis”
  - False results (usually +) with cross-reacting substances; Example: false + opiates with fluoroquinolone antibiotics
- Most labs set +/- cutoffs using federal guidelines → affects what will be detected

Confirmatory method
- More specific than screening method – only perform on positive specimens
- Most be analytically distinct from original method; Ex: if screening was done with an antibody, confirmation should not be antibody-based
- In general, more laborious and expensive - generally chromatography based
  - HPLC, GC, TLC
  - “Gold standard” = mass spectrometry and increasingly, tandem MS

Reporting protocols - two extremes
- Report drugs as “presumptive positive” without confirmation.
  - Retain specimen, confirmatory testing by clinician request
  - Can offer low cost testing and “pay as you go” confirmation
- Only report results that confirm
  - Required for results that can have legal consequences (pre-employment, conviction, parole, etc.)
  - No bad consequences from false positive
  - More time consuming and expensive

The Drug Testing Dilemma

High demand for rapid, accurate comprehensive toxicology
Patterns of substance abuse changing faster than we can respond
Limitations on resources
- Human and technical
- Financial, especially in the health care reform climate
Difficult to interpret results without knowing full protocol used, drug dosage, subject health, etc.

Reliance on Automation and Immunoassays for Drug Tests

Multifunctional analyzers that have drug testing on their menus popular for good reasons
Use of antibodies as analytical tools simple and cost effective
Limitations of immunoassays must be well known and understood by laboratorians; they are NOT generally well-understood by clinicians

Jenner and Cowpox- cross-reactivity of the immune response

Immunoassay Class Analysis

Many drugs and their metabolites have structural similarities within the same drug class
An antibody generated against one drug in the class, is likely to cross react with other drugs in the class
Mixed blessing- Abs allow one step “class analysis” but also suffer from cross reactivity
  - Antibodies to amine class can bind amphetamine/methamphetamine but also designer amines (MDMA) and OTC sympathomimetic amines (Pseudoephedrine)
Antibodies designed against natural opiates (morphine/codeine) bind opiates in foods with poppy seeds but can bind poorly to semi-synthetic and synthetic opiates.


Cross-reactivity of trazodone METABOLITE with amphetamine antibody illustrates why confirmation of immunoassay is so critical.

**Generalities of the “Big 5”**

See table in Quick References: “Overview of Common Urine Drug Tests” with citations for additional information.

**Ethanol**

Ethanol may not be part of “drug screen” since urine most common specimen.

Ethanol abuse poorly detected.

Detection of ethanol reliable but transient, only for acute abuse (7-12 hours).

Alcohol dehydrogenase most common method and is skewed toward ethanol.

Need gas chromatography for other alcohols.

Intoxication with other alcohols suggested by metabolic acidosis, high anion gap, high osmolar gap.

**Common Sources, Clinical Signs, and Analytical Methods for Alcohols**

[Table with details on common sources, osmolal gap, anion gap, and analytical methods for ethanol, ethylene glycol, isopropanol, methanol, propylene glycol, and their sources, clinical signs, and analytical methods.]

Sources of increased anion gap metabolic acidosis = MUDPILES

- methanol, uremia, diabetic ketoacidosis, propylene glycol, isoniazid, lactic acidosis, ethylene glycol, salicylates

Sources of increased osmolar gaps = ME DIE

- methanol, ethanol, diuretics (osmotic like mannitol), isopropanol, ethylene glycol

[Abbreviations: GC, gas chromatography; GC/MS, gas chromatography/mass spectrometry]
Mother / Neonate Drug Testing
Wexelblatt Study 2012-2013
- 5.4% all mothers have positive drug test on admission; 3.5% opioids
- 55-94% opioid-exposed infants develop withdrawal signs
- 30-80% opioid-exposed infants need pharmacologic therapy
ACOG recommends universal drug screening by history followed by urine testing for high risk history
Typical protocol
  - Obtain consent of mother → test her
  - Test baby if mother does not consent
Universal screening expensive with low yield
Specimen issues
  - Maternal drug use can vary through pregnancy
  - Maternal urine testing “snapshot” of one moment in time
Specimen types
  - Urine – fast, non-invasive plentiful
    - Most common maternal specimen
    - Difficult to obtain from neonates w/o contamination or invasive techniques
  - Hair
    - Second most common maternal sample- longer period of positivity, variance with hair color
    - Hair can be scant in neonates
  - Meconium or cord blood tissue
    - Increasingly popular for neonates
    - Limited window to collect, but abundant and non-invasive
    - Reflects neonatal exposure ~ last 2 trimesters
    - Meconium superior for exposure but cord faster to obtain
Testing
  - Best practice – NEVER report presumptive positive screen
  - Serious consequences of false positive
  - Some states moving to criminalize drug use during pregnancy
  - Confirm all positives with mass spectrometry
Identifying substance users/abusers
Increased used of drug testing
Those subject to drug testing widely expanding. Examples:
  - Employers – “for cause” and pre-employment
  - Substance abuse programs
  - Conditions of parole/probation/treatment
  - Athletes
Shift to legal/prescriptions substances
  - Perhaps less expensive
  - Legality makes purchase safer
MLO 2015- Opiate misuse ~25% with addiction rates ~10%
  “There may not be a drug dealer on every corner, but there is a pharmacy.”
Crackdown on prescriptions has contributed to re-emergence of heroin abuse- same effects and cheaper
Use of “not illegal” substances

More drug testing = pressure to quit or try to beat tests
Efforts to beat drug tests considered equal to failing drug test in most cases
Abuse of alternate substances to beat drug tests has flourished (see table in handout)
Can be more powerful – cocaine derivatives 100X stronger than cocaine
Variance from illicit labs - composition, dosage, adulterants (caffeine, acetaminophen, other drugs, etc.)
More difficult, specialized testing required

Emerging substance analysis is a big challenge - heterogeneous and constantly changing

Sample emerging substances

Synthetic marijuana (“K2”, “Spice”) - up to 700x more powerful than natural
Synthetic cathinones & methylone (“bath salts”)
Plants – khat, salvia
Caffeine/energy drink components
Dextromethorphan - “Robo tripping”
Ketamine analog animal tranquilizer
Others – constantly evolving “designed drugs”; manufacturers reluctant to allocate resources to create new tests when analytes changing rapidly

Marijuana - illegal or not?

Variance between states (23 states + DC some form of legalization)
Legal ≠ harmless
Safety issues in the workplace
Health/under the influence issues
Evidence of brain impairment/development disruption
High dose THC associated with schizophrenia

Traditional THC screening for major THC metabolite(s), not THC, in urine
Parent THC levels correlate with impairment, not inactive metabolites

Completely different testing, necessary for impairment/recent intake →
Blood
Oral fluid

THC levels reliably associated with impairment not available
EtOH hydrophilic → rapidly excreted
THC lipophilic → tissue sequestration
Chronic users - Constant THC in blood, users tolerant
Casual users - Less tolerant to THC, may be more impaired at same THC levels as chronic users

Legal limit of 5 ng/mL in Washington and Colorado for DUI, but “correct” level debated

Likely screening with oral fluid, confirmation with blood

Specimen Integrity for Drug Screens

Federal guidelines for protocols in accredited labs
Substance Abuse and Mental Health Services Administration
www.samhsa.gov

Information here based on their protocols

Giving a specimen for a drug test inherently hostile; an occasion when the subject may WANT an inadequate specimen

Specimen collection
From DONOR (not necessarily patient)
NOTE: Similar protocols for athlete testing
Establish identity with photo ID and fill out chain of custody form
Ideal - witness specimen

- U.S. Supreme Court stated witnessing urine specimen was invasion of privacy
- Reserved for special circumstances - “for cause” drug screens

Protocols for non-witnessed urine specimen

- Can’t take purses, coats, etc. into bathroom
- Ideally, person strips and puts on exam gown; If clothed, minimal clothing on and empty pockets
- Witness that hands are washed to eliminate adulterants under finger nails
- No running water in sink/blue water in toilet

- Immediately check specimen temperature- 32-38 °C within 4 minutes of collection
- Chain of custody documentation
- Split specimen- one for testing, one for re-testing
- 30 mL usual minimum; 60 mL desirable

Four categories of specimen problems
- Adulterated- introduction of chemical to urine to interfere with analysis
- Substituted- another person’s urine or another substance submitted
- Invalid- does not meet the criteria for acceptability (volume, temperature at collection, appearance, etc.)

Dilute- addition of water, use of diuretic or detox agent or subject drinks lots of fluid

Specimen problems, as above, are usually considered the same as positive for a drug

Laboratory specimen validation

- No signs of tampering such as broken seals, split specimens that look different, etc.
- Physical appearance and odor
- Urine specific gravity must be >1.0010 to rule out dilution and < 1.0200 to rule out added substance
- Urine pH must be 5-9; <3 or >11 likely adulterated
- Creatinine must be >2 mg/dL to prove fluid is urine
- Absence of adulterants demonstrated by testing
  - Bleach, nitrites, pyridine, glutaraldehyde, etc.
  - Add internal standard which should test positive if no adulterants present

Special case: drug testing for therapy compliance

- Users must test positive for a drug as a condition of their treatment program
- May abstain from fluids to concentrate drug in urine

False positives
- Color based immunoassays that detect higher absorbance → Signaled by color and high specific gravity
- User “spiked” urine with drug → no metabolites

Non- specimen problems

Non- specimen problems can cause drug test “failure” for toxicology or athletes

Examples

- Refusing to submit a sample
- Failure to show up for testing/disclose location
- Tampering with collection process
- Possessing or selling banned substance

Medical Review Officer

MRO- Person with medical knowledge needs to review case and interpret results
Examples of “false” positives

- Poppy seeds cause false positive opiates
  - Measure levels - morphine or codeine >15,000 ng/mL NOT consistent with poppy seeds
  - Look for metabolites - 6 monoacetyl morphine = heroin specific metabolite
- Cocaine in coca leaf tea
- Cocaine as legal local anesthetic
  - Patients can be positive up to 72 hours
  - Physician who performed procedure can be positive!
- Marinol - legal synthetic form of THC given to cancer patients for nausea

Issues of positive drug testing

- Use is not necessarily abuse
- Use is not necessarily impairment
- Specimens not always perfect
- Testing not always perfect
- Interfering substances
- Quality medical review officer to differentiate drugs from testing issues
- Inappropriate retention/discharge treatment program

Issues of negative drug testing

- Failure to identify abusers
  - Continued negative consequences
  - Delay in treatment
  - Crime
- Harm from impaired individuals
  - Economic/health
  - Accidents
  - Crime
- Inappropriate retention/discharge from treatment program
- Change in abuse patterns
  - Historically, abused substances illegal and testing skewed toward those drugs
    - Opiate testing for heroin, not pain killers
    - Poor detection of some legal opiate substances such as Oxycontin® and Vicodin®
    - Abuse of OTC drugs and other substances
    - THC causes impairment but legal limits not yet in place
- Abuse of substances not detected by drug screens
  - Synthetic cannabinoids
  - Khat
  - Bath salts and many others

So now what? Changes on their way to drug testing?

Increased incidence of drug abuse and novel abuse patterns will put pressure on labs.
Changes to testing protocols probably just a matter of time.
However.........I know we can do it.

HAPPY 2016 NATIONAL MEDICAL LABORATORY PROFESSIONALS WEEK!!