A Laboratorian’s Guide to Pre-Analytical Variables to Prevent Drug Testing Results from Getting *Burned*

KAMISHA JOHNSON-DAVIS PHD, DABCC

UNIVERSITY OF UTAH & ARUP LABORATORIES
SALT LAKE CITY, UTAH
Learning Objectives

1. Describe the most common pre-analytical variables that affect drug testing results

2. Compare the advantages and disadvantages of different specimens for drug testing

3. Discuss the importance of the timing of specimen collection for drug detection

4. Discuss examples of drugs that are susceptible to various pre-analytical variables
# Why is Drug Testing Necessary?

<table>
<thead>
<tr>
<th>Therapeutic Drug Monitoring (TDM)</th>
<th>Forensic Toxicology</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Guide/optimize dosing</td>
<td>• Death Investigation</td>
</tr>
<tr>
<td>◦ Failure to respond to treatment</td>
<td>• Child custody</td>
</tr>
<tr>
<td>• Monitor patient compliance</td>
<td>• Pre-employment drug testing</td>
</tr>
<tr>
<td>• Identify drug-drug interactions</td>
<td>• Professional sports</td>
</tr>
<tr>
<td>◦ Adverse drug reactions (ADR)</td>
<td>• Identify drugs involved in clinical signs and symptoms</td>
</tr>
<tr>
<td>• Monitor decontamination</td>
<td>(overdose/poisoning)</td>
</tr>
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</table>
Adverse Drug Reactions

- Adverse drugs reactions (ADRs) account for 41% of all hospital admissions (Nebeker et al. 2005)
  - Inappropriate dose or prescription
  - Drug-drug interactions
  - Allergic reactions

- ADRs kill ~100,000 patients in US hospitals each year (Kohn et al., 1999)
  - ~2 million Americans are affected by ADRs

- ~70% of medical decisions are based on laboratory results

- Quality results are important for drug analysis
Phases of Analysis

Common Pre-Analytical Errors

- Misidentification of Patient
- Mislabelling of Specimen
- Improper Specimen Mixing
  - Blood clots, prevent anticoagulation, hemolysis
- Improper Specimen
- Wrong Collection Tube
- Improper Timing of Specimen Collection
- Other Pre-Analytical Factors

What’s the Best Specimen for Drug Detection?

- Acute Exposure?
- Chronic Exposure?
- In Utero Drug Exposure?
- Roadside Drug Testing?
- Postmortem Drug Analysis?
Client called ARUP laboratories due to an unexpected negative result for oxycodone.

- Client suspects drug diversion by the nurse
- Patient records state oxycodone was administered
- Oxycodone administration was excessive

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 pm – 15 mg</td>
<td>8 pm – 25 mg</td>
</tr>
<tr>
<td>9 pm – 20 mg</td>
<td>9 pm – 25 mg</td>
</tr>
<tr>
<td>11 pm – 25 mg</td>
<td>11 pm – 25 mg</td>
</tr>
<tr>
<td>2 am – 25 mg</td>
<td>1 am – 25 mg</td>
</tr>
<tr>
<td>3 am – 20 mg</td>
<td>3 am – 20 mg</td>
</tr>
</tbody>
</table>
Case of the Unexpected Negative Result

- Blood collection – performed on Day 4

- What could have caused this negative result?
  - Non-compliance
  - Drug Diversion
  - Wrong specimen collected

- Oxycodone half-life: 4 – 6 hr
- 95-99% of drugs are eliminated within 5-7 half-lives
- Oxycodone would have been eliminated from blood: 20 – 42h
Wrong specimen

Urine specimen has a wider detection window for drugs
What is the detection window?

- Depends on
  - Specimen
  - Pattern of drug use
  - Dose
  - Concomitant medications
  - Clinical status of the patient
  - Individual metabolism and elimination kinetics of each drug
  - Sensitivity of the analytical techniques
    - cutoff concentrations?

- False negative results – Wrong specimen
Specimens

- Breath
- Oral Fluid
- Blood
- Urine
- Sweat/Tears
- Breast milk
- Hair/Nails
- Meconium
- Tissue (umbilical cord, liver)
- Vitreous – Postmortem
Detection Windows of Specimens

Detection Windows

Approximate drug detection periods for various specimen types.

From: Handbook of Drug Monitoring Methods
Edited by: Amitava Dasgupta
Humana Press
Blood

- Collections are observed

- Adulteration difficult

- Best specimen for correlation of clinical signs and symptoms (impairment) with drug use

- Monitor decontamination
  - Overdose situation
Blood

- Useful for people that cannot provide urine
  - Dialysis patients

- Represents only recent use (short window of detection)

- Specimen errors
  - Use of gels separator tubes
  - Requires prompt removal of plasma or serum from the clot
Blood

- Whole blood specimen is not used for all drugs
  - Lipophilic drugs can partition in RBCs
    - Partitioning can reach equilibrium
  - Enzymes in RBCs can metabolize drugs - Antipsychotic (haloperidol)
  - Drugs can bind to:
    - Cellular membrane, hemoglobin, binding proteins in cytosol of RBCs

- Consequently, drugs are assayed in serum/plasma
  - Centrifuged from RBCs within 2 hr

NACB Guidelines for TDM Services, 1999
Urine

- Easy to collect for adults
  - not so easy for neonates and young children

- Detects drug use/exposure over the past few days (most drugs)
  - Drug metabolites - provides strong evidence that the drug was in the body

- Actual concentrations are of limited value
  - Do not correlate with impairments
  - Will not identify amount of drug taken
  - May not detect recent use if compound(s) is metabolized to more than one drug
Strategies for “beating” the test

- Easy to adulterate or substitute when collections are not observed
  - Over-hydration
  - Diuretics
  - Substitution
    - Synthetic urine
    - Catheterization
  - Additives
    - Sodium chloride, Bleach, Soap, Drano, Lemon juice, Nitrites (Urine Luck), Vitamin C, Visine (eyedrops), Glutaraldehyde, Peroxidase (Stealth)

http://www.iatdmct.org/index.php/publisher/articleview/frmArticleID/30/
SAMHSA Guidelines for Adulterated Specimen

- **Normal**
  - pH 5.0 – 8.0
  - Creatinine: >20 mg/dL
  - Specific gravity: 1.005 – 1.030 g/mL

- **Diluted**
  - Creatinine < 20 mg/dL; SG: 1.001 – 1.003 kg/L

- **Substituted**
  - Creatinine <2 mg/dL; SG: < 1.001

- **Adulterated**
  - pH ≤ 3 or ≥ 11; Nitrite > 500 mg/L
Meconium

- Begins to form at ~12 wks gestation
- Detects drug exposure during ~the last trimester of pregnancy
- Low risk of adulteration
- Relatively easy to collect if available
  - May not pass for several days after birth, particularly for premature or sick infants
  - May be lost in utero
Challenges with Meconium

- Difficult matrix for drug extraction
  - Composition heterogenous

- Testing is not widely available
  - No standardization

- Requires confirmations testing in most cases
  - High false positive rate by immunoassay (cocaine, amphetamines)

- Interpretation of results may be vague
  - Cannot predict drug dose
  - The frequency of use
  - Drug stability can vary
Oral fluid

- **Collection**
  - Observed, non-invasive
  - Many commercial collection devices

- **Composition**
  - <1% protein; recovery of highly protein-bound drugs may be poor

- **Drug detection window – similar to blood**
  - Many metabolites not present in oral fluid
  - Drug concentrations is dependent on pH of the saliva and drug pKa

- **Adulterants (mouthwashes) ineffective after ~15min**

- **Smoked drugs can contaminate oral cavity**
  - May not reflect blood concentrations

Collections are non-invasive
- Used for all age groups

Wider window of drug detection
- Head hair: 1 cm represents ~1 month
- Chronology of drug use

Not all drugs are found in hair
Concerns with Hair Testing

- Bias in drug binding
  - Dark hair vs light hair

- Drug concentration doesn’t correlate dose or time of administration

- Sampling errors
  - Insufficient sample collection
  - Need ~200 hairs

- Analytical methods for detection
  - cutoffs
Concerns with Hair Testing

False Negative Results
- Extensive washing > remove drug from hair

False positive Results
- Failure to remove external contamination
- Effectiveness of “De-tox” shampoos is questionable
# Collection Tubes

<table>
<thead>
<tr>
<th>Closure Color</th>
<th>Collection Tube</th>
<th>Mix by Inverting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>BD Vacutainer® Blood Collection Tubes</strong> <em>(glass or plastic)</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Blood Cultures - SPS</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td></td>
<td>• Citrate Tube*</td>
<td>3 to 4 times</td>
</tr>
<tr>
<td></td>
<td>• <strong>BD Vacutainer® SST</strong>&lt;sup&gt;®&lt;/sup&gt; Gel Separator Tube</td>
<td>5 times</td>
</tr>
<tr>
<td></td>
<td>• Serum Tube <em>(glass or plastic)</em></td>
<td>5 times (plastic) none (glass)</td>
</tr>
<tr>
<td></td>
<td>• Heparin Tube</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td></td>
<td>• <strong>BD Vacutainer® PST</strong>&lt;sup&gt;®&lt;/sup&gt; Gel Separator Tube With Heparin</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td></td>
<td>• EDTA Tube</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td></td>
<td>• Fluoride (glucose) Tube</td>
<td>8 to 10 times</td>
</tr>
</tbody>
</table>
Case of the Discrepant Result

- A patient was diagnosed for depression and a serum specimen was sent to the laboratory to monitor compliance for Tricyclic antidepressants.
- Physician received the result and called the lab because the value was 40% lower than the previous 3 months of testing.
- Physician requested repeat testing and the result was still the same – and questioned if the laboratory made an error.
Case Scenario

- The run was evaluated – quality control values were “in range”.

- Supervisor call physician to inquire about changes in dose or specimen collection.

- Physician stated that blood specimen was collected in a gel separator tube and stored refrigerated (24h) before shipment.
Collection Tubes can affect Drug Concentration

- **Citrate/Oxalate Tubes**
  - Decrease drug concentration of Anticonvulsants (Phenytoin/Valproic acid)

- **Gray top tube - sodium fluoride preserves alcohol concentration**
  - Ethanol, methanol, isopropanol, acetone

- **Heparin tubes**
  - Should not be used to measure free (unbound) drug concentration
  - Can increase free (unbound) drug concentration
  - Activates lipoprotein lipase → fatty acids displace drug from albumin
## Gel Separator Tubes

### Can Cause Low Drug Recovery (Lipophilic drugs)

<table>
<thead>
<tr>
<th>Cardiac Drugs</th>
<th>Free Drug analysis for Anticonvulsant</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Flecainide</td>
<td>- Phenytoin</td>
</tr>
<tr>
<td>- ↓ 40% upon contact with gel</td>
<td>- Carbamazepine</td>
</tr>
<tr>
<td>- Quinidine</td>
<td>- Valproic acid</td>
</tr>
<tr>
<td>Tricyclic Antidepressants</td>
<td>Phenobarbital (sedative, anticonvulsant)</td>
</tr>
<tr>
<td>- Amitriptyline, Nortriptyline Desipramine</td>
<td>- Lidocaine (anesthesia)</td>
</tr>
</tbody>
</table>
Results Upon Investigation

Wrong specimen container
Specimen Collection for Therapeutic Drug Management

TIMING OF SPECIMEN COLLECTION
Case of the Critical Value for Digoxin

- 55 y.o. male was admitted to the ED due to chest pain
- ECG results showed irregular heart beats
- Patient was administered digoxin
- Serum specimen was collected post dose to assess digoxin concentration
Case scenario

- Therapeutic range: 0.8 – 2.0 ng/mL
- Toxic: > 2.4 ng/mL
- Patient’s result – 2.6 ng/mL

- Specimen was collected 4 hours after dose
- Digoxin has a long distribution phase
- TDM must occur at least 8 hours after the last dose
Results Upon Investigation

Wrong timing of specimen collection
Timing of Specimen Collection

- Specimens are drawn at either –
  - Pre-dose (Trough), peak, or random

- Majority of drugs are collected at trough
  - Most therapeutic ranges are for trough collection

- Peak collection
  - Drugs administered intravenously
  - Patient experiences signs of toxicity after dose

- For drugs with a long distribution phase – patients must be at steady state before collecting specimen
  - Random specimen is collected (digoxin – cardiac)
Steady state ($C_{ss}$):

amount of drug in = amount of drug out, requires 5-7 $t_{1/2}$

Note: plot is for illustrative purposes; drug does not have to be given at the half-life to predict $C_{ss}$.
Pre-analytical Variation Can Alter Drug Results

OTHER FACTORS
**Drug Stability**

### Rapid Metabolism

- **Fosphenytoin** (anticonvulsant)
  - Rapid metabolism to phenytoin
  - Half-life: 15 min
  - Specimen collection – Critical Frozen

- **Prazepam** (antianxiety)
  - Metabolizes to nordiazepam

- **Mycophenolic Acid** (immunosuppressant)
  - Undergoes metabolism in test tube
  - Refrigerate specimen

### Drug Degradation

- **Bupropion** (antidepressant)
  - Specimen collection – Critical Frozen

- **Olanzapine** (antipsychotic)
  - Specimen collection – Critical Frozen

- **Busulfan** (anticancer)
  - Specimen collection – on ice or frozen
<table>
<thead>
<tr>
<th>Light Sensitive</th>
<th>Heat sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Amiodarone (antiarrhythmic)</td>
<td>• Plasma concentrations of “free” drug – affect plasma protein binding</td>
</tr>
<tr>
<td>• Methotrexate (anticancer)</td>
<td>○ Phenytoin (anticonvulsant)</td>
</tr>
<tr>
<td>• Librium (antianxiety)</td>
<td>○ Valproic acid (anticonvulsant)</td>
</tr>
<tr>
<td>• Carbamazepine (anticonvulsant)</td>
<td>○ Total Carbamazepine (anticonvulsant)</td>
</tr>
<tr>
<td>• Chlorpromazine (antipsychotic)</td>
<td>• Lithium (mood stabilizer)</td>
</tr>
<tr>
<td>• Fluoxetine (antidepressant)</td>
<td></td>
</tr>
<tr>
<td>• Haloperidol (antipsychotic)</td>
<td></td>
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</tbody>
</table>
Pre-Collection Variables

- Acute phase reactants – may affect drug binding to proteins

Drug Binding to Plasma Proteins

- Normal protein binding
- Increased protein binding
- Decreased protein binding

↓ therapy

↑ toxicity

NACB Guidelines for TDM Services, 1999
Pre-Collection Variables

- **Exercise**
  - Cause transient changes in analyte concentration
  - Alcohol – breathalyzer test

- **Diurnal variations** (circadian rhythm changes)
  - Affect analyte concentrations
    - Valproic acid, carbamazepine, aminoglycosides
  - Drug monitoring performed at consistent time each day

- **Smoking**
  - Decreases serum drug concentration
    - hydrocodone
    - (Ackerman & Ahmad, J Ark Med Soc. 2007)
  - Induces drug metabolism
    - Theophyline, Caffeine, imipramine, haloperidol, propranolol, flecainide
    - (Zevin & Benowitz, Clin Pharmacokinet. 1999)
  - Increases clearance
    - heparin

NACB Guidelines for TDM Services, 1999
Post-collection Causes of Variation

- Specimen Storage conditions are drug-dependent
  - Refrigeration—slows metabolism, degradation, bacterial growth (urine)
    - Can cause hemolysis
  - Freezing for labile analytes

- Analyte concentration in blood/urine may change due to:
  - Adsorption to tube (THC)
  - Protein denaturation (affect concentration of free (unbound) drug
  - Evaporation

- Evaluate other conditions of specimen collection and handling
  - preservatives, heat, light, freeze/thaw, etc.
Summary

Pre-analytical variables can affect the validity of drug testing results

Specimen type
Specimen collection
Specimen handling
Timing of specimen collection


NACB Guidelines for Therapeutic Drug Monitoring Services

www.iatdmct.org/index.php/publisher/articleview/frmArticleID/30/


Case of the Elevated Immunosuppressant Result

- Patient was experiencing adverse affects from immunosuppressant drug
- A pre-dose (trough) specimen was collected once the patient reached steady state concentration.
- A specimen was sent to a laboratory for sirolimus/cyclosporine quantification
Case Scenario

- The laboratory alerted the Physician because the test results was higher than the therapeutic range
- Patient was also prescribed antifungal drugs
- Dose adjustment was made to lower blood concentration
Results Upon Investigation

Drug-Drug Interaction
Drug-Drug Interactions

- **Drugs that inhibit CYP450 system**
  - Increase blood concentrations of drugs (may lead to toxicity)
    - Antibiotics, steroids, antifungals, nicardipine, midazolam (antianxiety, anticonvulsant)

- **Drugs that will induce the CYP450 system**
  - Lower blood concentrations of drugs and therapeutic effect
    - Anticonvulsants (phenobarbital, phenytoin, carbamazepine), antibiotic (rifampin)

- **Reduce clearance and elimination of drugs**
  - Lead to elevated serum/plasma concentration