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?

| Therapeutic Drug Monitoring (TDM)	Forensic Toxicology

• Guide/optimize dosing
   ○ Failure to respond to treatment
 	
• Monitor patient compliance
 	
• Identify drug-drug interactions
   ○ Adverse drug reactions (ADR)
 	
• Monitor decontamination
	• Death Investigation
	
	• Child custody
	
	• Pre-employment drug testing
	
	• Professional sports
	
	• Identify drugs involved in clinical signs and symptoms (overdose/poisoning)

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
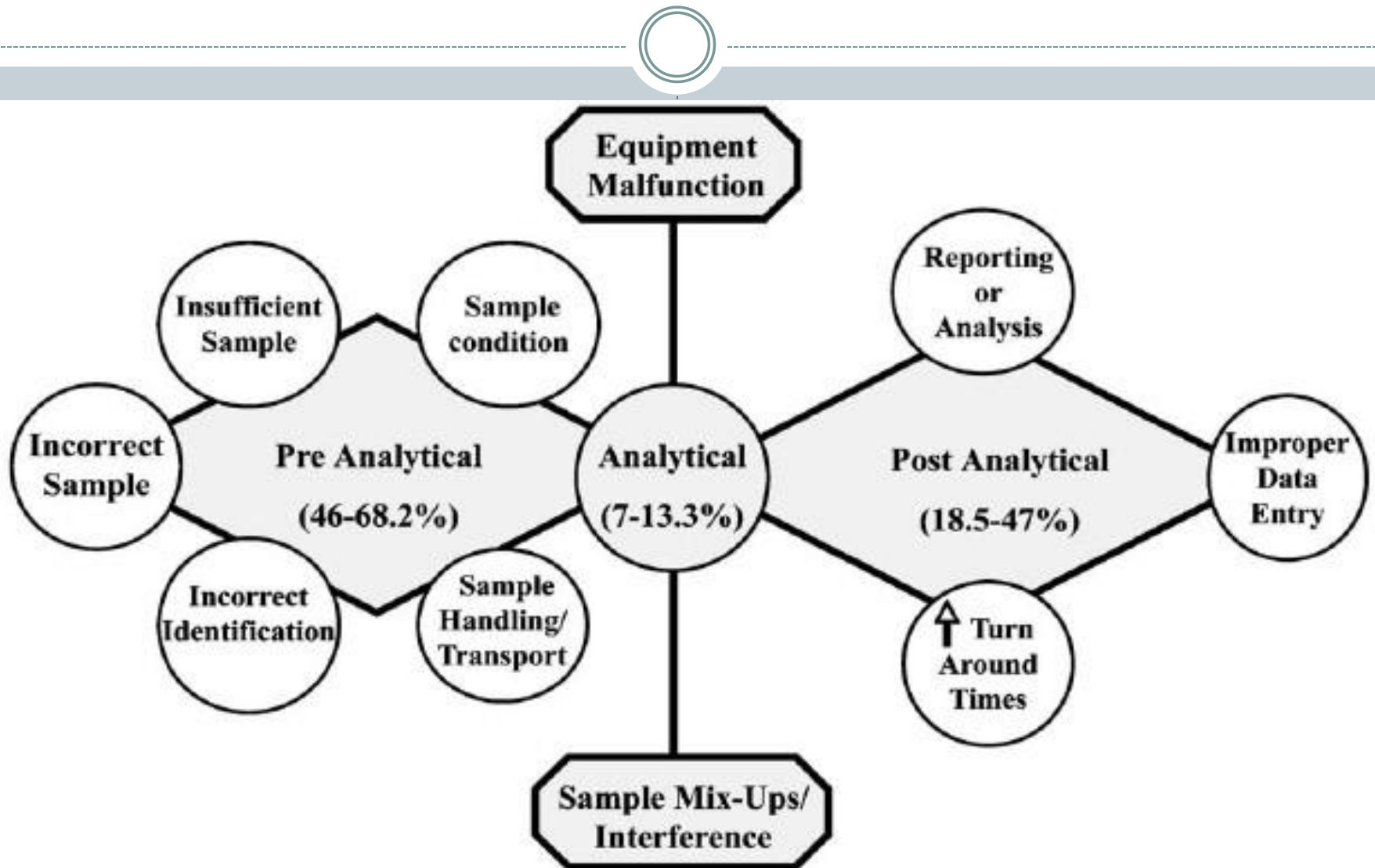
Common Pre-Analytical Errors

MISIDENTIFICATION OF PATIENT

MISLABELING 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?

Case of the Unexpected Negative Result

- 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

Day 1	Day 2
8 pm – 15 mg	8 pm – 25 mg
9 pm – 20 mg	9 pm – 25 mg
11 pm – 25 mg	11 pm – 25 mg
2 am – 25 mg	1 am – 25 mg
3 am – 20 mg	3 am – 20 mg

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

Results Upon Investigation

Wrong specimen

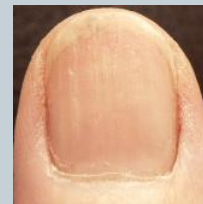Urine specimen has a wider detection window for drugs
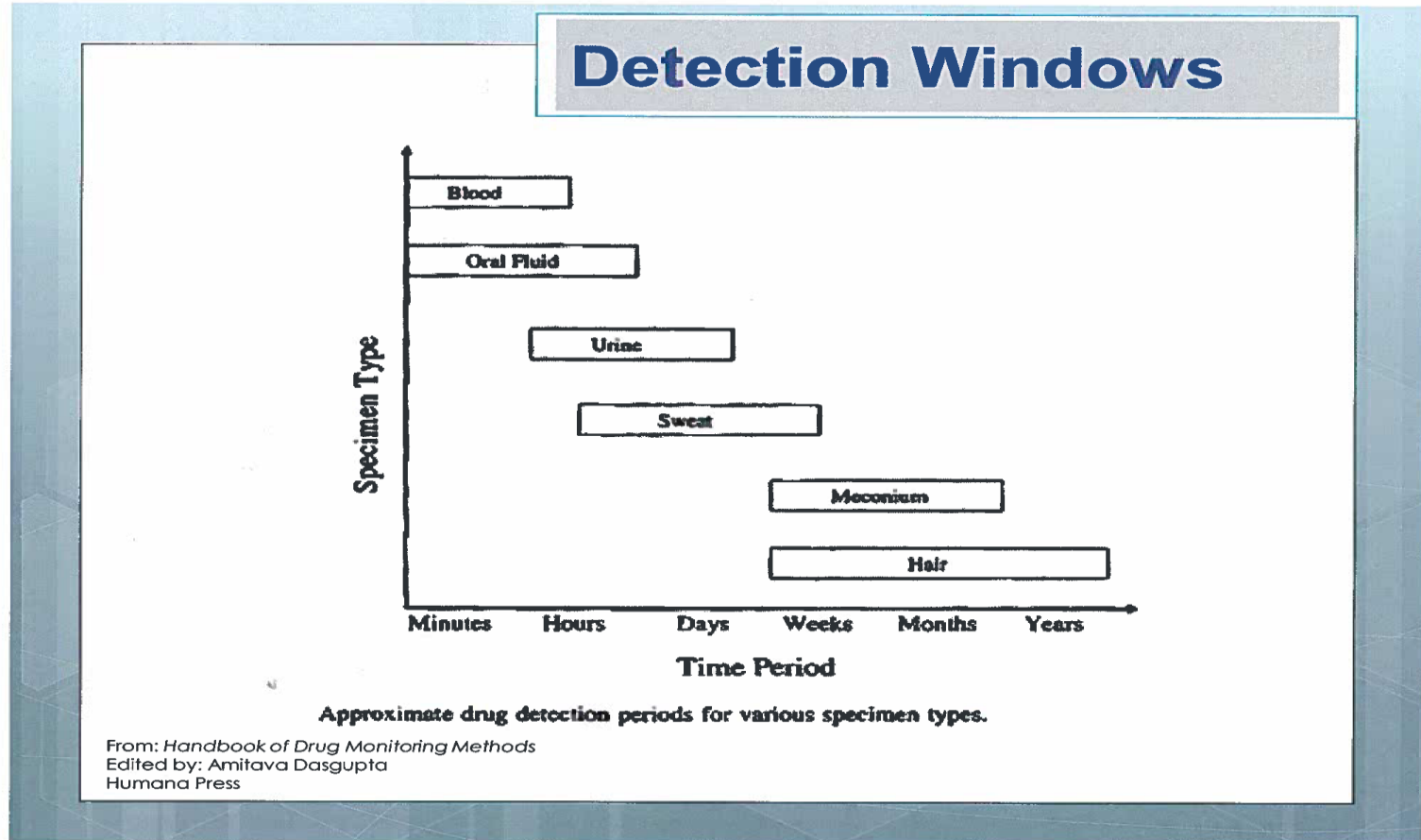
What is the detection window?

- 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

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)

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 $\leq$ 3 or $\geq$ 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
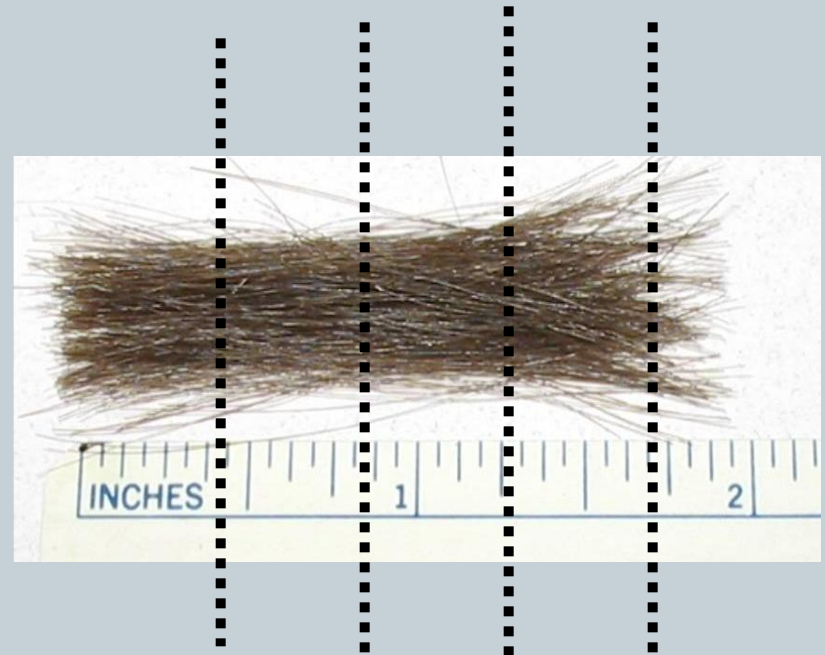
- ➤ Adulterants (mouthwashes) ineffective after ~15min

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

Bosker and Huestis (2009) Clin Chem 55:1910-1931

Hair

➤ Collections are non-invasive

  ➤ Used f or 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
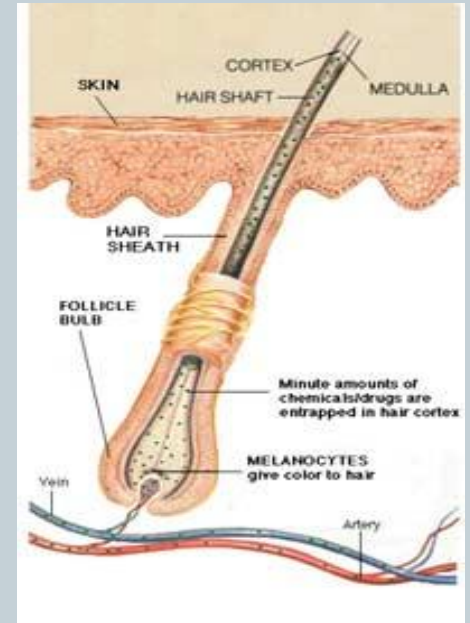
Concerns with Hair Testing

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

Closure Color	Collection Tube	Mix by Inverting
BD Vacutainer® Blood Collection Tubes *(glass or plastic)*		
	• Blood Cultures - SPS	8 to 10 times
	• Citrate Tube*	3 to 4 times
or	• BD Vacutainer® SST™ Gel Separator Tube	5 times
	• Serum Tube *(glass or plastic)*	5 times (plastic) none (glass)
	• Heparin Tube	8 to 10 times
or	• BD Vacutainer® PST™ Gel Separator Tube With Heparin	8 to 10 times
or	• EDTA Tube	8 to 10 times
	• Fluoride (glucose) Tube	8 to 10 times

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
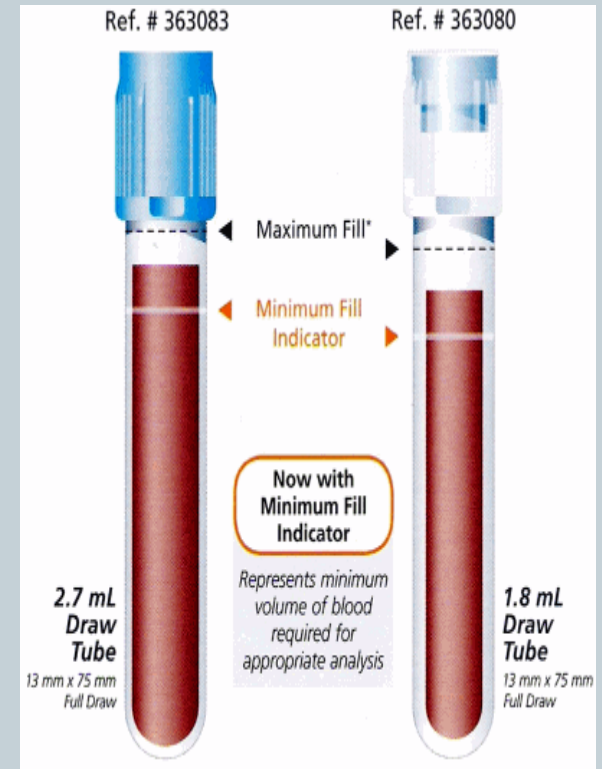
Case Scenario

- 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

Ref. # 363083   Ref. # 363080

Maximum Fill*

Minimum Fill Indicator

Now with Minimum Fill Indicator

Represents minimum volume of blood required for appropriate analysis

2.7 mL Draw Tube
13 mm x 75 mm Full Draw

1.8 mL Draw Tube
13 mm x 75 mm Full Draw

Gel Separator Tubes

Can Cause Low Drug Recovery (Lipophilic drugs)

- Cardiac Drugs
  - Flecainide
    - ↓ 40% upon contact with gel
  - Quinidine

- Tricyclic Antidepressants
  - Amitriptyline,
  - Nortriptyline
  - Desipramine

- Free Drug analysis for Anticonvulsant
  - Phenytoin
  - Carbamazepine
  - Valproic acid

- Phenobarbital (sedative, anticonvulsant)

- Lidocaine (anesthesia)

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 $t_{1/2}$
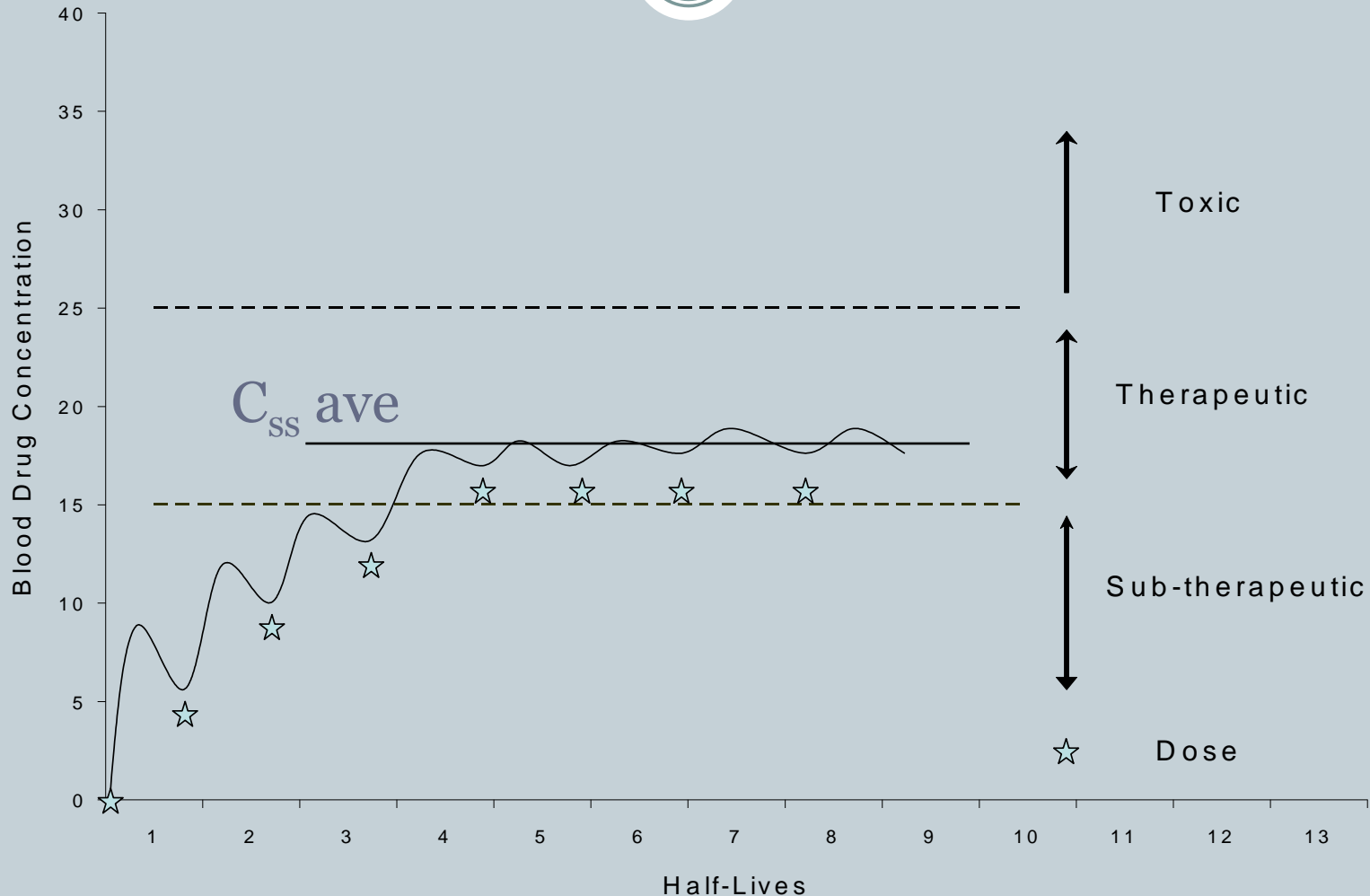
Note: plot is for illustrative purposes; drug does not have to be given at the half-life to predict C_{ss}

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

- Buproprion (antidepressant)
  - Specimen collection – Critical Frozen

- Olanzapine (antipsychotic)
  - Specimen collection – Critical Frozen

- Busulfan (anticancer)
  - Specimen collection – on ice or frozen

Labile Drugs

Light Sensitive

- Amiodarone (antiarrhythmic)
- Methotrexate (anticancer)
- Librium (antianxiety)
- Carbamazepine (anticonvulsant)
- Chlorpromazine (antipsychotic)
- Fluoxetine (antidepressant)
- Haloperidol (antipsychotic)

Heat sensitive

- Plasma concentrations of "free" drug – affect plasma protein binding
  - Phenytoin (anticonvulsant)
  - Valproic acid (anticonvulsant)
  - Total Carbamazepine (anticonvulsant)

- Lithium (mood stabilizer)
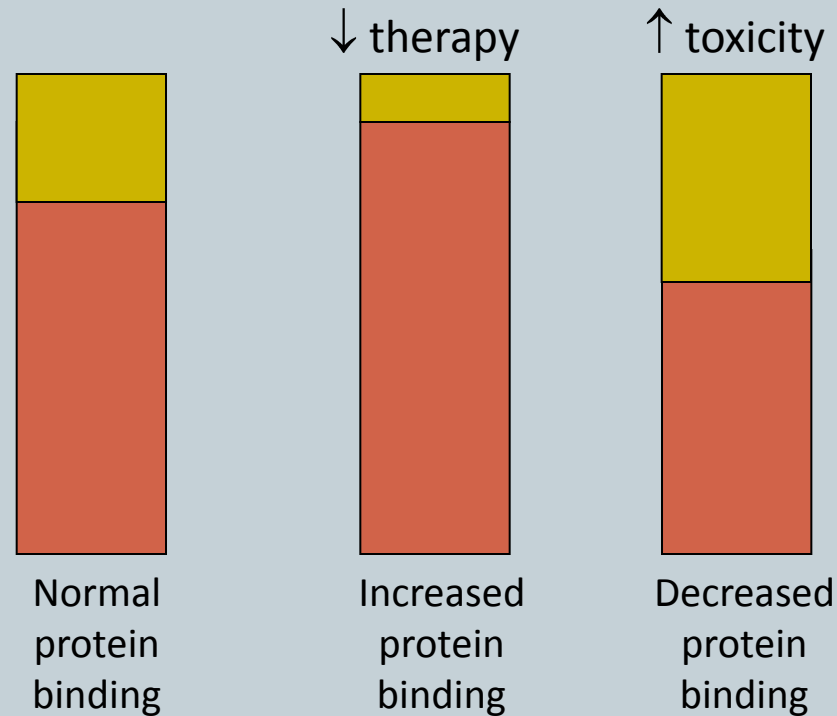
Pre-Collection Variables

- Acute phase reactants – may affect drug binding to proteins

Drug Binding to Plasma Proteins

↓ therapy   ↑ toxicity

Normal protein binding   Increased protein binding   Decreased protein binding

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

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

References

- Kohn KT, Corrigan JM, Donaldson MS. To Err Is Human: Building a Safer Health System. Washington, DC: National Academy Press; 1999.

- Nebeker JR, et al. 2005. High rates of adverse drug events in a highly computerized hospital. Arch Intern Med; 165:1111-1116.

- Plebani M. 2006. Errors in clinical laboratories or errors in laboratory medicine? Clin Chem Lab Med; 44:750-759.

- http://www.specimencare.com/main.aspx?cat=711&id=3031

- NACB Guidelines for Therapeutic Drug Monitoring Services

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

- Bosker WM, Huestis MA. 2009. Oral fluid testing for drugs of abuse. Clin Chem 55:1910-1931.

- Zevin S, Benowitz NL, 1999. Drug interactions with tobacco smoking: an update. Clin Pharmacokinet. 36:425-438.

- Ackerman WE, Ahmad M. 2007. Effect of cigarette smoking on serum hydrocodone levels in chronic pain patients. J Ark Med Soc. 104:19-21.

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