

Laboratory Drug Testing: Methods, Concepts and Case Studies

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- Gain general knowledge of the technology available for drug testing along with each technology's benefits and limitations
- Understand how drug concentration is impacted by the testing matrix (or specimen type), biological clearance rates, and dose vs. collection time
- Understanding and interpreting lab results when they are inconsistent with expectations

- No conflicts to disclose



Laboratory Methods

- *Immunoassays*
- *Mass spectrometry*
- *Strengths and Limitations*

Screen vs. Confirm

- *Differences between screen and confirm results*
- *When to screen and when to go straight to confirm*
- *Benefits and Limitations*

Timing and Types of Sample Collection

- *Mini-review on pharmacokinetics*
- *Detection windows*
- *Sample type*

Benzodiazepines Case Study

- *Metabolism pathways*
- *Result patterns and interpretations*
- *Screen results vs. confirm results*

Opioids Case Study

- *Metabolism pathways*
- *Result patterns and interpretations*
- *Screen results vs. confirm results*

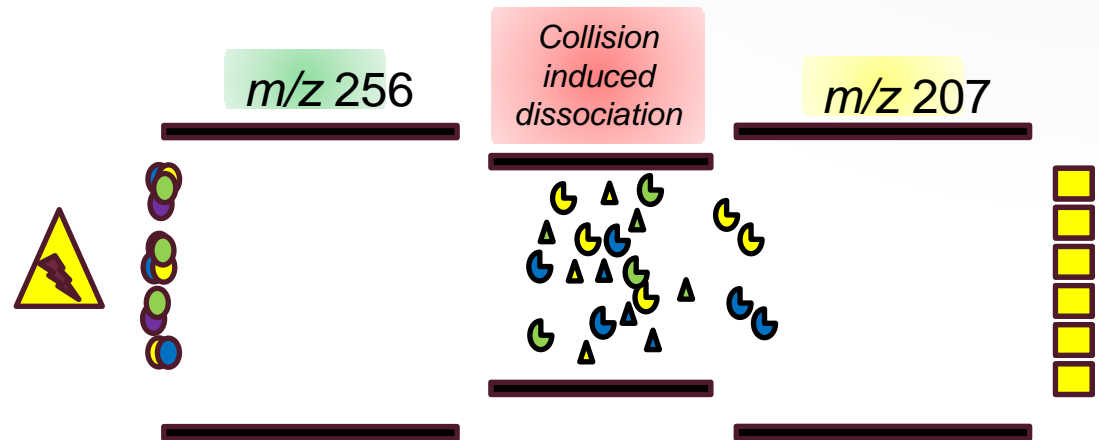
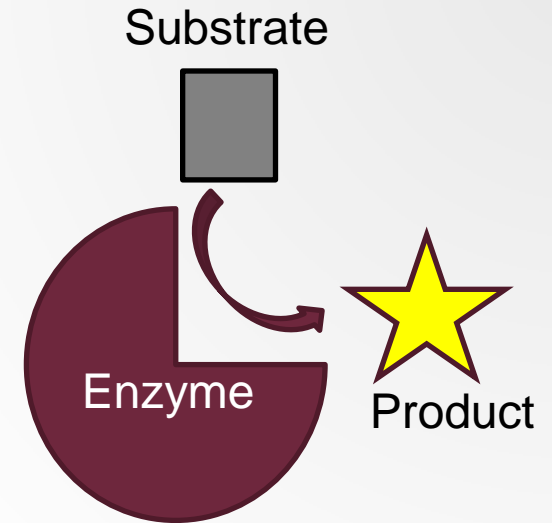
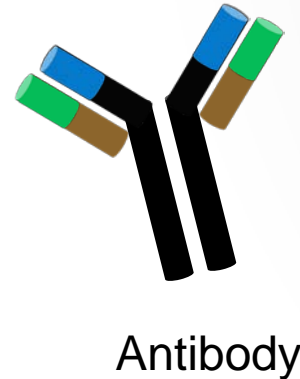
Amphetamine Case Study

- *Metabolism pathways*
- *Amphetamine False Positive*
- *Unexpected Negative Results*

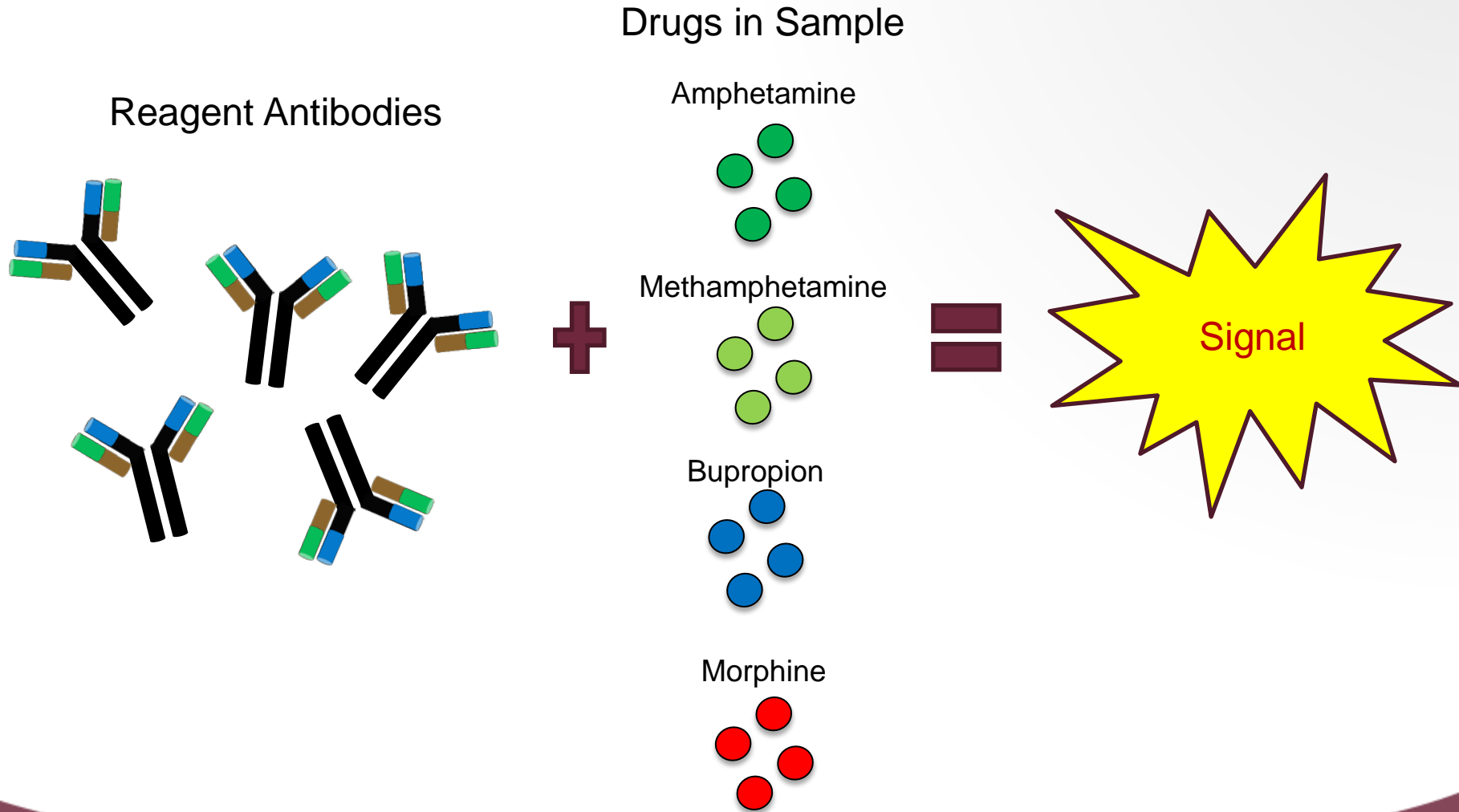
Laboratory Methods to Support Pain Management Testing



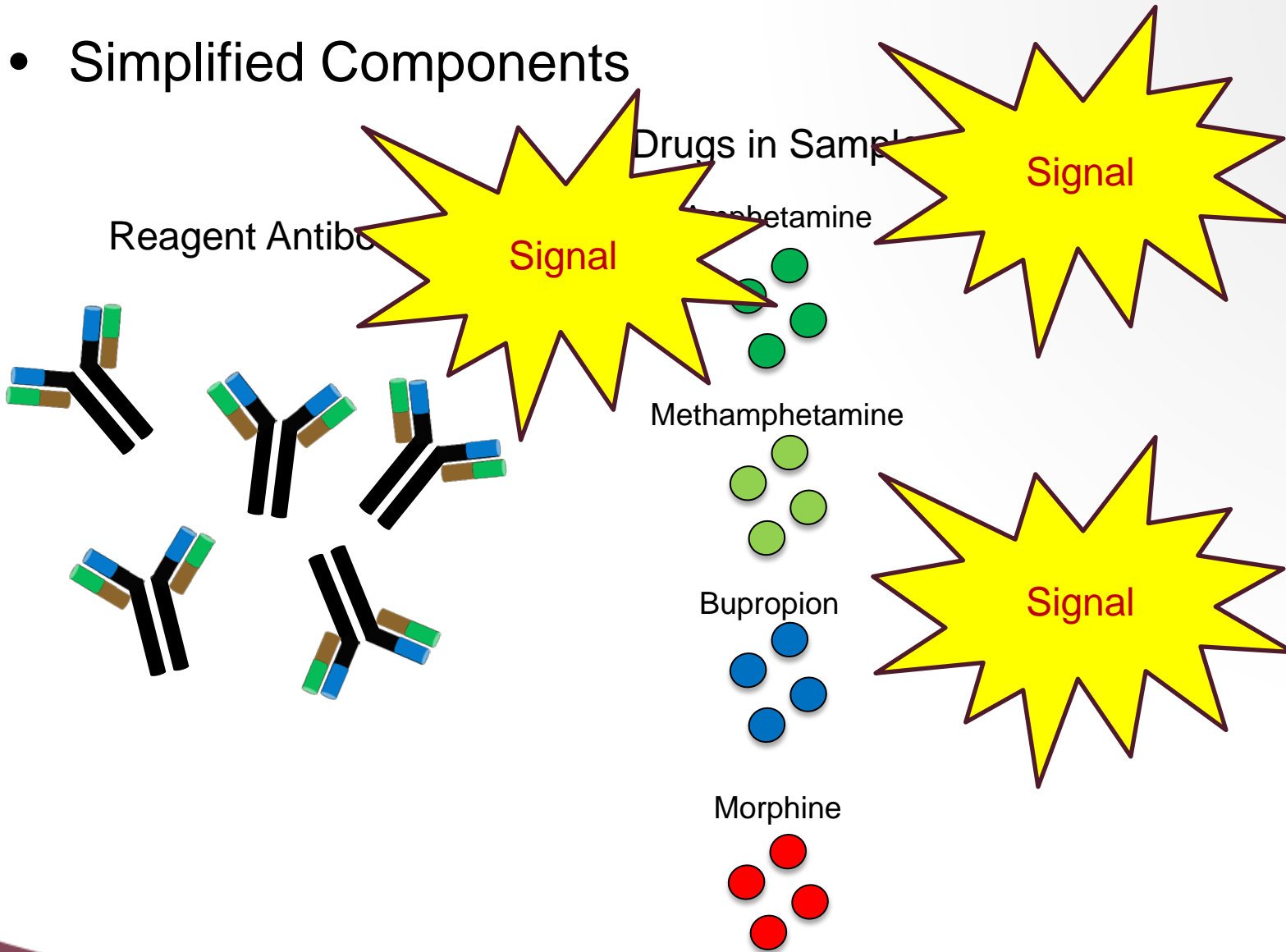
- Immunoassays
- Enzymatic assays
- GC-MS
- LC-MS
- LC-MS/MS
- LC-TOF MS



- Simplified Components



- Simplified Components



Key Points

- Cutoff is based on a “representative” compound
- Cross-reactivity allows for structurally related compound detection
- Cross-reactivity allows for false positives

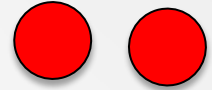
Table 7 — Concentrations (ng/mL) of Opiate Compounds That Produce a Result Approximately Equivalent to the 300 ng/mL Cutoff

Compound	Concentration (ng/mL) at 300 ng/mL Cutoff
Codeine	102–306
Dihydrocodeine	291
Hydrocodone	247
Hydromorphone	498
Levallorphan	>7500*
▶ Levorphanol	1048
Meperidine	>50000†
6-Acetylmorphine	435
Morphine-3-Glucuronide	626
Nalorphine	9862*
Naloxone	828139
▶ Oxycodone	2550
Oxymorphone	>20000

Therapeutic doses of ofloxacin (Floxin) or levofloxacin (Levaquin), non-opiates, may produce positive results with this assay. A positive result from an individual taking ofloxacin or levofloxacin should be interpreted with caution and confirmed by another method.

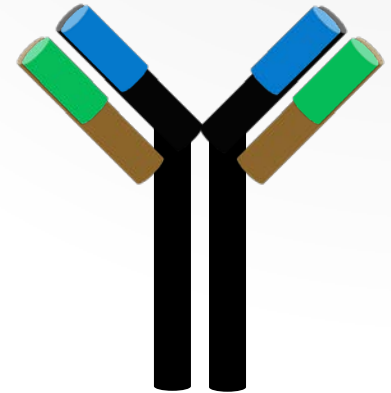
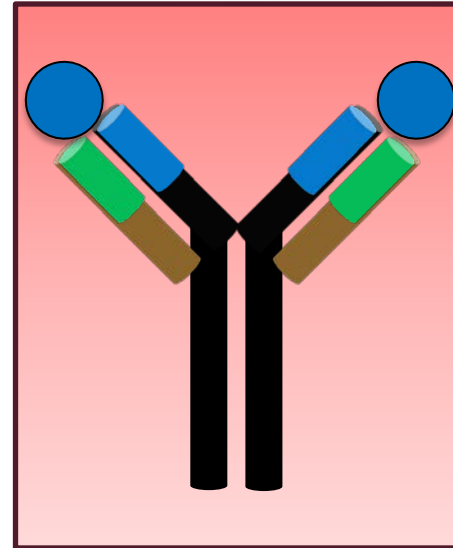
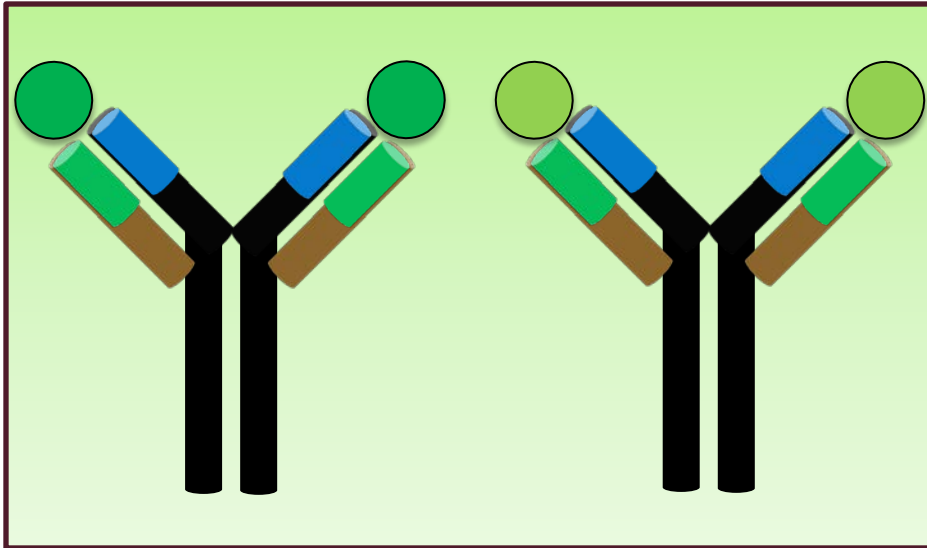
- Key Points about Immunoassays
 - Good & Bad Cross-reactivity (*sensitivity*)
 - **Can be different with different vendors**

Morphine



Amphetamine
Methamphetamine

Bupropion



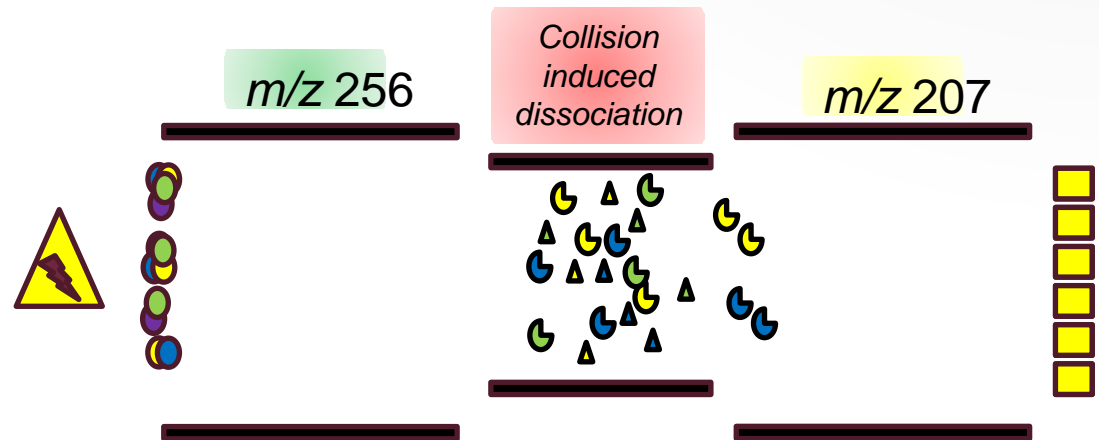
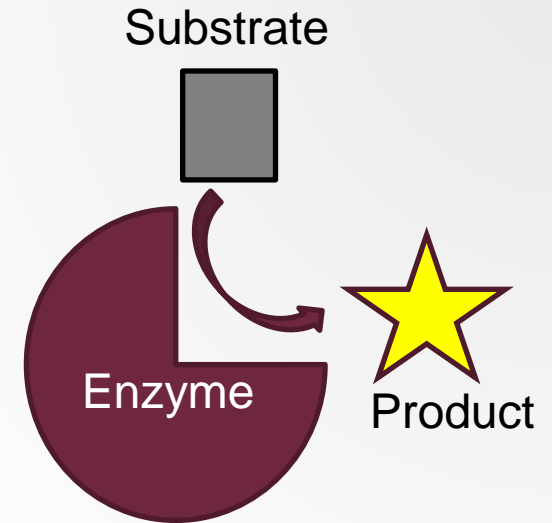
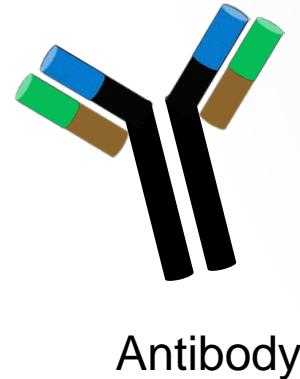
✓ Immunoassays

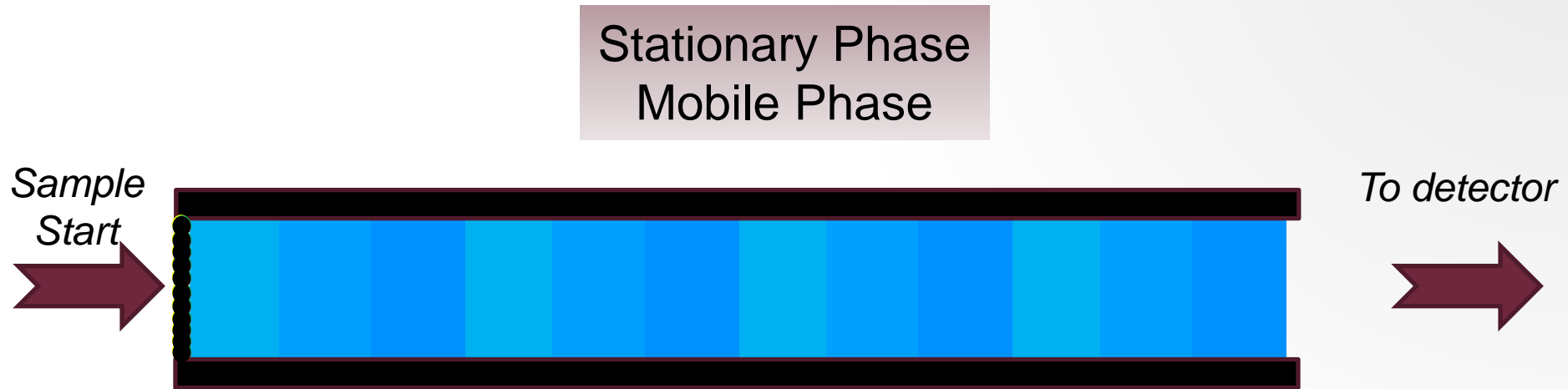
❖ Enzymatic assays

GC-MS

LC-MS/MS

LC-TOF MS





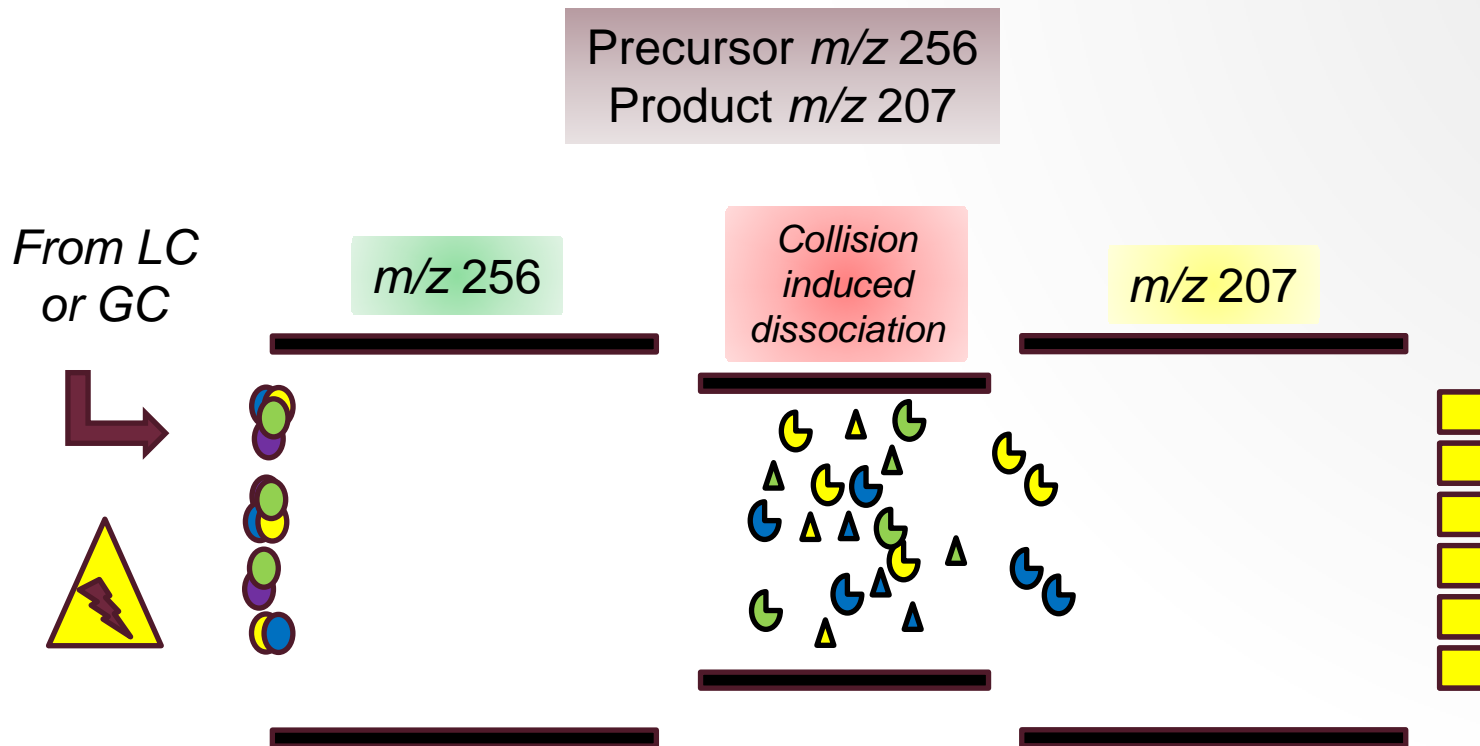
Key Concepts

1. Everything starts at the same time
2. Mobile phase moves in one direction
3. Compounds repeatedly “choose” mobile phase or stationary phase
4. Less stationary phase interaction results in early elution
5. More stationary phase interaction results in late elution

Selective for m/z 256From LC
or GC

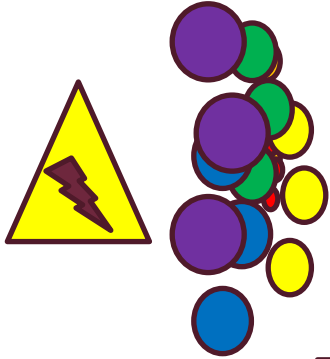
Key Concepts

1. Gas phase ions a must
2. Ion Flight Stabilization



Key Concepts

1. Precursor and Product Ion Flight Stabilization
2. Only subsets of ions get through



Key Concepts

1. Also based on m/z
2. Everything starts at the same time
3. Everything gets the same amount of “push”
4. Smaller goes faster
5. Bigger goes slower
6. Everything (eventually) gets to the detector

- Immunoassay

Good

- Detects classes of compounds
- Signal is a combination of all compounds detected – can boost sensitivity
- Fast
- Relatively inexpensive
- Point of Care Testing possible

Bad

- Cross-reactivity with unrelated compounds
- Inability to differentiate detected compounds
- Usually qualitative
- Results can differ between vendors

❖ GC or LC-MS/MS

Good

- Individual compounds identified
- Quantitation is possible
- High Specificity
- High Sensitivity

Bad

- Longer TAT
- Interferences can still occur
- Relatively more expensive

❖ LC-TOF MS

Good

- Individual compounds identified
- High Specificity
- High Sensitivity
- Reduces need for reflexive confirmation

Bad

- Longer TAT
- Interferences can still occur
- Relatively more expensive
- Not available for all sample types – yet!



Timing and Types of Sample Collection



Urine

Good	Bad
Naturally concentrated	Easier to adulterate
Metabolites can enhance detection	Dose determination NOT possible
Longer window of detection	Not appropriate for dialysis patients

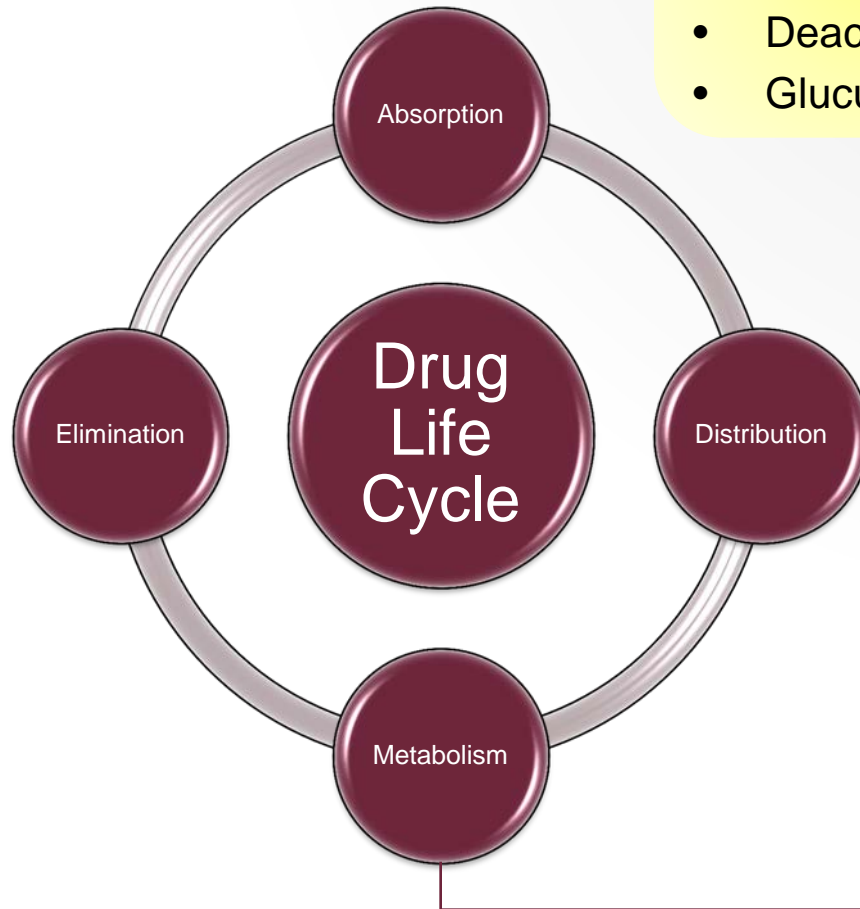
Serum/Plasma

Good	Bad
Parent drugs often present	More invasive
Pharmacokinetics can be determined	Collection timing is critical
Difficult to adulterate	Shorter window of detection
Equates dose with effect	
Appropriate for dialysis patients	

Pharmacokinetics:

What the body does to a drug

- Age
- Co-medications
- Genetics
- Clinical status
- Dosing pattern
- Drug delivery mechanism
- Food-drug interactions



- Oxidation
- Reduction
- O-Demethylation
- N-Demethylation
- Deacetylation
- Glucuronidation



Detection Windows

Drug	Plasma half-life	Urine Detection Window
Amphetamine	7 to 34 hours	3 to 5 days
Codeine	1.9 to 3.9 hours	2 to 3 days
Amobarbital	15 to 40 hours	4 to 6 days
Clonazepam *7-aminoclonazepam	19 to 60 hours 30 to 92 hours	2 to 4 days
THC (metabolite)	4 to 12 hours	1 to 45 days



Usually
measured
in HOURS

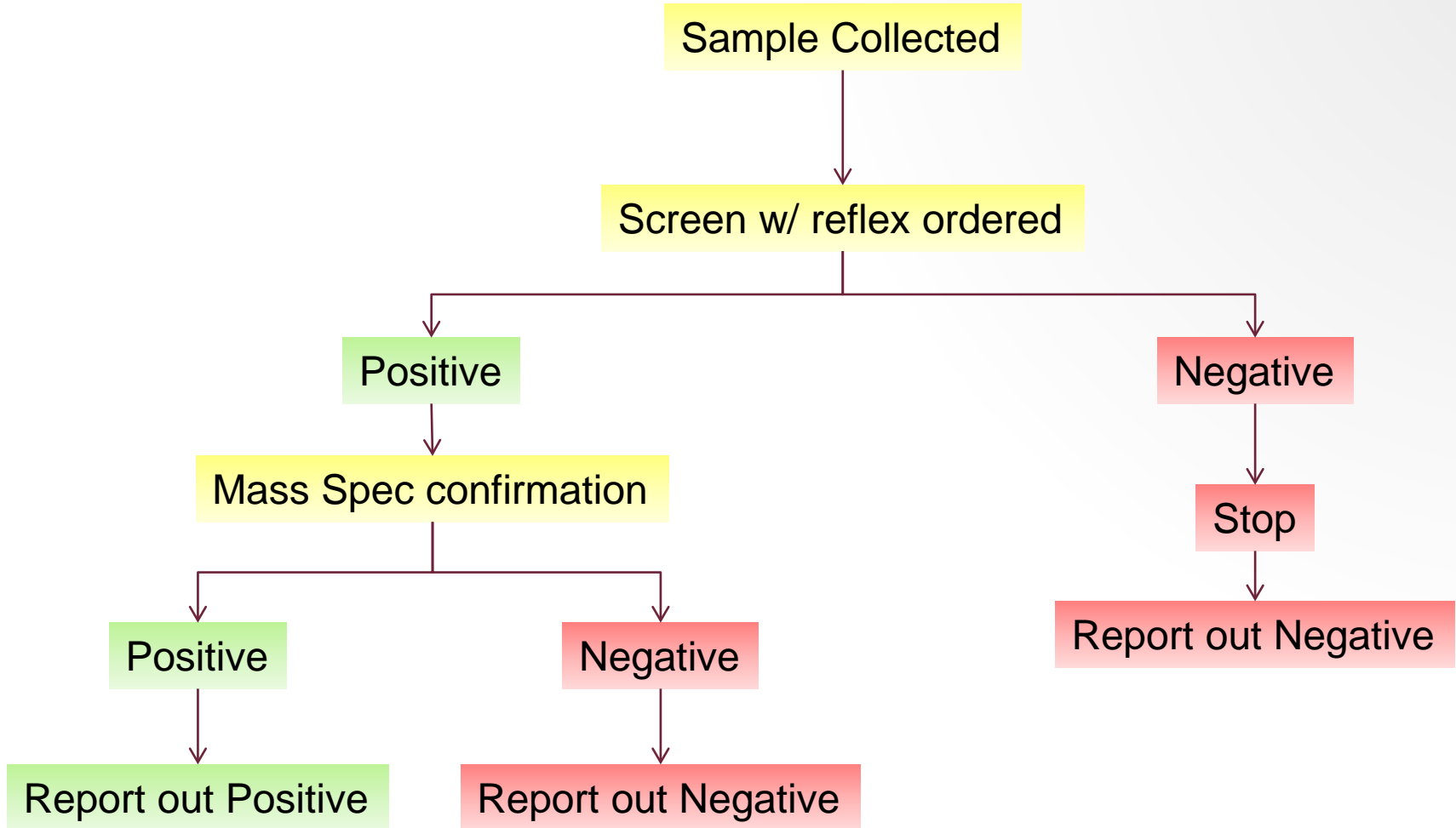


Usually
measured
in DAYS

Screen vs. Confirm



Typical Testing Workflow



- Often immunoassay based
 - *Single representative compound as target**
- Qualitative
 - *Positive or Negative Only**
- Compound classes reported

Example Results: UDS

AMPHETAMINE	NEGATIVE
BARBITURATES	NEGATIVE
BENZODIAZEPINES	NEGATIVE
COCAINE	NEGATIVE
<u>OPIATES</u>	H <u>POSITIVE</u>
PCP	NEGATIVE
PROPOXYPHENE	NEGATIVE

Possible Interpretations

- ✓ Morphine
- ✓ Codeine
- ✓ Hydrocodone
- ✓ Heroin
- ✓ Levofloxacin (Levaquin)

“Which Lab” makes a big difference!

ARUP, Drugs of Abuse 0090453

Drugs
Marijuana
Cocaine
Opiates
Oxycodone
Phencyclidine
Amphetamines
MDMA (Ecstasy)
Barbiturates
Benzodiazepines
Methadone
Propoxyphene

Lab “L”, Drug Abuse Profile

Drugs
Marijuana
Cocaine
Opiates
Ethanol
Phencyclidine
Amphetamines
Barbiturates
Benzodiazepines

Lab “M”, Drug of Abuse Screen

Drugs
Marijuana
Cocaine
Opiates
Phencyclidine
Amphetamines
MDMA (Ecstasy)
Barbiturates
Benzodiazepines
Methadone
Propoxyphene

- Different method than the method used to screen the specimen
- Different aliquot of the same sample
- Typically Quantitative
- Mass spectrometry most common (LC-MS/MS)

Example Results: Urine Opioid Confirmation

Hydrocodone	= 897 ng/mL
Hydromorphone (free)	= 6 ng/mL
Dihydrocodeine	(qualitative only)
Unable to identify Oxycodone (free) due to interfering substances in the specimen	

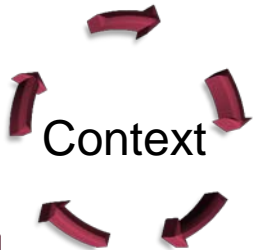
Possible Interpretations

- ✓ Hydrocodone
- ✓ Codeine

Is Confirmation Testing Needed?

Tests that usually don't require confirmation

- Amp w/o meth
- Barbs
- Cocaine
- Marijuana
- Methadone
- Meth w/ amp
- PCP
- Propoxyphene
- TCAs



- Screen alone
 - Sometimes concentration is not needed
 - False positives are low
 - Results consistent with expectations

- Screen w/ Reflex to Quantitative confirmation
 - Opiates and oxycodone
 - Benzodiazepines
 - Screen results unexpected

- Drugs not included in screening panel
 - Buprenorphine
 - Fentanyl

Benzodiazepine Case Study



- Age: 61
- Gender: F
- Relevant medications
 - Clonazepam

Problem

Repeatedly NEGATIVE urine screens for benzos

Compliance

- Drug wasn't taken
- Drug taken wrong
- Adulteration

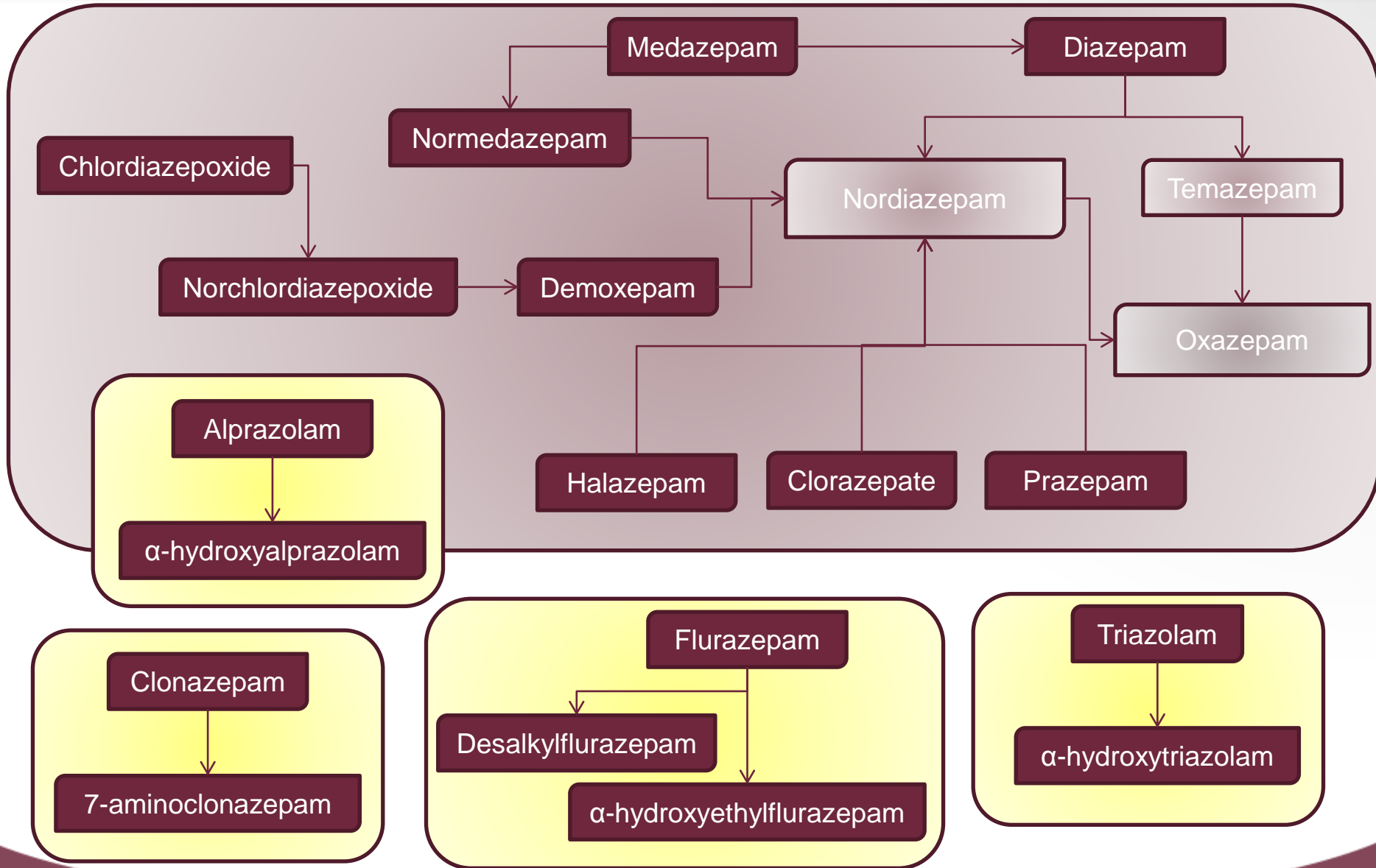
Physiology

- Drug not absorbed
- Fast metabolizer

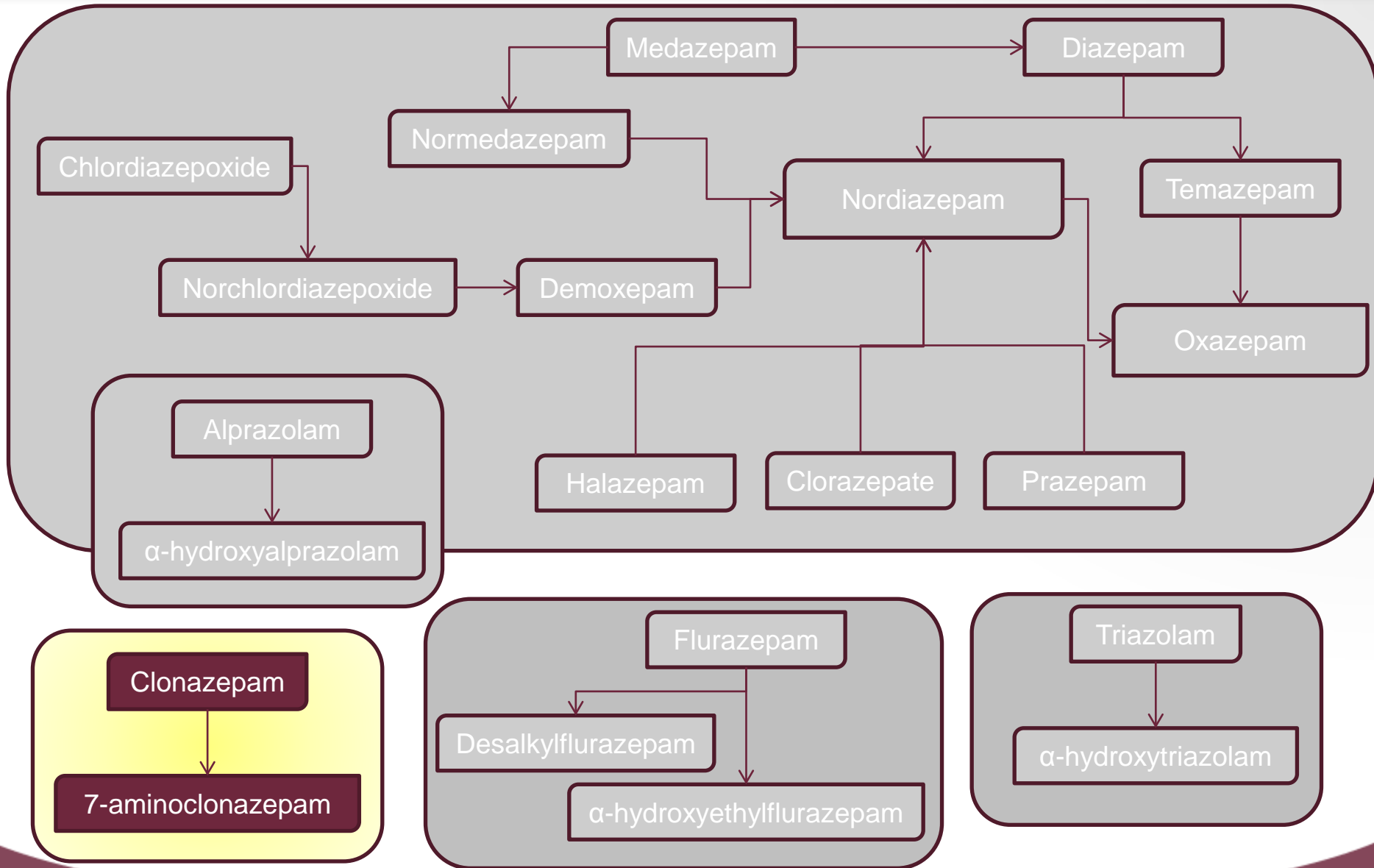
Testing

- Specimen timing wrong
- Specificity/Sensitivity inadequate
- Mix-up

Benzodiazepine Metabolism



Benzodiazepine Metabolism



What is the assay target? ►

- ARUP
 - EMIT II Plus Benzodiazepine
 - Lormetazepam as representative target
 - 200ng/mL cutoff

The Benzodiazepine Assay has two cutoffs: 200 ng/mL and 300 ng/mL Lormetazepam.

Positive – The drugs listed are in ng/mL at which they will cross-react equivalent to the Lormetazepam cutoff.

	200 Cutoff	300 Cutoff
Alprazolam	65	79
7-Aminoclonazepam	5700	11000
7-Aminoflunitrazepam	590	1400
7-Aminonitrazepam	365	1000
Bromazepam	630	1400
Chlordiazepoxide	3300	7800
Clobazam	260	350
Clonazepam	260	500
Clorazepate	#	#
Clotiazepam	250	420

Clonazepam Facts

- Detection Time of 1 – 10 days in Urine
- Predominately excreted as 7-aminoclonazepam
- Little to no clonazepam excreted

- ✓ Multiple negative benzo screens
 - Consistent with assay performance
 - Assay looking for clonazepam
 - Urine likely contains 7-aminoclonazepam

1. Skip the screen and go straight to confirm

- More specific assay
- 7-aminoclonazepam measured directly
- More sensitive

Screen vs. Confirm ►

2. Order screen and benzo confirm regardless of screen result

- Same reasons as #1
- Identify abused drugs if clinical suspicion is high

3. Test blood

- More likely to find parent drug
- ARUP assay is directed against clonazepam



Opioids Case Study



- Age: 53
- Gender: M
- Relevant medications
 - Percocet (Oxycodone w/ Acetaminophen)

Problems

- 1st urine screen POSITIVE for opiates
- Reflex confirm POSITIVE for hydrocodone, hydromorphone, dihydrocodeine
- 2nd urine screen NEGATIVE for opiates

Compliance

- Drug was taken
- Drug added to urine
- Drug abuse
- Incorrect prescription

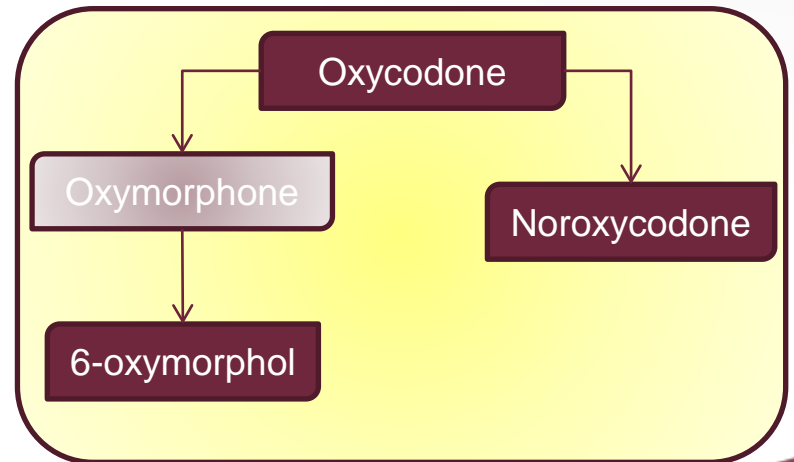
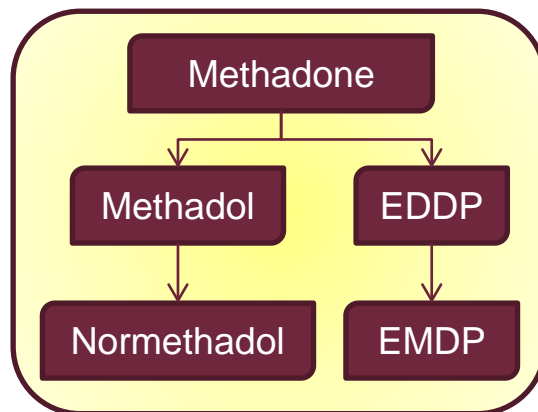
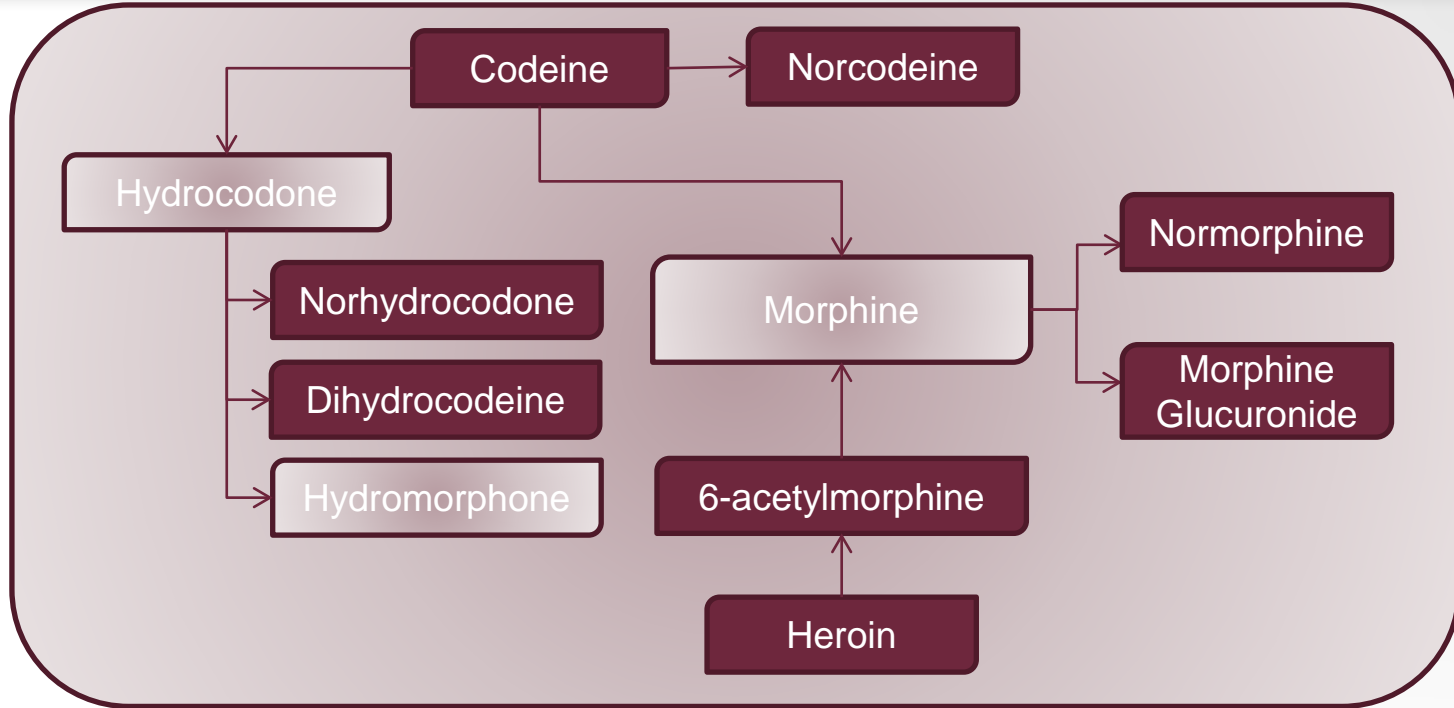
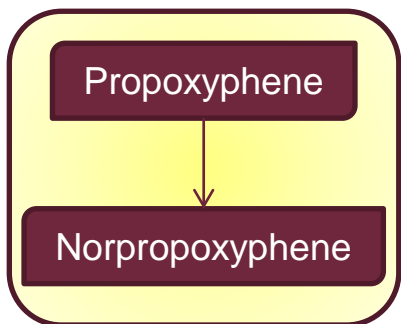
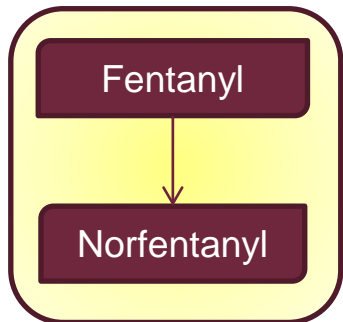
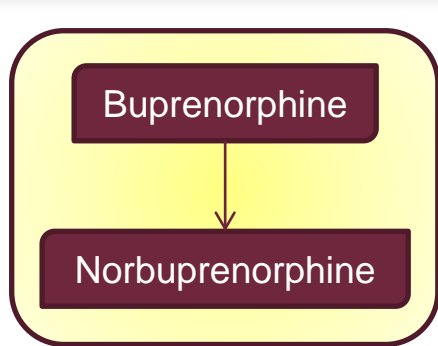
Physiology

- Drug is a metabolite of the prescribed medication
- Fast metabolizer

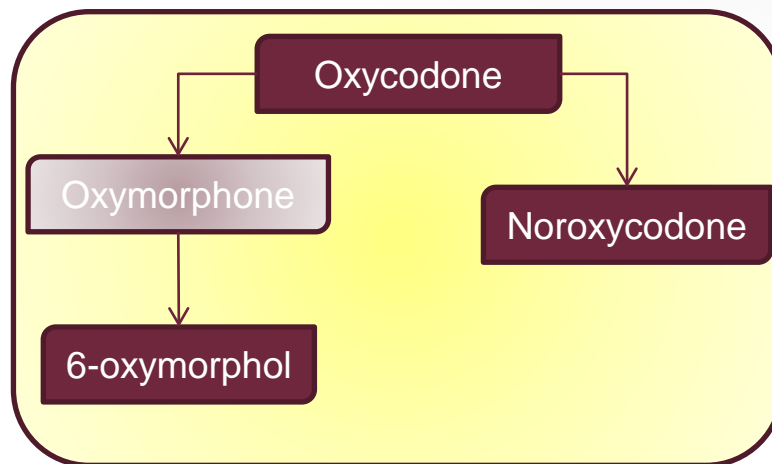
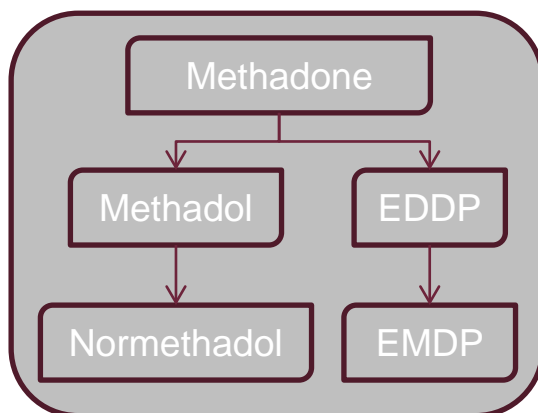
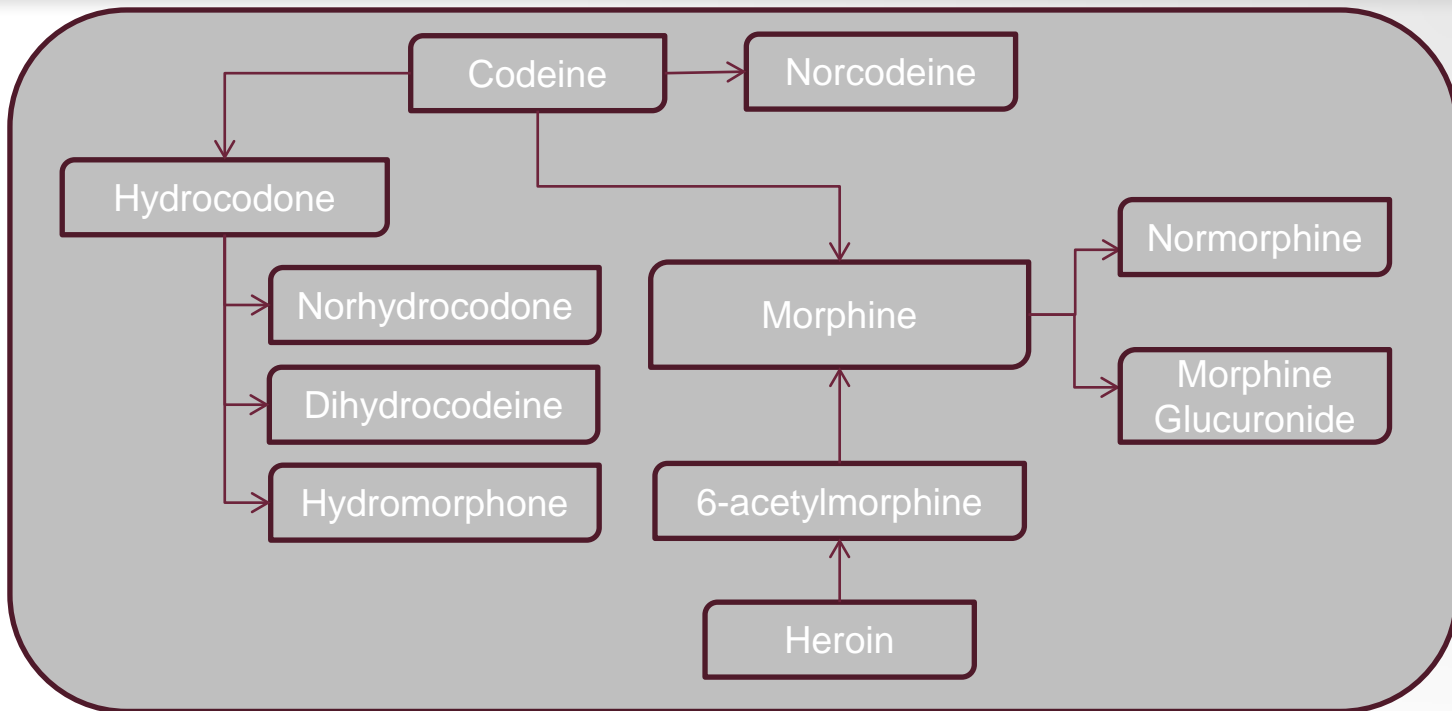
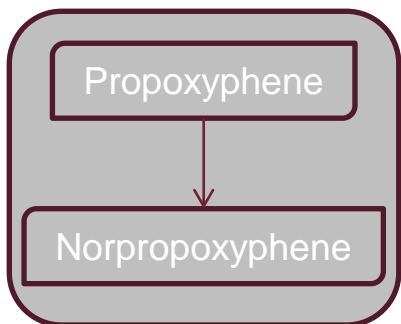
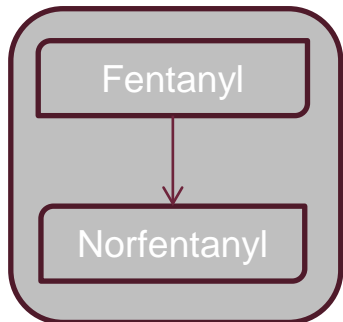
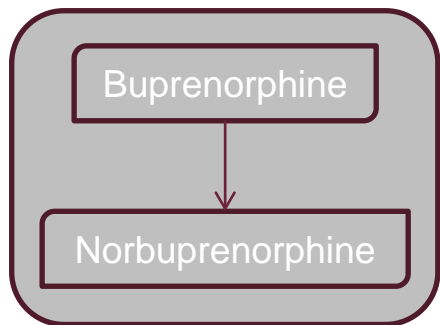
Testing

- Specimen timing wrong
- Specificity inadequate
- Mix-up

Opiate & Opioid Metabolism



Opiate & Opioid Metabolism

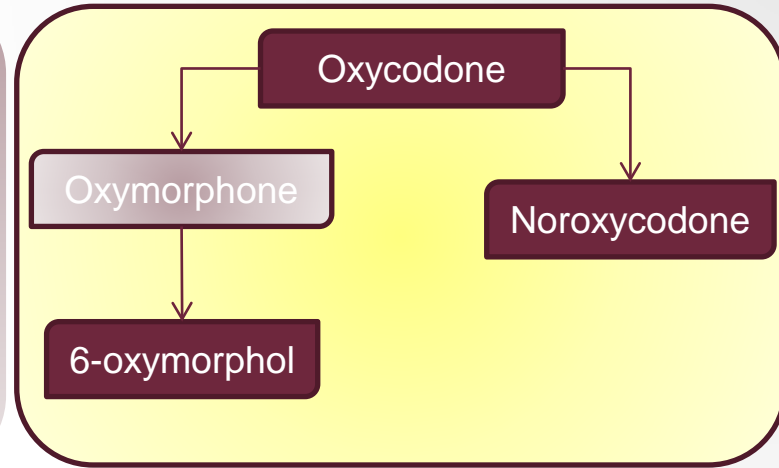


1st Opiate Screen and Confirm

- **ARUP**

What lab performed the screen? ►

- EMIT II Plus Opiate
- Morphine as representative target
- 300ng/mL cutoff



Positive – The drugs listed are in ng/mL at which they will cross-react equivalent to the morphine cutoff.

	300 Cutoff	2000 Cutoff
6-Acetylmorphine	435	4182
Codeine	102–306	660–1980
Dihydrocodeine	291	1872
Hydrocodone	247	1545
Hydromorphone	498	5349
Levofloxacin	125000	–
Levorphanol	1048	4700
Morphine-3-Glucuronide	626	6167
Nalorphine	5540	(see below)
Naloxone	11000	(see below)
Normorphine	1200	–
Ofloxacin	330	–
Oxycodone	1500	(see below)
Pholcodine	320	1400

Confirm Results - ARUP

POSITIVE
 Confirmed **POSITIVE** by LC-MS/MS for the following opiate(s):

- Hydrocodone = 897 ng/mL
- Hydromorphone (free) = 6 ng/mL
- Dihydrocodeine (qualitative only)

Unable to identify Oxycodone (free) due to interfering substance(s) in the specimen.

Compliance

- Drug wasn't taken
- Drug taken wrong
- Adulteration

Physiology

- Drug not absorbed
- Fast metabolizer

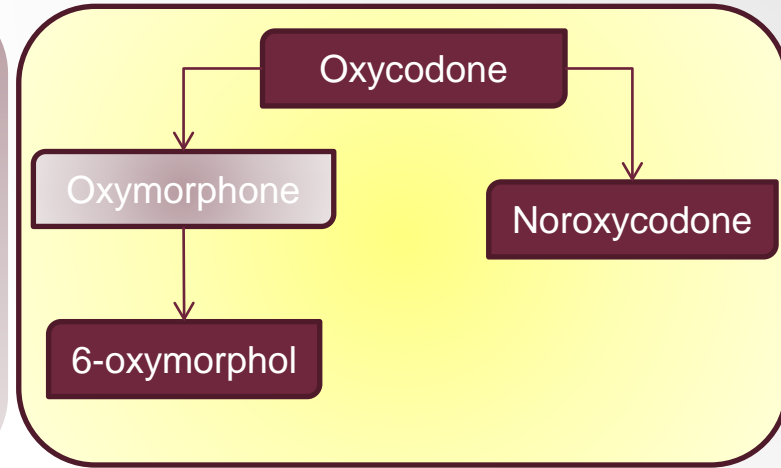
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- **ARUP**

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Naloxone	11000	(see below)
Normorphine	1200	–
Ofloxacin	330	–
Oxycodone	1500	(see below)
Pholcodine	320	1400

✓ 1st screen w/ reflex confirmation

- Inconsistent w/ Oxycodone ingestion alone
- Ingestion of hydrocodone containing product highly likely

✓ 2nd screen

- Incorrect screening test most likely (Oxycodone might be there but the ordered test couldn't find it)

1. Ensure drug screen is targeted to drugs of interest
 - Opiate screen will not reliably find oxycodone
 - Separate oxycodone screening assay is needed
2. Order oxycodone screen alone
 - No clinical concern for abuse of other drugs
3. Order opiate & opioid confirmation directly
 - Provides individual drugs with quantitation
 - No clinical concern for abuse of other drugs
4. Counsel/confront patient and provide an opportunity for re-testing with a new sample to avoid the possibility of sample mix-up



Screen vs. Confirm ▶

What is the assay target? ▶

Amphetamine Case Study



- Age: 64
- Gender: F
- Relevant medications
 - Tylenol w/ Codeine, Wellbutrin (Bupropion)

Problem

POSITIVE amphetamine screen w/ negative confirmation

Compliance

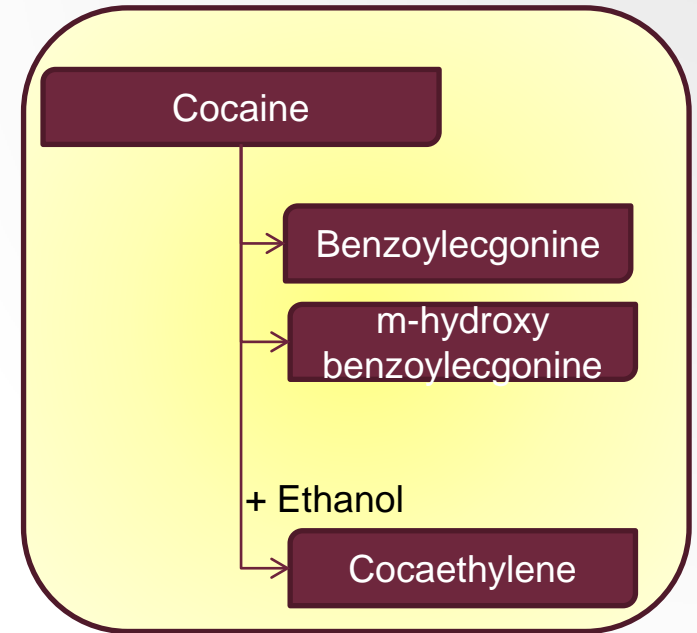
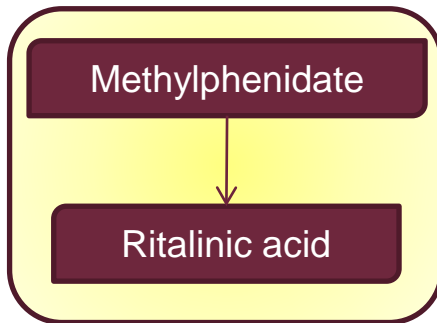
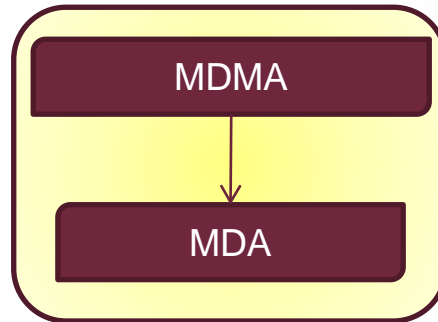
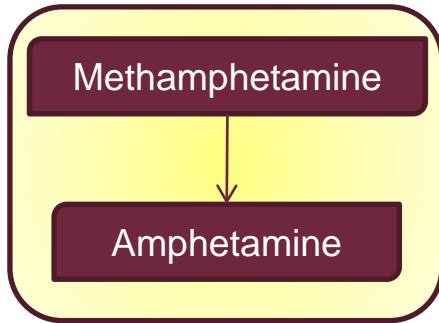
- Drug was taken
- Drug added to urine
- Drug abuse
- Incorrect prescription

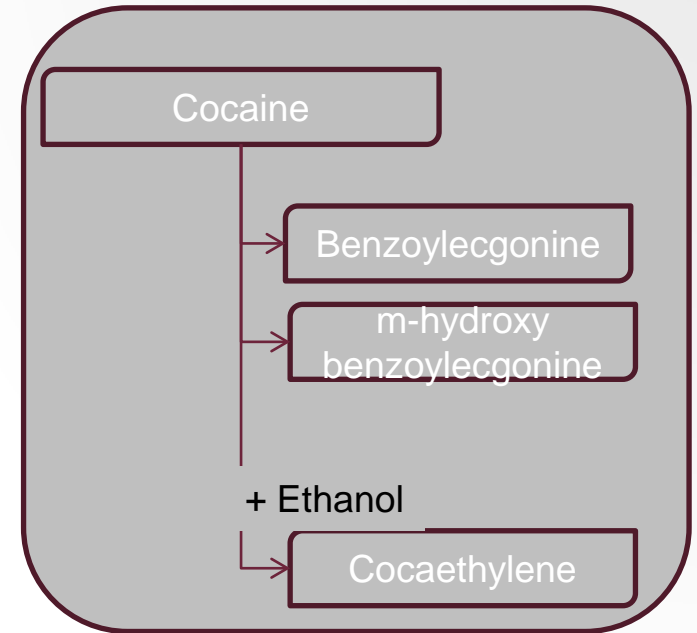
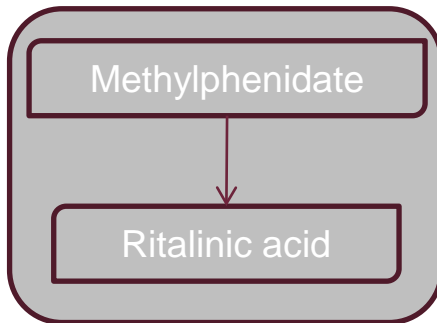
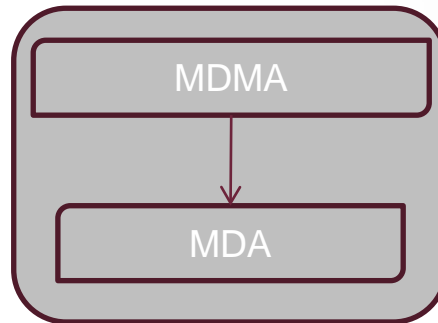
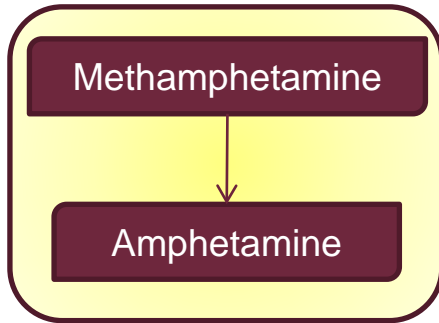
Physiology

- Drug is a metabolite of the prescribed medication
- Fast metabolizer

Testing

- Specimen timing wrong
- Specificity inadequate
- Mix-up





- ARUP
 - EMIT II Plus Amphetamines
 - d-Methamphetamine as representative target
 - 300ng/mL cutoff

The Amphetamines Assay has three cutoffs: 300 ng/mL, 500 ng/mL, and 1000 ng/mL d-Methamphetamine.

Positive– The drugs listed are in ng/mL at which they will cross-react equivalent to the d-Methamphetamine cutoff.

	300 Cutoff	500 Cutoff	1000 Cutoff
d,l-Amphetamine	625	1050	2150
l-Amphetamine	3450	3750	11500
Benzphetamine *	400	700	1000
d,l-Methamphetamine	450	700	2100
l-Methamphetamine	725	1325	3650
MDA (Methylenedioxyamphetamine)	1100	1700	(see below)
MDEA (Methylenedioxyethamphetamine)	4400	6800	(see below)
MDMA (Methylenedioxymethamphetamine)	5200	9150	(see below)
Phenmetrazine	2300	3500	13000
Selegiline	#	#	#

Common Issues

- Vicks inhaler
- D/L isomers
- Selegiline metabolite
 - AMP/MAMP
- Adderall
- Vyvanse

Negative — Structurally Related – The drugs listed are in $\mu\text{g}/\text{mL}$ at which they will cross-react equivalent to the d-Methamphetamine cutoff.

	300 Cutoff	500 Cutoff	1000 Cutoff
Bupropion	250	500	2220
Cathinone	>100	>100	>100
4-Chloramphetamine	2.6	4.5	12.2
Chloroquine	2100	2200	4500
l-Ephedrine	400	800	3500
Fenfluramine	25	40	150
MDA (Methylenedioxyamphetamine)	(see above)	(see above)	6.5
MDEA (Methylenedioxyethamphetamine)	(see above)	(see above)	27.2
MDMA (Methylenedioxymethamphetamine)	(see above)	(see above)	34.3
Mephentermine	8	15	60
Methcathinone	>100	>100	>100
Methoxyphenamine	90	160	360
Phentermine	5.8	9	25
Phenylpropanolamine	700	1000	2000
PMA (p-Methoxyamphetamine)	4	7	34
PMMA (p-Methoxymethamphetamine)	8	14	81
Propranolol	100	125	500
d,l-Pseudoephedrine	1400	2600	8300
nor-Pseudoephedrine	40	70	170
Quinacrine	2500	3800	16500
Tranlycpromine	30	60	200
Tyramine	150	200	600

- ✓ Positive amphetamine screen
 - Consistent w/ bupropion ingestion

What cross-reacts in the assay? ►

- ✓ Negative amphetamine confirmation
 - Consistent w/ bupropion ingestion

Screen vs. Confirm ►

1. Expect the amphetamine positive and ignore
 - Low clinical suspicion of abuse
2. Skip the screen and go straight to confirm for opiates/opioids and/or amphetamines
 - More specific assay
 - Methamphetamine and amphetamine do not interfere with opioid confirm
 - Codeine (and metabolites) measured directly
3. Order screen and amphetamine confirm regardless of screen result
 - Same reasons as #2
 - Identify abused drugs if clinical suspicion is high





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