

## Complexities of urine drug testing for opioids and other drugs commonly used for chronic pain management

Bridgit Crews, PhD, DABCC

Chemistry & Toxicology

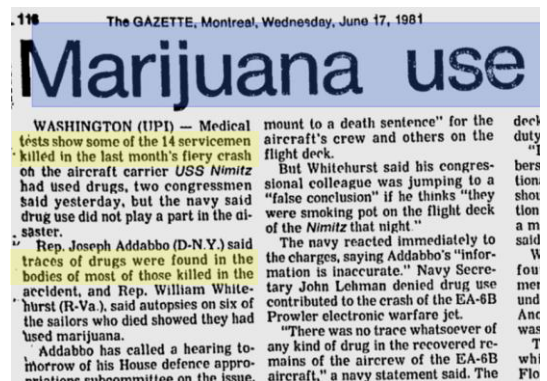
Kaiser Permanente Regional Laboratories

## Heroin Epidemic of 1960's

- From 1950-1961 the US death rate from heroin increased 5-fold.
- Heroin became the leading cause of death in NYC for adults age 15-35. The average age of death from heroin was 29 years old.
- Methadone maintenance was developed as a medical treatment for opioid addiction.
  - First US program was opened in 1958, as of 1998 there were about 180,000 people in MM (18-36% of heroin users in US)

## History of Urine Drug Testing (UDT)

- By 1970 the Fed. Gov. implemented specific, mandatory testing requirements for methadone treatment programs licensed by the FDA.
- In 1960's and 70's Military recognized increase in heroin use in personnel expanded testing to active duty personnel using urine testing - positives were treated as a "medical cases" (no punishment).



## Federal Oversight of Drug-testing

- 1986 Federal Drug-Free Workplace Program.
- 1989 Department of Transportation required testing of 7 million private sector workers.

U.S. Department of Health & Human Services was given the authority to develop guidelines to be used by the federal workplace drug-testing programs.

## SAMHSA

Substance Abuse and Mental Health Services Administration (Branch of HHS)

SAMHSA oversees Federal Workplace Testing programs **and** Opioid Management Treatment programs.

*NO FEDERAL TESTING GUIDELINES FOR CLINICAL DRUG TESTING PROGRAMS (OTHER THAN LABORATORY REQUIREMENTS PERTAINING TO CLIA)*

## SAMHSA Testing Guidelines

### Forensic (Workplace)

- Urine
- Chain-of-custody procedures
- Extensive validity testing
- GC/MS (LC-MS/MS)
- Test for "Federal 5"
- Established cutoffs
- Labs must be SAMHSA certified
- Results must be reviewed by a MRO

### Clinical (Substance Abuse Treatment)

- Urine, oral fluid, ??
- Basic sample ID protocols
- Validity testing may be performed
- GC/MS, LC-MS/MS, LC-HR/MS
- No set testing panels
- No set cutoffs
- Labs must be CLIA certified
- Results must be reviewed by CLS if testing is not CLIA-waived.

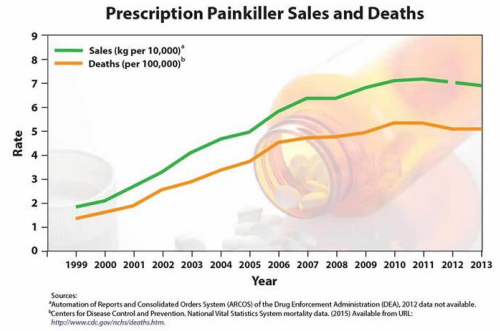
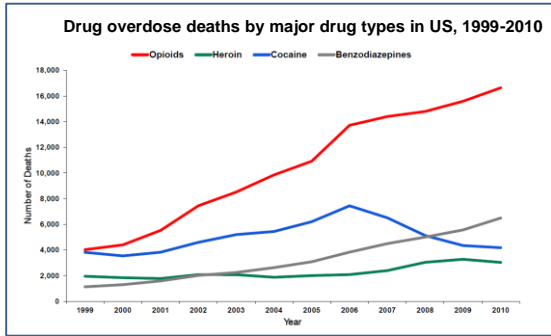
## Clinical Drug Testing

### Populations Tested:

- Emergency department
- OB/GYN, PED (Kaiser Early Start Program)
- Pain management with chronic opioid therapy
- Addiction and chemical dependency rehabilitation

### Prescription Drug Abuse:

Prescription drugs account for the 2<sup>nd</sup> most commonly abused drugs, behind marijuana and ahead of cocaine, heroin, methamphetamine.



**Risk Factors for Prescription Painkiller Abuse and Overdose**

- Obtaining overlapping prescriptions from multiple providers and pharmacies.
- Taking high daily dosages of prescription painkillers.
- Having mental illness or a history of alcohol or other substance abuse.
- Living in rural areas and having low income.

### Root Cause Analysis of Opioid Related Deaths

Most opioid decedents had **multiple substances** in their blood at the time of death

80% of opioid-associated deaths (West Virginia)

60% of methadone-related deaths (Utah)

- Alcohol is most common
- Benzodiazepines (17%)

*Pain Medicine 2011; 12: S26-S35*

Street market for prescription pain drugs

Drug	Street price	Legal price (w/prescription)
Oxycodone	\$10-80	\$6.00
Hydrocodone	\$5-25	\$1.50

Diversion is also a real concern.

CNNMoney June 1, 2011

Most recent guidelines for opioid prescribing agree that urine drug testing as one risk mitigation strategy

American Academy of Pain Medicine:

"Monitoring of compliance is a critical aspect of chronic opioid prescribing, using such tools as random urine drug screening, pill counts, and where available, review of prescription monitoring data base reports."

American Pain Society and American Academy of Pain:

"clinicians should obtain urine drug screens or other information to confirm adherence"

Institute for Clinical Systems Improvement:

"Random drug screens are one tool to monitor compliance with the opioid regimen [...], Check for diversion, Check for drugs of abuse, Test for the presence of the prescribed drug"

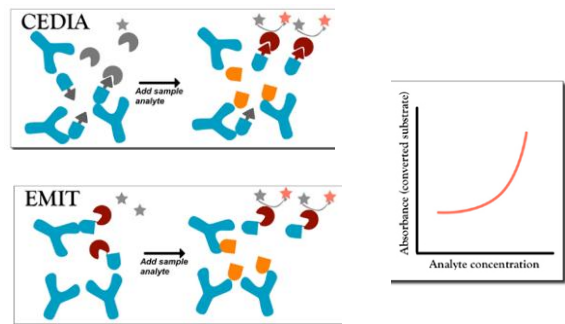
Most clinical urine drug testing is modeled after workplace drug testing (Forensic Model)

Developed in the late 70's to 80's:

- Amphetamines, Cocaine, Morphine, PCP, Marijuana.
- Most workplace specimens (98%) are negative.

Immunoassay  $\xrightarrow{\text{If Positive, Confirm}}$  GC-MS, LC-MS/MS

Cloned Enzyme Donor Immunoassay



Enzyme Multiplied Immunoassay Technique

### Cross-Reactivity of Opiates Immunoassays

Listed concentrations (ng/mL) give a positive signal equivalent to 300 ng/mL morphine

OPIATES	Emit II Plus	ONLINE DAT II	CEDIA	DRI and MULTIGENT
morphine	300	300	300	300
morphine glucuronide	626	552	370-638	270-340
codeine	102-306	224	240	150
hydromorphone*	498	1,425	526	1,400
hydrocodone	247	1,086	625	650
oxycodone	1500	> 75,000	>10,000	10,500
oxymorphone	9300	> 100,000	>20,000	37,000
6-acetylmorphine	435	386	370	280

\* Cross-reactivity is not reported for hydromorphone-glucuronide.

### DRI® Immunoassay vs. LC-MS/MS

Analyte	EIA cutoff (ng/mL)	LCMS cutoff (ng/mL)	% Missed
<b>Opiates</b>	<b>300</b>		
Codeine	150	50	30
Hydrocodone	650	50	23
Hydromorphone	1400	50	69
Morphine	300	50	12

*Perform 400 UDAP P per day*  
*280 screen positive (and confirm positive)*  
*~ 120 screen negative*  
*~ 16% of these are FALSE NEGATIVES!*

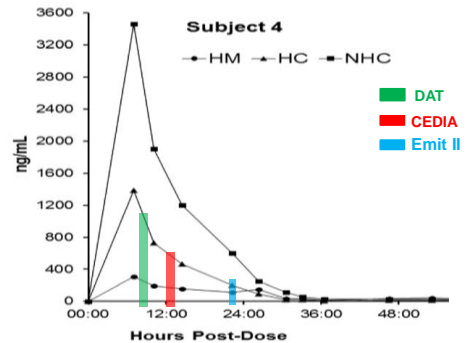
Pain Physician 2010; 13:273-81, Pain Physician 2010; 13:71-81, Ther. Drug Mon. 2009; 13:746-8

### Detection Time Windows Depend on Cut-offs (and many other variables)

Drug	Window of Detection	Cut-off (ng/mL)
Buprenorphine	Up to 4 days	0.5
Codeine	1 - 2 days	300
Morphine	1 - 2 days	300
Hydrocodone	1 - 2 days	100
Hydromorphone	1 - 2 days	300
Oxycodone (immediate)	1 - 1.5 days	100
Oxycodone (controlled)	1.5 - 3 days	100
Methadone	3 - 11 days	300

Baselt RC and Cravey RH. Disposition of toxic drugs and chemicals in man  
 White RM and Black ML. Pain management testing reference

### Urinary hydrocodone, hydromorphone, and norhydrocodone after 10 mg dose



Adapted from: JAT 2012; 36, 507-514

Cross-Reactivity of Benzodiazepine Immunoassays

BENZODIAZEPINES	Emit II Plus	DAT Plus	CEDIA	CEDIA HS**	DRI and MULTIGENT
$\alpha$ -hydroxylprazolam	100	228	160	120	NR
$\alpha$ -hydroxylprazolam glucuronide	110	370	NR	NR	NR
7-aminoclonazepam	5700	288	> 400	210	2,500
lorazepam	600	341	250	180	1,000
lorazepam glucuronide	>10,000	>20,000	>10,000	450	NR
nordiazepam	NR	200	150	120	NR
oxazepam	250	259	280	160	200
oxazepam glucuronide	>10,000	NR	>10,000	800	NR
temazepam	140	256	210	220	125
temazepam glucuronide	6900	>30,000	>10,000	800	NR
** High sensitivity CEDIA assay which includes incubation with beta-glucuronidase.					

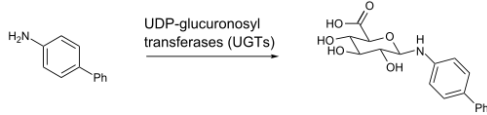
DRI Benzodiazepine Immunoassay vs. LC-MS/MS

Analyte	EIA cutoff (ng/mL)	LCMS cutoff (ng/mL)	% Missed
<b>Benzodiazepines</b>	<b>200</b>		
Lorazepam		40	18
Nordiazepam		40	40
Oxazepam		40	25
Clonazepam		40	76

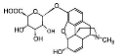
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Glucuronidation: Increase water-solubility for improved excretion through the urine and feces (via bile)

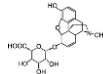
Opioids, Benzodiazepines, THC, Irinotecan, Ethanol, etc.



Morphine-3- $\beta$ -D-glucuronide



Morphine-6- $\beta$ -D-glucuronide



Cross-Reactivity of Benzodiazepine Immunoassays

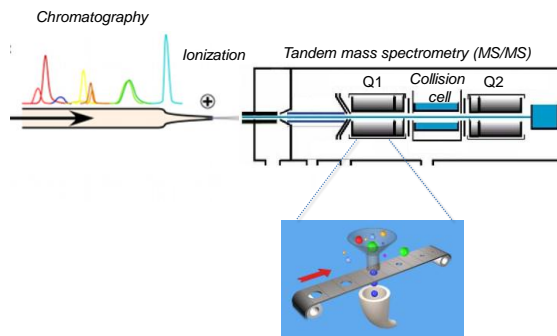
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temazepam glucuronide	6900	>30,000	>10,000	800	NR
** High sensitivity CEDIA assay which includes incubation with beta-glucuronidase.					

Approximately 17% of pain management patients prescribed clonazepam will have urinary 7-aminoclonazepam concentrations below 200 ng/mL.

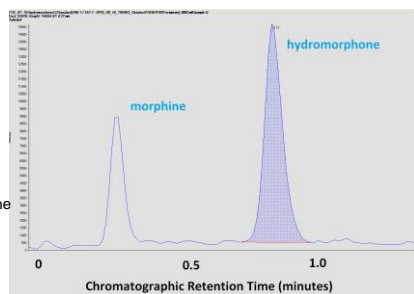
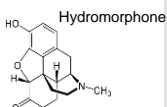
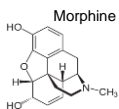
### Medication Monitoring

- 1) Need the ability to detect therapeutic levels
- 2) Most specimens are expected to be positive
- 3) Need to identify presence of specific drugs, not just class of drugs.

### Liquid chromatography tandem mass spectrometry

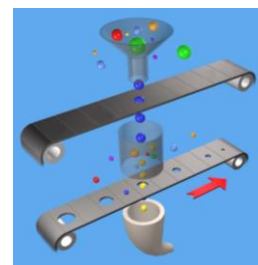


### Liquid Chromatography

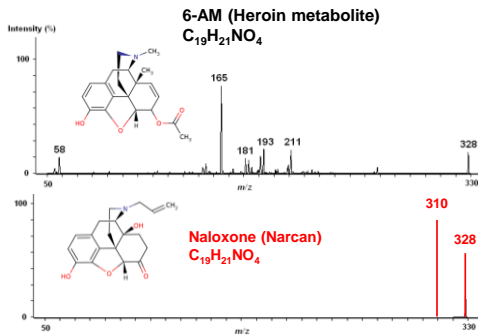


### Tandem Mass Spectrometry

#### Triple Quadrupole



## Tandem Mass Spectrometry (MS/MS)



## Mass Spectrometry

### Advantages

- Excellent Specificity
- Ability to differentiate many drugs
- Lower detection limits - ability to detect therapeutic use

### Disadvantages

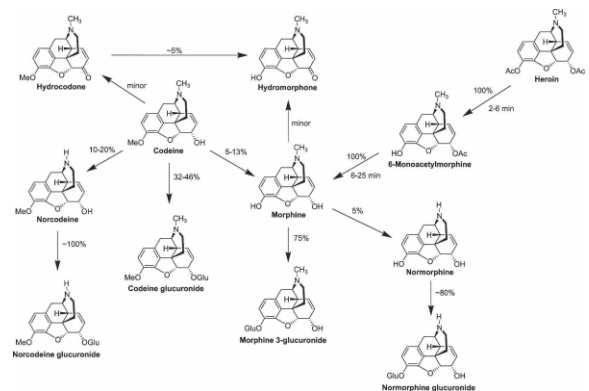
- High instrument cost (\$200,000 – \$400,000)
- High complexity
- Laboratory developed tests
- Need highly trained users
- Require more technical instrument maintenance

## Interpretation is *STILL* Challenging

- Complex metabolic pathways
- Inter-individual variation
- Patients who try to “beat the test”
- Process impurities
- Other biological/chemical processes?

*Can mass spec methods be too sensitive??*

## Opiate Metabolic Pathways





**MRO Advisory: Critical Pre-Publication Information for MROs on Opiate Interpretations**

An important poster was presented at a recent pain management meeting that showed there are known impurities found in prescription opiates that can lead to misinterpretations of opiate drug test results. In the gross manufacture of commercial opiates there are low levels of "impurities" that include other opiate drugs. The relevant impurities are shown in the table below.

**Acceptable Opioid Process Impurities  
In Commercial Drug Substances**

NB: New Methods Eliminate These Impurities for Hydrocodone and Hydromorphone; Both Varieties Are Currently Available

Commercial Active Pharmaceutical Ingredient (API)	Process Impurities	Allowable Limit (%)	Typical Observed (%)
Codeine	Morphine	0.15	0.01 – 0.1
Hydrocodone	Codeine	0.15	0 – 0.1
Hydromorphone	Morphine	0.15	0 – 0.025
	Hydrocodone	0.1	0 – 0.025
Morphine	Codeine	0.5	0.01 – 0.05
Oxycodone *	Hydrocodone	1.0	0.02 – 0.12
Oxymorphone	Hydromorphone	0.15	0.03 – 0.1
	Oxycodone	0.5	0.05 – 0.4

Information from API Manufacturers' Certificates of Analysis

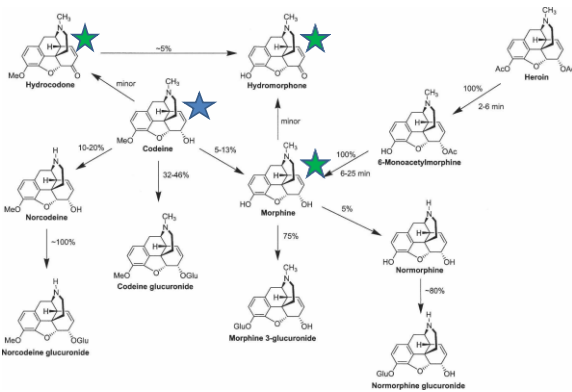
**Opiates –Drugs and Expected  
Metabolites**

Drug	Drug (generic)	Metabolites	Impurities
MS Contin	Morphine	Hydromorphone	Codeine
Codeine	Codeine	Morphine, Hydromorphone, Hydrocodone	
Norco	Hydrocodone	Hydromorphone	Codeine
Dilaudid	Hydromorphone		
Heroin	Heroin	Morphine, 6-AM, hydromorphone	Codeine
Oxycontin	Oxycodone	Oxymorphone	Hydrocodone**
Opana	Oxymorphone		Oxycodone, Hydromorphone

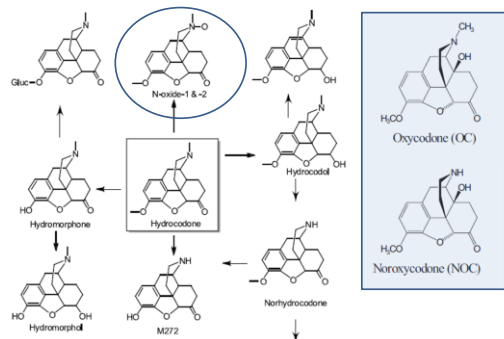
If Oxycodone is > 100,000 ng/mL, hydrocodone should be < 1,500 ng/mL.  
If Oxycodone is < 100,000 ng/mL, hydrocodone should be < 500 ng/mL.

Clin Chim Acta. 2011 Vol. 412 Pp. 29-32

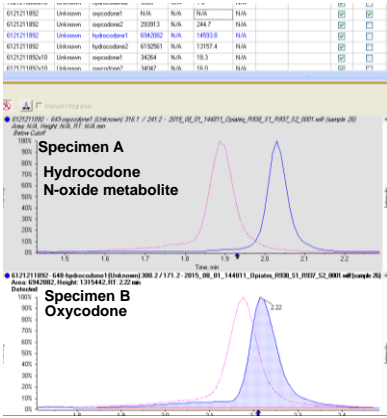
**MAJOR Opiate Metabolic Pathways**



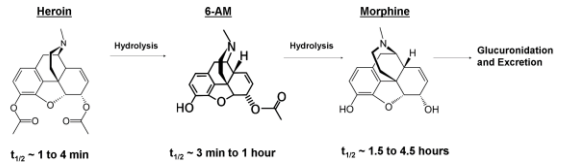
**MINOR Opioid Metabolic Pathways**



Li, A, et al. (Teva Pharmaceuticals) Xenobiotica 2012; Early Online: 1-9



### Unique Metabolism of Heroin Detected by LC-MS/MS



Heroin use is typically *screened* for using immunoassays targeting morphine.  
**Federal Guidelines:**  
 "Test for 6-AM When Morphine  $\geq 2,000$  ng/mL"

### Quantitation of Morphine and 6-AM in over 500,000 Urines

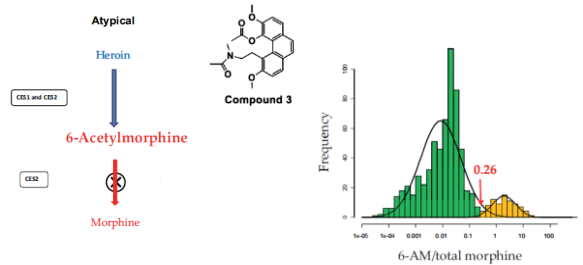
Year	Total Samples	Number of 6AM positive samples	Number of 6AM positive, morphine negative (< 50 ng/mL) samples	Percentage of 6AM positive samples negative for morphine
2008	16935	35	7	20%
2009	234069	437	36	8%
2010	384300	1081	134	12%

The Federal Register states: "At most "only" 6 out of a million will test positive for 6AM and not have morphine present" *Quest's Data*

However in our population there is closer to 280 out of a million...



### Possible mechanism for inhibition of morphine formation from 6-AM?

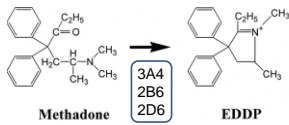


O Beck et al. *Forensic Sci Int* 2015 150-156

Case 1: Very Unique Metabolism?

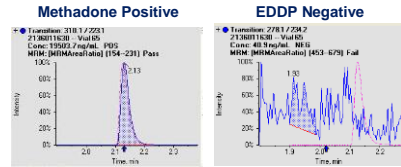
Methadone is a synthetic opioid that is used as an analgesic and also as an anti-addictive medication for patients with opioid dependency.

It stabilizes patients by mitigating opioid withdrawal and at higher doses can block the euphoric effects of heroin.



EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidene

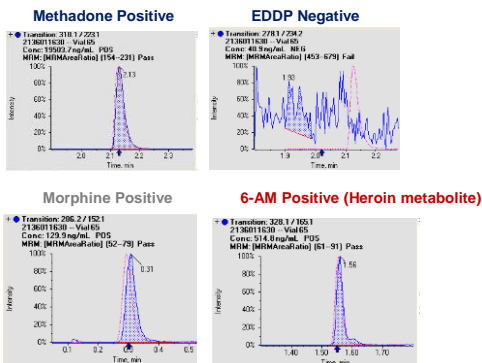
Very Unique Metabolism?



A study of 1093 methadone maintenance patients showed 100% had urinary EDDP > 100 ng/mL (JAT 2003, 27, 332-341)

Typical Human Urine

- pH 4.5-8.0 (pH can increase to 10 under elevated storage temperatures)
- Specific gravity 1.003-1.040
- Creatinine ≥ 20 mg/dL
- Temperature 90°F -100°F (within 4 minutes)
- Oxidants
- Glutaraldehyde
- Nitrites



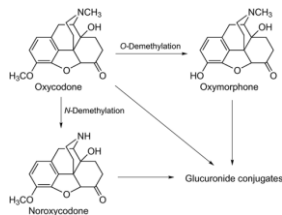
Common Household Chemicals	Detection by Adulteration Tests	Drugs Affected
Sodium Chloride	↑ specific gravity	Amphetamine, barbiturates, benzoylcegonine, cannabinoids, opiates,
Vinegar	↓ pH	Cannabinoids
Liquid hand soap	↑ pH	Cannabinoids, barbs, benzodiazepines
Detergents/laundry soap	↑ pH	Cannabinoids, barbs, amphetamines
Sodium Bicarbonate	↑ pH	Opiates
Sodium Hypochlorite (bleach)		Cannabinoids, Benzodiazepines
Visine Eye Drops	Cannot be detected	Cannabinoids

## Reg Lab Sample Adulteration

- If a urine sample has a creatinine concentration < 20mg/dL, we reflex to specific gravity.
- SG < 1.003 indicates diluted specimen.

## Case 2: Unique Metabolism?

- Oxy screen T'Follow (Oxycodone = 1924.0 )
- No trace of Noroxycodone or Oxymorphone
- Prior tests show similar metabolic pattern (oxycodone with no metabolites)



## Case 2: cont.

- Past test also shows THC metabolite.
  - Physician notes states they have informed patient that they will not refill opiates if the patient tests positive for THC again.
  - Next test shows Ur Creatinine < 5 mg/dL, reflex to Specific Gravity.
  - Specific Gravity = 1.00
- AND Oxycodone is present without metabolites!*

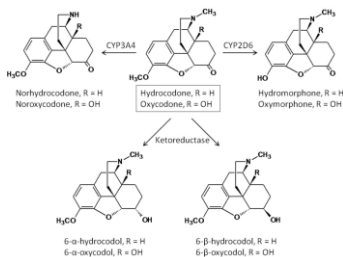
### Case 3: Unprescribed use of Oxycodone?

- Patient positive for Oxycodone and Oxymorphone.
- Physician contacted us because the patient is only prescribed Opana ER (Oxymorphone).
- Oxymorphone = 11,851 ng/mL
- Noroxycodone = 74 ng/mL

### Case 3: cont.

### Case 4: High hydrocodone and no metabolites!

- Hydrocodone = 162711 ng/mL
- No Metabolites!



### Case 4: High hydrocodone and no metabolites!

- Hydrocodone = 162711 ng/mL
- No Metabolites!
- 6-AM = 29 ng/mL
- Morphine = 26 ng/mL (Cutoff = 50 ng/mL)
- Script for Norco.
- In Past: DETECTED for hydrocodone, norhydrocodone, and hydromorphone.

## Summary

- Medication monitoring for pain management requires a unique testing approach.
- Interpretation of opioid UDT is not straight forward. Individuals need to be highly trained to interpret UDTs.
- Laboratorians need to work toward better ways relay UDT interpretations to physicians.
- Test anomalies and odd results should not be overlooked or disregarded!