The Role of the Clinical Laboratory in the Current Opioid Epidemic

Skyler J. Simpson, MD

PGY-1 Pathology Resident

University of Utah and ARUP Laboratories

Conflicts of Interest

No conflicts of interest to disclose

Learning Objectives

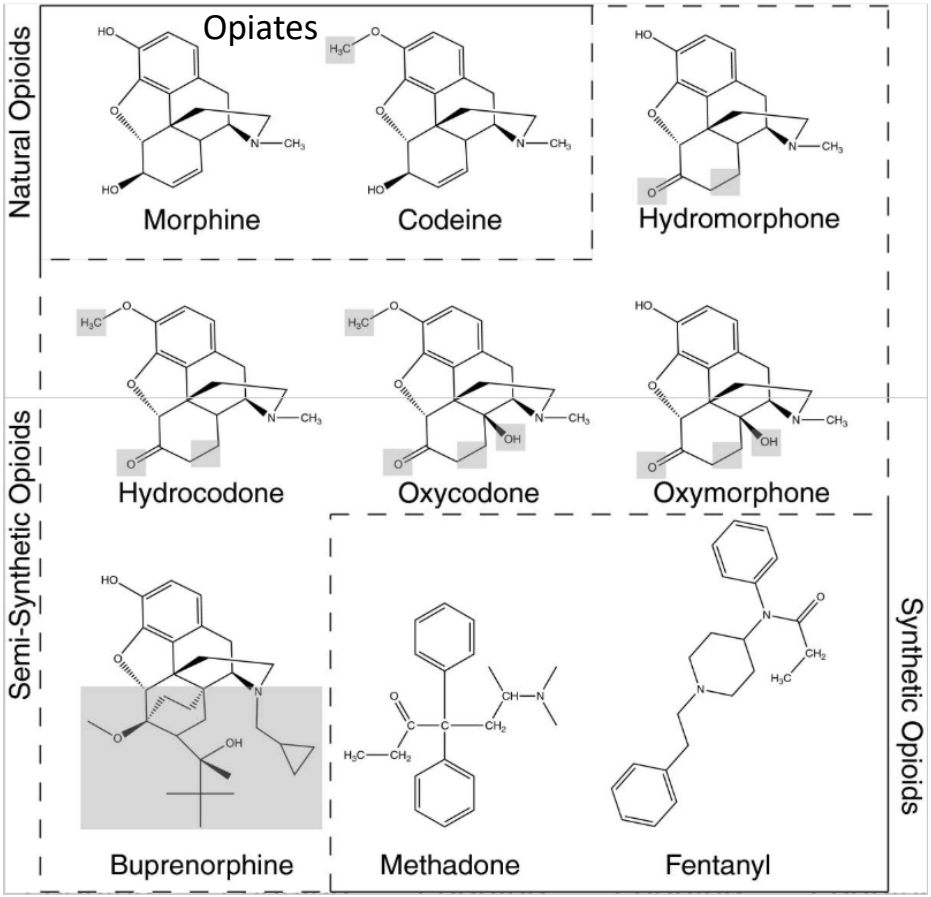
1. Explain what opioid medications are and their clinical uses
2. List the potential short- and long-term consequences of opioid use
3. Discuss the different laboratory tests for opioids and their uses

Outline

- **Opioids and their clinical use**
- Short-term effects and the opioid epidemic
- Long-term effects of opioid use
- Opioid laboratory testing
  - Immunoassays
  - LC-MS/MS
- Difficulties in laboratory result interpretation
- Clinical Cases
- Summary

What are opioid medications?

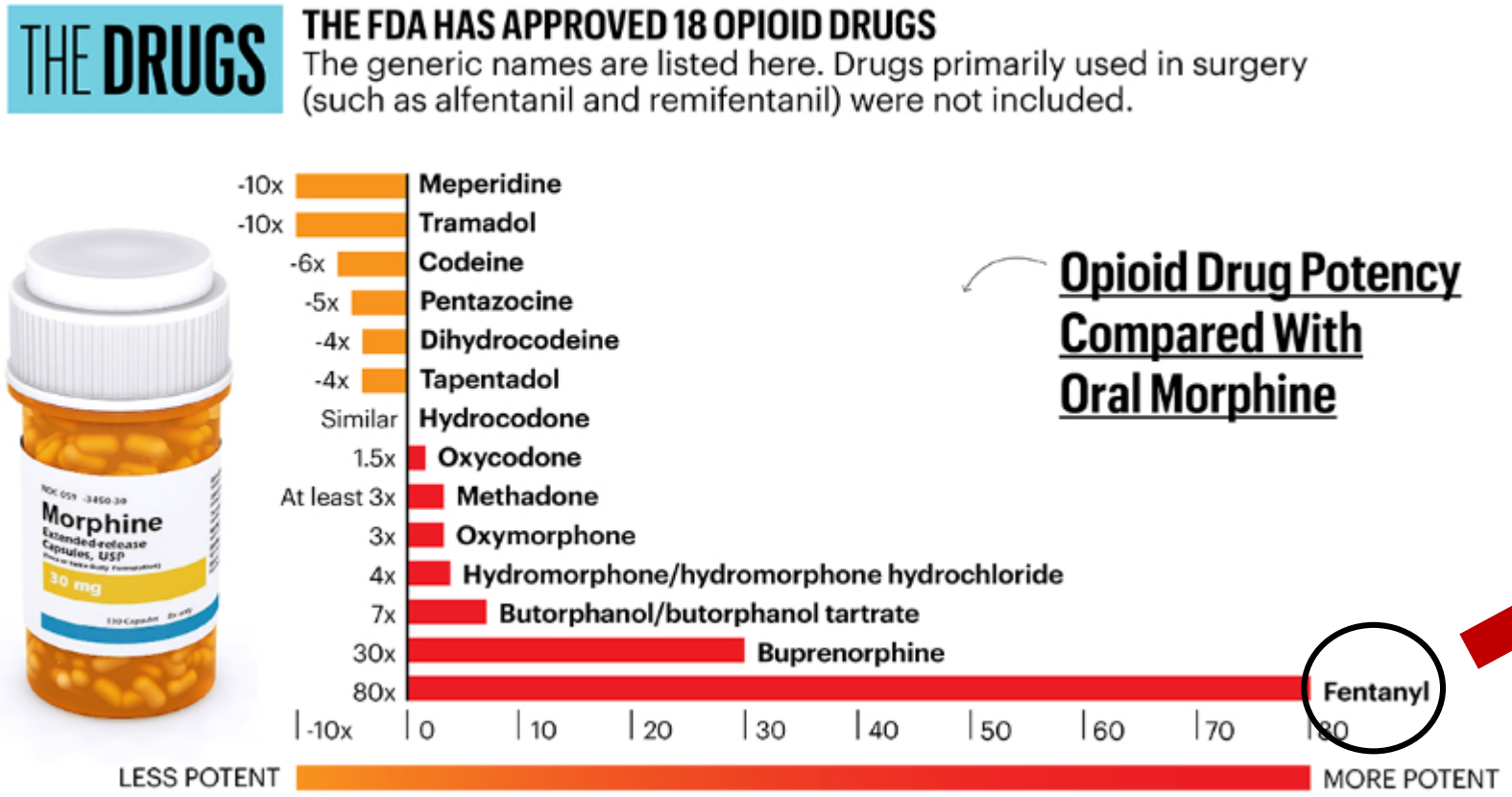
- Opium is an extract of the juice of the poppy *Papaver somniferum* that has been used for thousands of years
- In 1806 Friedrich Sertürner first isolated morphine from opium
- Since that time, many other opioids have been synthesized



[1]Pathan H, Williams J. Basic opioid pharmacology.
[2]Milone MC. Laboratory testing for prescription opioids. 2012

Flower image from Wikipedia.org

Relative Potency of Opioid medications



- Carfentanil- used as an elephant sedative, is 100 times more potent than fentanyl (~10,000x morphine). Heroin has been found laced with it.

Image from https://www.aarp.org/health/drugs-supplements/info-2017/opiates-prescription-pain-medication-information.html

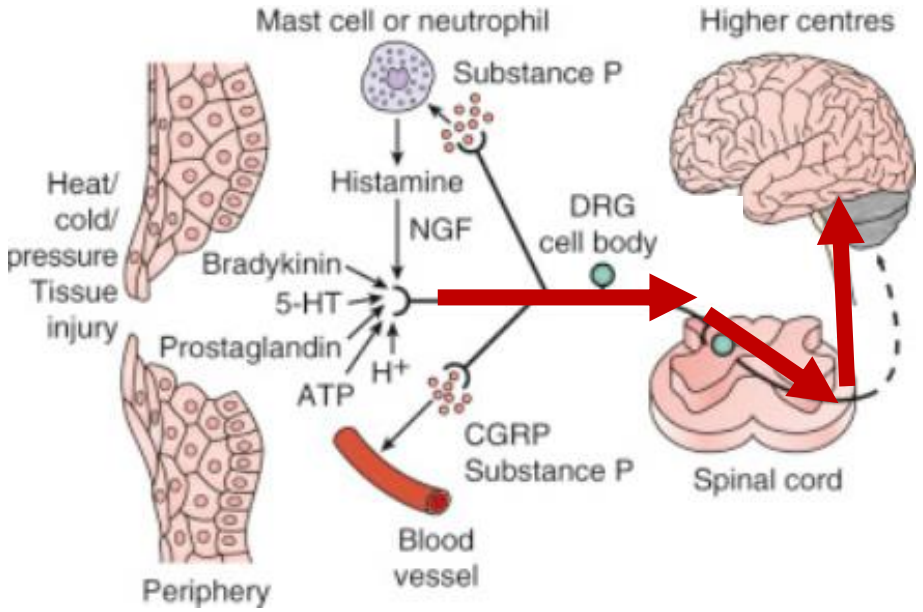
Clinical use for opioid medications

- Opioids are mainly used for acute and chronic pain.
- Not great evidence for neuropathic pain relief.
- Other uses: cough suppressant, anti-diarrheal medication (loperamide)

[3]Ritter, JM et al. Rang and Dale's Pharmacology. 9th edition.

What is pain?

- Pain signals are generated by the release of certain chemicals when tissue is damaged.
- These signals are then sent to the brain for interpretation
- Pain has a protective function



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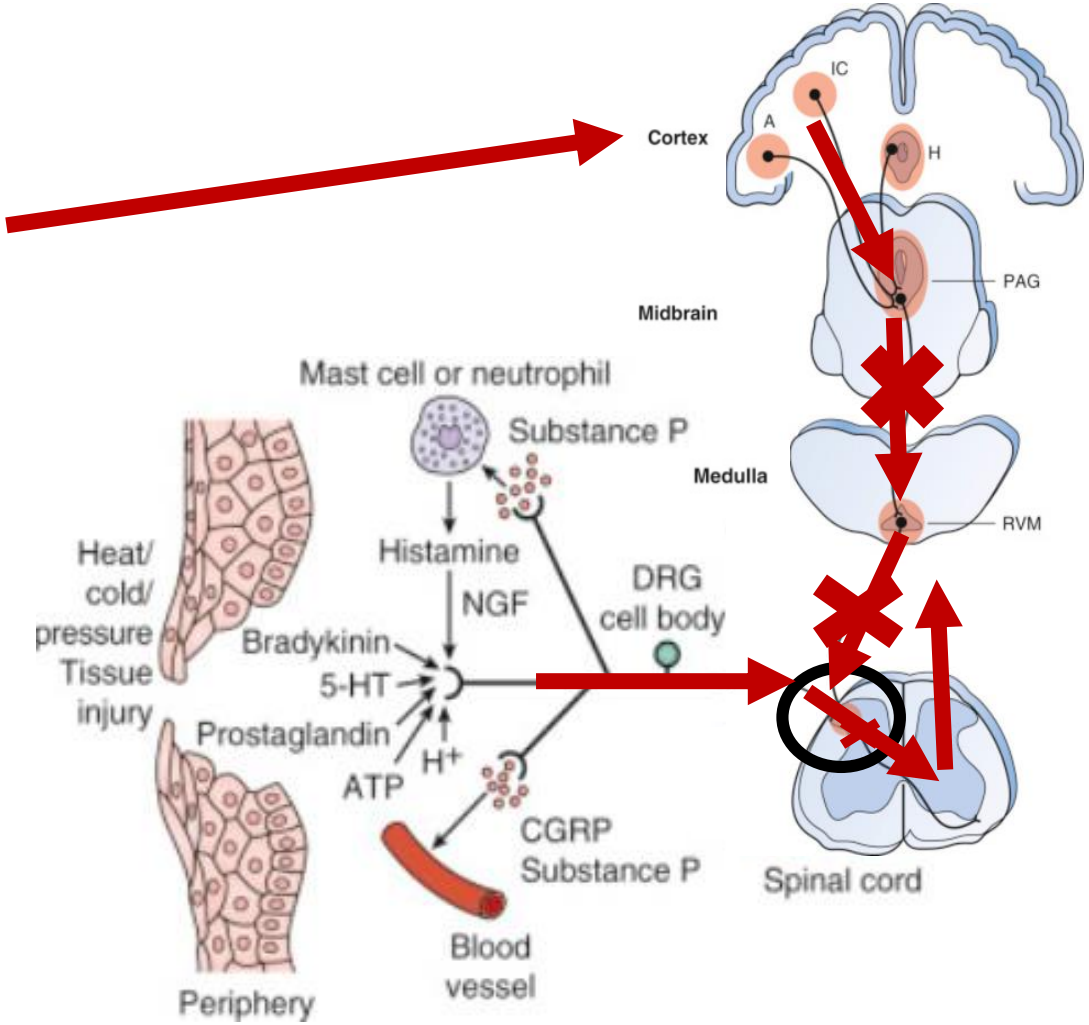
Opioid medications mechanism of action

- Act on same receptors as our “natural opioids”- enkephalins and endorphins
- Act mainly in the central nervous system to provide analgesic effects through interactions with the mu-opioid receptor



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Acute effects of opioid use

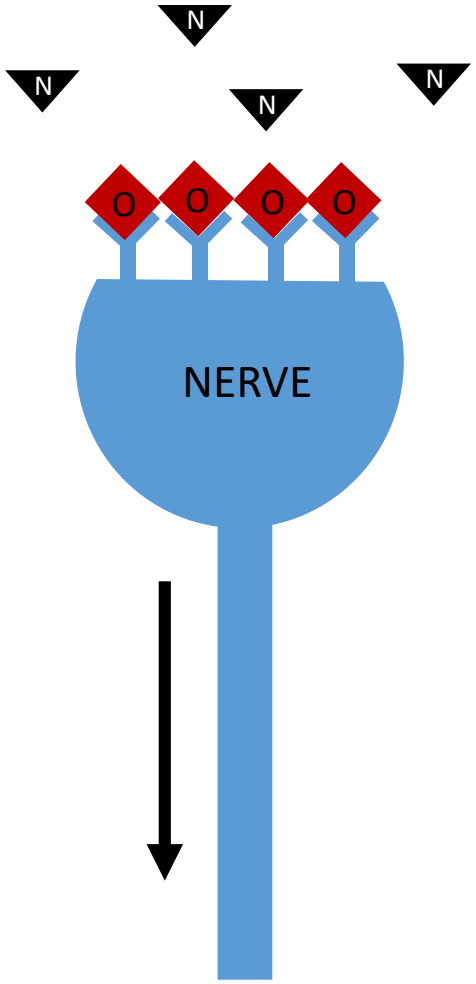
- Some are desired, others are not
- Other uses: Reduced GI motility and cough suppression
- Unwanted effects: Nausea/vomiting, respiratory depression, hallucinations
- Euphoric effects lead to psychologic dependence

Receptor	μ	δ	κ	NOP
[redacted]				
Supraspinal	+++	—?	—	Anti-opioid [a]
Spinal	++	++	+	++
Peripheral	++	—	++	—
Respiratory depression	+++	++	—	—
Pupil constriction	++	—	+	—
Reduced gastrointestinal motility	++	++	+	—
Euphoria	+++	—	—	—
Dysphoria and hallucinations	—	—	+++	—
Sedation	++	—	++	—
Catatonia	—	—	—	++
Physical dependence	+++	—	—	—

[3]Ritter, JM et al. Rang and Dale's Pharmacology. 9th edition.

Opioid intoxication

- Clinical signs and symptoms of opioid overdose:
  - **Respiratory depression (major cause of death)**
  - **Sedation**
  - **Pupillary constriction**
  - Constipation
  - Nausea/vomiting
- Opioid Overdose Reversal: Opioid antagonist **Naloxone**



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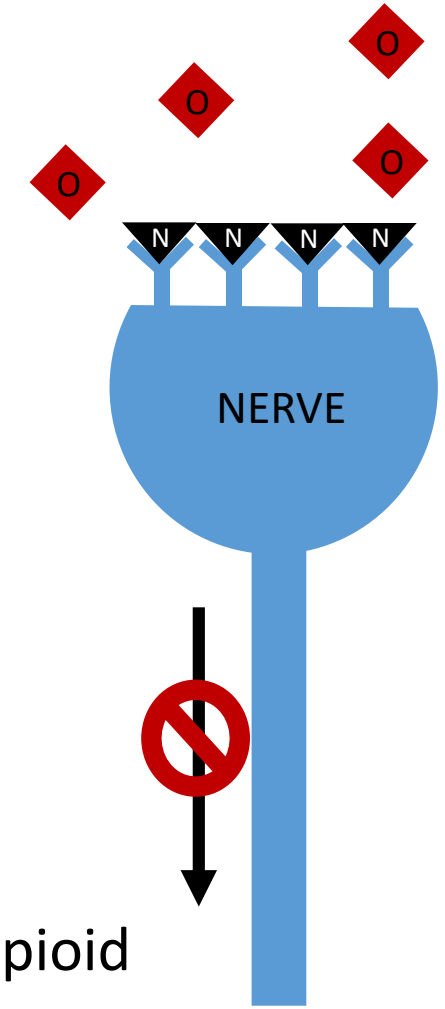
- Opioid Overdose Reversal: Opioid antagonist **Naloxone**
  - May need higher concentrations of Naloxone depending on opioid



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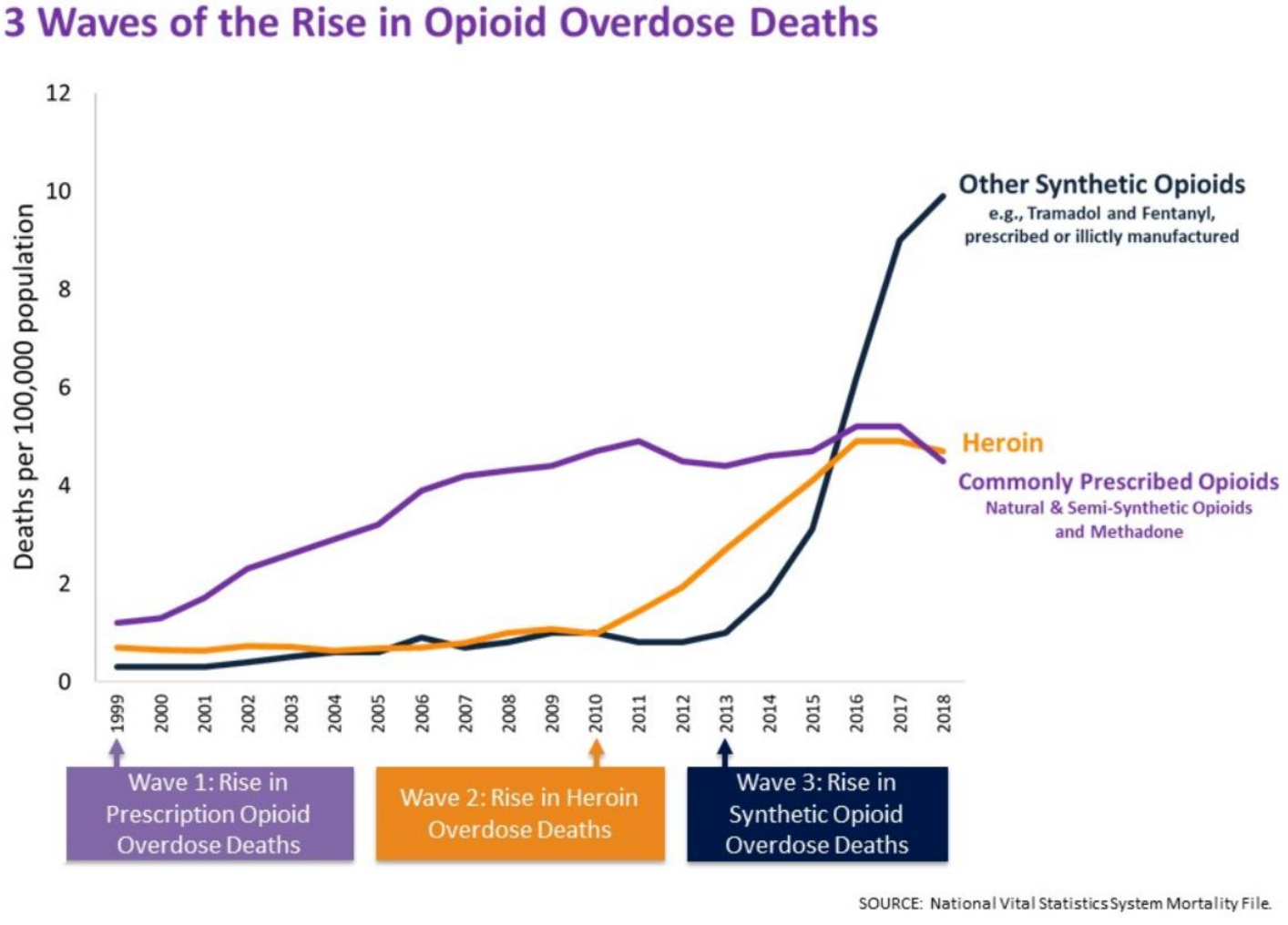
Opioid Epidemic

- During 2016, an estimated 48.5 million reported use of illicit drugs or misuse of prescription drugs within the past year.
- Between 1999 and 2018, ~450,000 opioid drug overdose deaths in the US.
- In 2018, ~67,000 people died from drug overdose, 70% were opioid related.



[4]https://www.cdc.gov/drugoverdose/epidemic/index.html

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Long term effects of chronic opioid use

- Increased tolerance- Desensitization of the mu-opioid receptors (higher doses of drug are needed to produce the same effects)
  - Can be seen within even a few days of repeated administration

- State of hyperalgesia (increased sensitivity to pain) with prolonged exposure

[3]Ritter, JM et al. Rang and Dale's Pharmacology. 9th edition.

Opioid Withdrawal

- Similar to tolerance, physical dependence can develop after use for only a few days.
- Abrupt cessation of opioid medications leads to the withdrawal symptoms (can last a few days):
  - Increased irritability
  - Body shakes/restlessness
  - Diarrhea, nausea, vomiting
  - Excessive yawning
  - Dilated pupils
  - Rhinorrhea (runny nose)
  - Piloerection (hair standing up)
  - Sweating, tachycardia (fast heart rate)

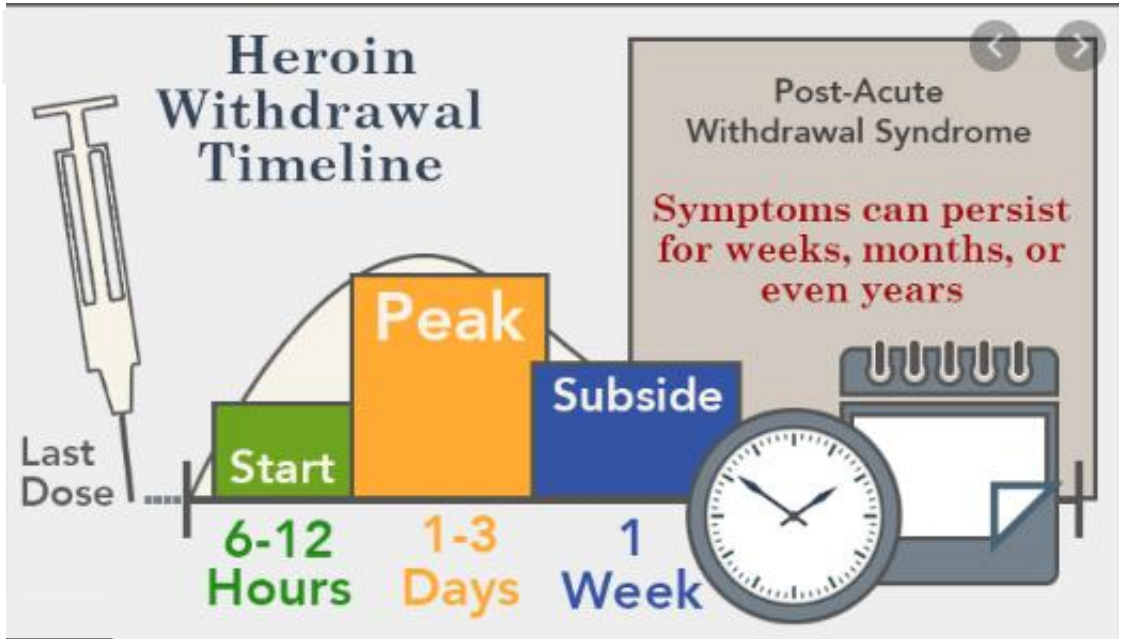

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Image from https://americanaddictioncenters.org/withdrawal-timelines-treatments/heroin

Opioid Withdrawal

- Treatment for withdrawal: More opioid medications!
  - Severe withdrawal can be dangerous and potentially life threatening
- Goal in treatment: Gradual cessation of opioid medications
  - Methadone, Buprenorphine
- Also of note, opioid overdose reversal agents like naloxone can precipitate withdrawal by blocking opioid effects.

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Reasons for Clinical Laboratory Drug Testing

- Clinical setting:
  - Suspicion for prescription or illicit drug use in a patient (screen)
  - Detect the use of non-prescription opioids (i.e. heroin)
  - Determine if patient is following drug regimen (pain management)
  - Patient in rehab programs
  - May be needed for organ transplantation or medically-related activities.
- Social Services: Testing mom or baby for the presence of drugs
- Other
  - Work drug screens for employment
  - Forensics: Determine drug related deaths, criminal prosecution (i.e. vehicle homicide)

[2]Milone MC. Laboratory testing for prescription opioids. 2012

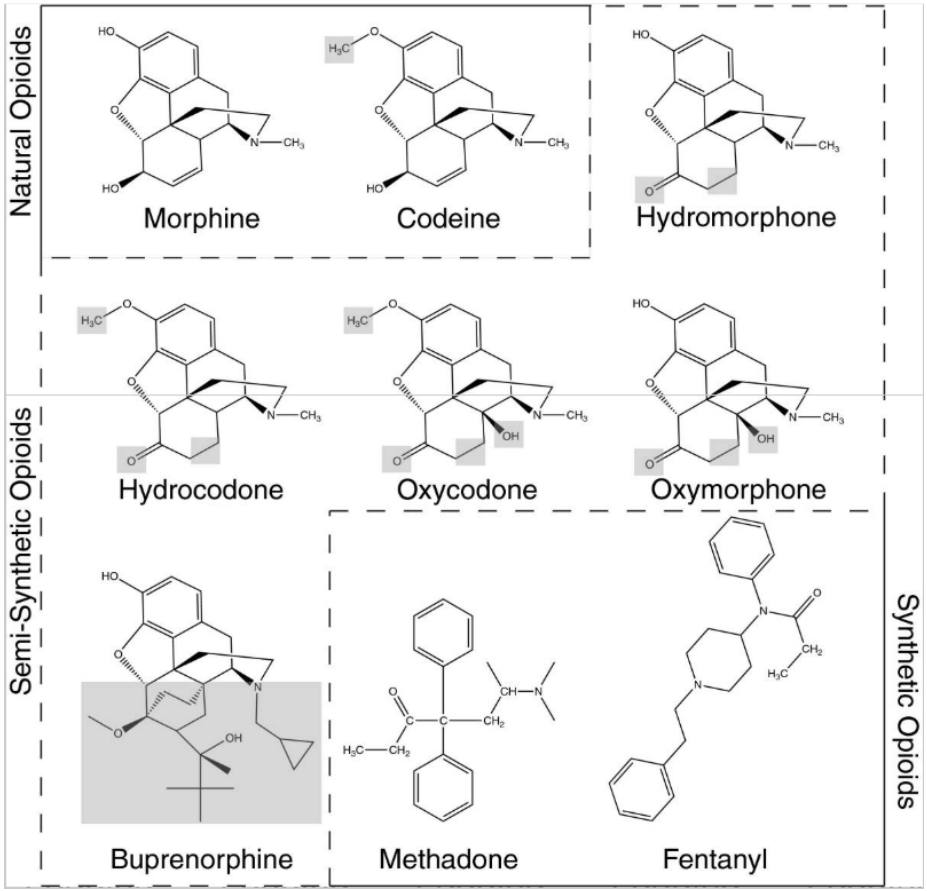
Types of Laboratory Specimens

- Adults
  - Whole blood, serum, or plasma (More reliable, but shorter window)
  - Urine (Can be dilute and yield false negatives, but longer detection window)

- Neonates (newborns)
  - Urine and blood (not as good, only detects recent drug use)
  - Umbilical cord tissue (easy to collect, but can have quality issues)
  - Meconium (first stool, harder to collect and can also have quality issues)

Laboratory Testing for Opioids

- **Opioid Immunoassays:**
  - Recognizes structure of drugs
  - Decent test for screening (rule out)
    - False positives and negatives
  - Not good for confirming drug presence
- **LC-MS/MS:**
  - Separates drugs via chromatography and uses mass spectrometry to look at molecular weights and fragmentation
  - Confirmatory testing
  - Essential testing when there are discrepancies



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Opioid Immunoassays

- Clinical setting use
  - Quick and cheap (point of care)
- Different immunoassays:
  1. Opiates (morphine)
  2. Oxycodone
  3. Buprenorphine
  4. Methadone
  5. Meperidine
  6. Fentanyl
  7. Tramadol
  8. Tapentadol
- Standard immunoassay in ED may miss some opioids depending on which immunoassays are available

Detection of commonly prescribed opioids in three different commercially available opiate immunoassays using a 300 ng/mL cutoff concentration for morphine

Drug	Siemens (Syva) EMIT[a]	Microgenics CEDIA[b]	Abbott FPIA[c]
Buprenorphine	>1,000,000	>100,000	
Codeine	102-306	300	237
Dihydrocodeine	291	300	626
Fentanyl	> 1,000,000	>100,000	
Hydrocodone	247	300	643
Hydromorphone	498	300	
Levorphanol	1,048	100,000	926
Meperidine	>15,000	150,000	
6-Acetylmorphine	435	300	746
Morphine-3-Glucuronide	626	300	643
Nalorphine	5,540	100,000	
Naloxone	36,000	6,000	
Oxycodone	1,500	10,000	2,857
Oxymorphone	9,300	20,000	5,000

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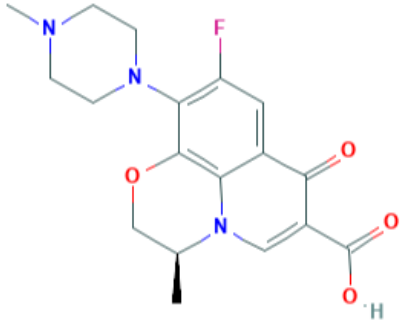
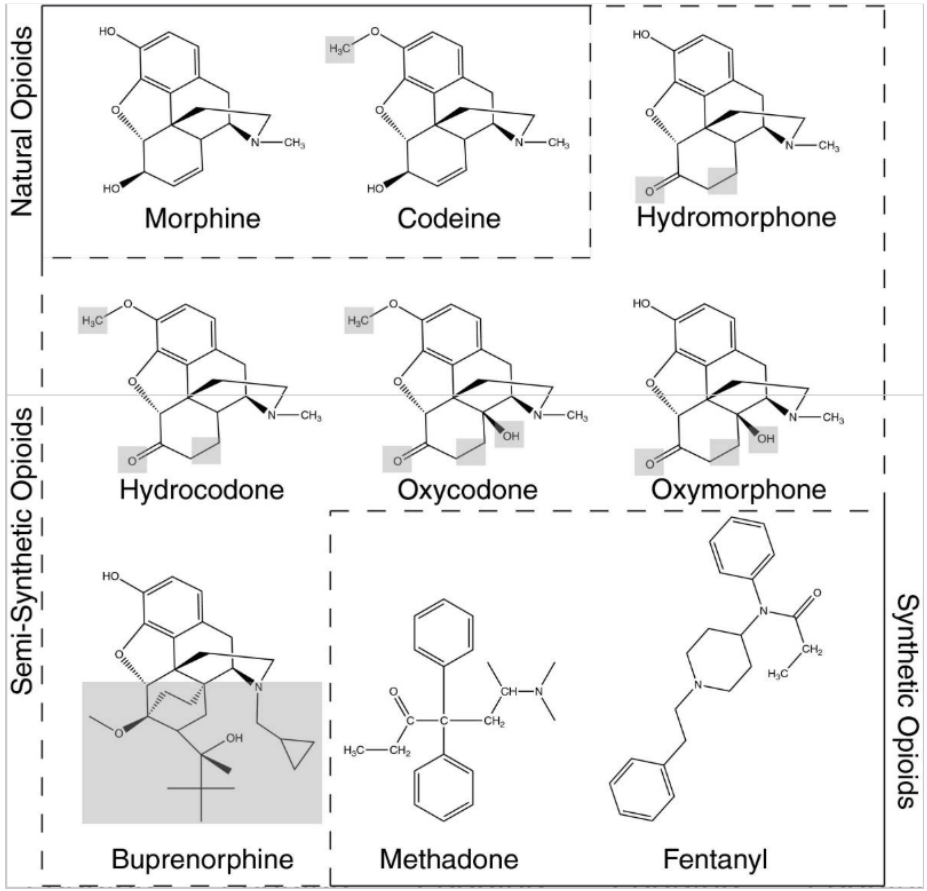
Accuracy of Opioid Immunoassays

- Opiate Immunoassay False positives:
  - Fluoroquinolones (class of antibiotics)



Levofloxacin

  - Rifampin (antibiotic)
  - **Poppy seed consumption (not really a false positive as they contain opiates)**
    - This is why most immunoassays use the 300 ng/mL morphine cutoff and 2,000 ng/mL for federal workplace threshold



[2]Milone MC. Laboratory testing for prescription opioids. 2012
[5]de Paula M, Saiz LC, González-Revaldería J, Pascual T, Alberola C, Miravalles E. Rifampicin causes false-positive immunoassay results for urine opiates. 1998.

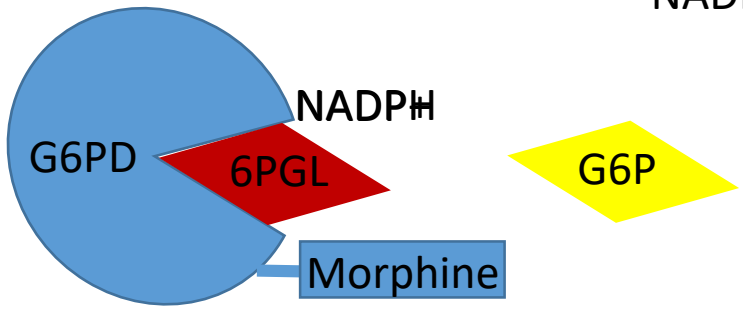
Image from Pubchem.ncbi.nlm.hih.gov

Opiate Immunoassay Method

- Emit II Plus Opiate Assay, 300 ng/mL morphine cutoff
  - Polyclonal antibody to the drug (morphine)
  - Use the bacterium *Leuconostoc mesenteroides* enzyme glucose-6-phosphate dehydrogenase (G6PD) with bound drug

$$\text{Glucose 6 Phosphate} \xrightarrow{\text{G6PD}} 6-\text{Phosphogluconolactone}$$

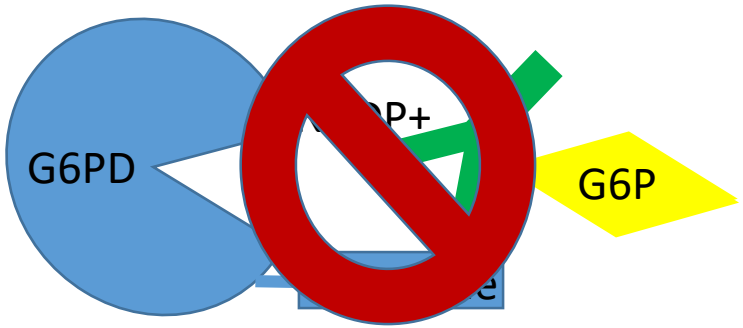
NADP+ NADPH



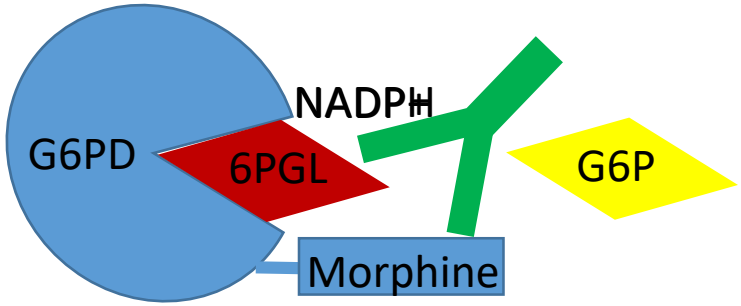
NADP+ and NADPH (340 nm) have different absorbance that can be measured via spectrophotometry

Opiate Immunoassay Method

With no drug in urine:



With drug in urine



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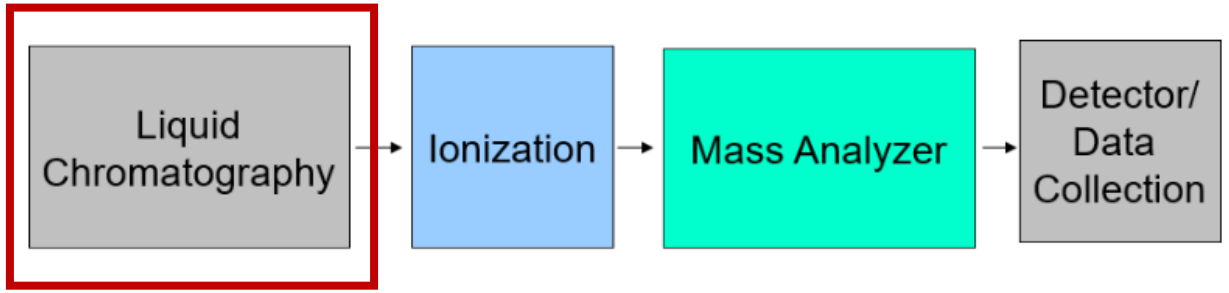
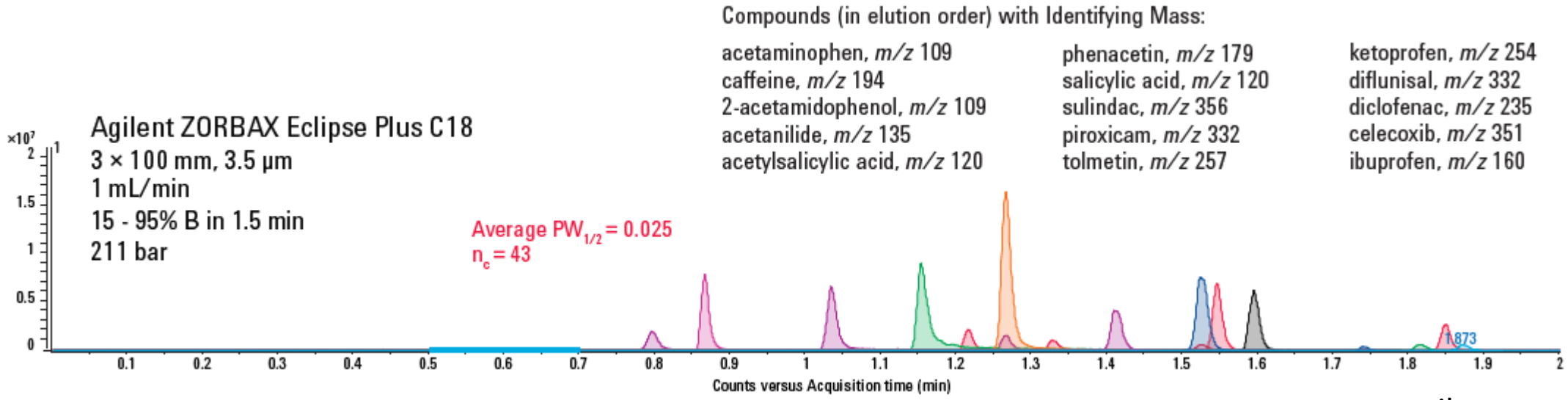
Opioid LC-MS/MS



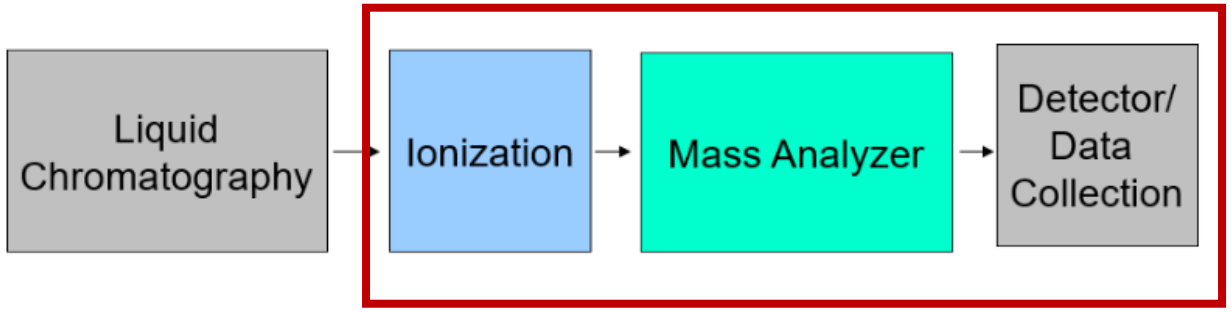
- Liquid Chromatography is used to separate out compounds in the urine based on size and polarity.



www.agilent.com

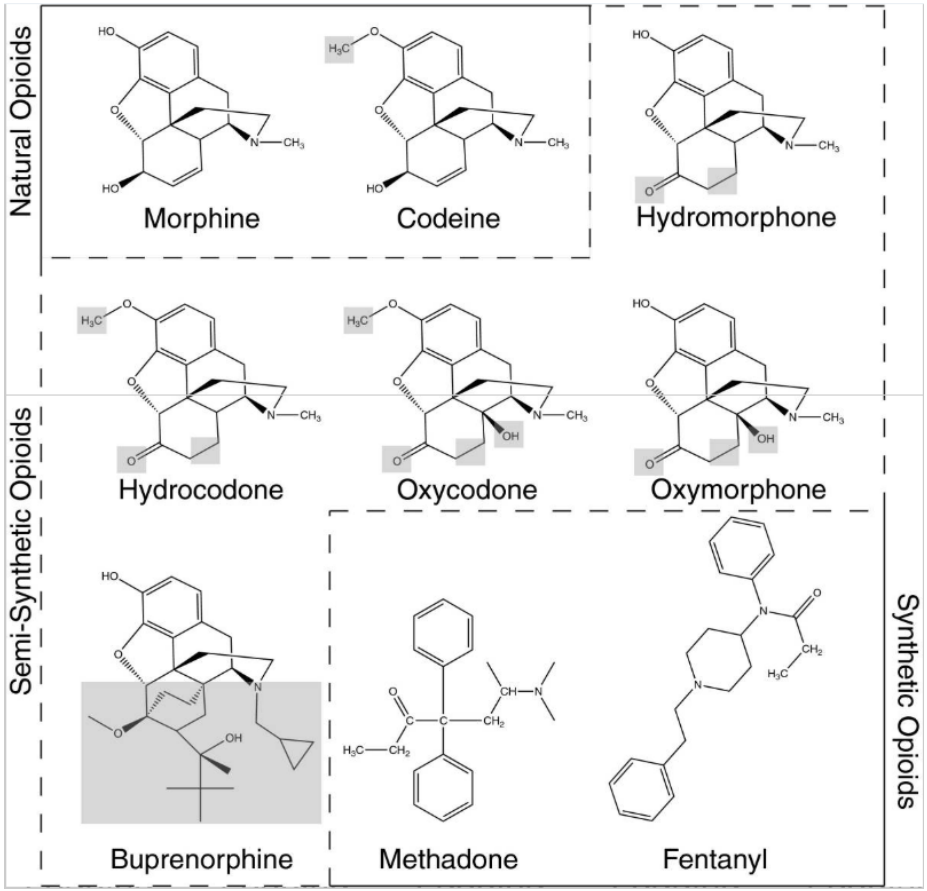
LC-MS/MS image from Dr. Lisa Johnson at ARUP laboratories

Opioid LC-MS/MS



- Mass-Spectrometry Overview
  - Ionization: Positive mode electrospray ionization (ESI) is one method used
    - Need charge for the mass spec instrument to analyze the molecule (mass-to-charge ratio, m/z)
  - Mass Analyzer: Separates ions by mass and charge before the detector
  - Detector: Identify opioid medications and metabolites based on molecular weight and fragmentation

Analyte	Q1 Mass (m/z)	Q3 Mass (m/z)	Retention Time (min)
Morphine	286.1	165.2	1.36
Hydromorphone	286.1	157.2	1.89
Hydrocodone	300.3	171.2	3.27
Codeine	300.3	215.2	2.48



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LC-MS/MS image from Dr. Lisa Johnson at ARUP laboratories

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Interpretation of Lab Results

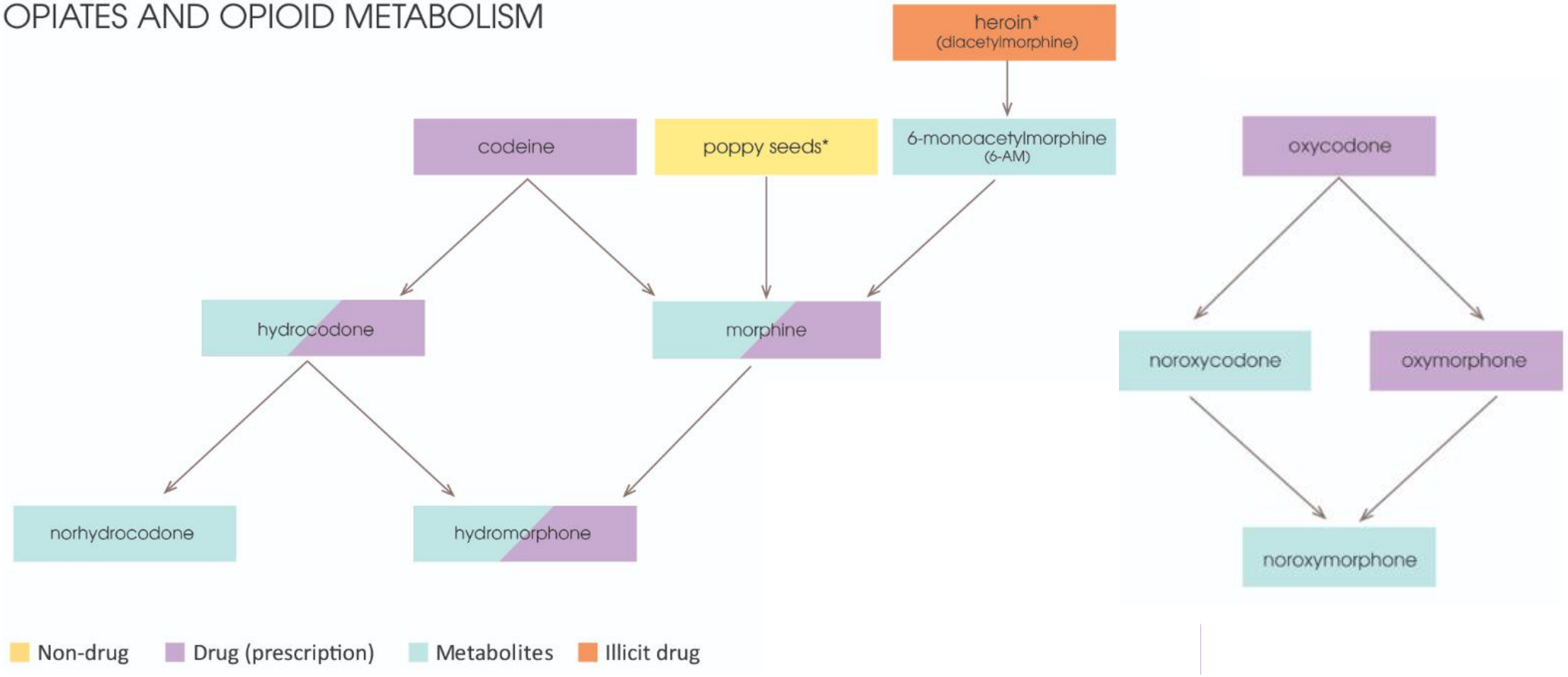
- This is where the laboratory has an impact in patient care as we try to help clinicians with interpretation of results

- Immunoassays:
  - Have to think about the false positives and false negatives

- Mass spectrometry based assays:
  - Can be difficult because some drugs are metabolites of others
    - For example, morphine is a metabolite of codeine, making it difficult for us to say sometimes whether a patient is only taking one drug or multiple drugs

ARUP LABORATORIES

OPIATES AND OPIOID METABOLISM



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ARUP LABORATORIES

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Case #1
Is my patient Dr. House?

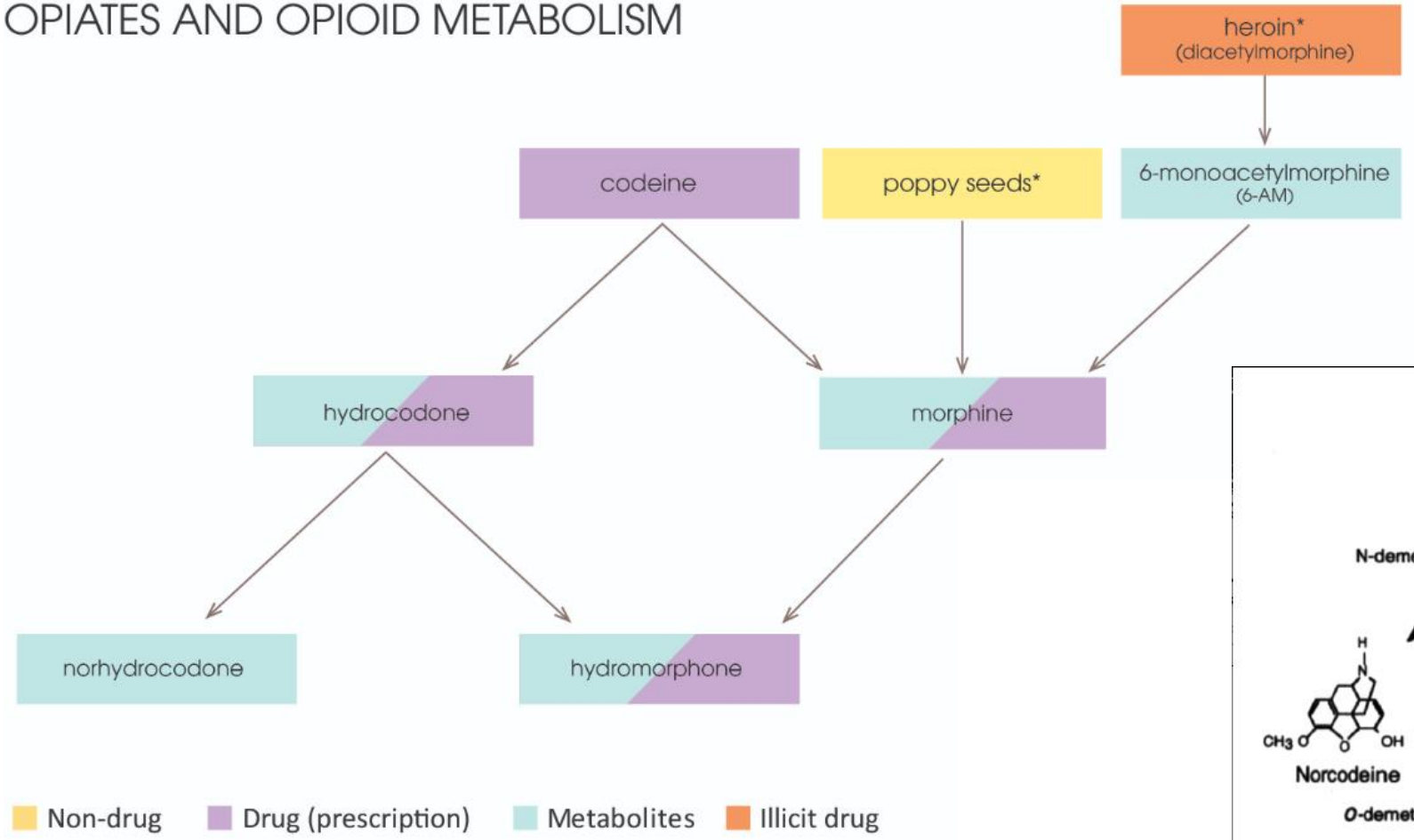
Case Clinical Information

- 54-year-old female with a long standing prescription for Tylenol #3 (Acetaminophen and Codeine)
- Clinician was screening the patient for any other drug use
- Test: Qualitative pain hybrid panel (Mass-spectrometry)

Codeine (cutoff 40 ng/mL)	**Present**
Morphine (cutoff 20 ng/mL)	**Present**
Hydrocodone (cutoff 40 ng/mL)	**Present**

- Question: Patient claims she has had false positive results for hydrocodone before, is this possible?

OPIATES AND OPIOID METABOLISM



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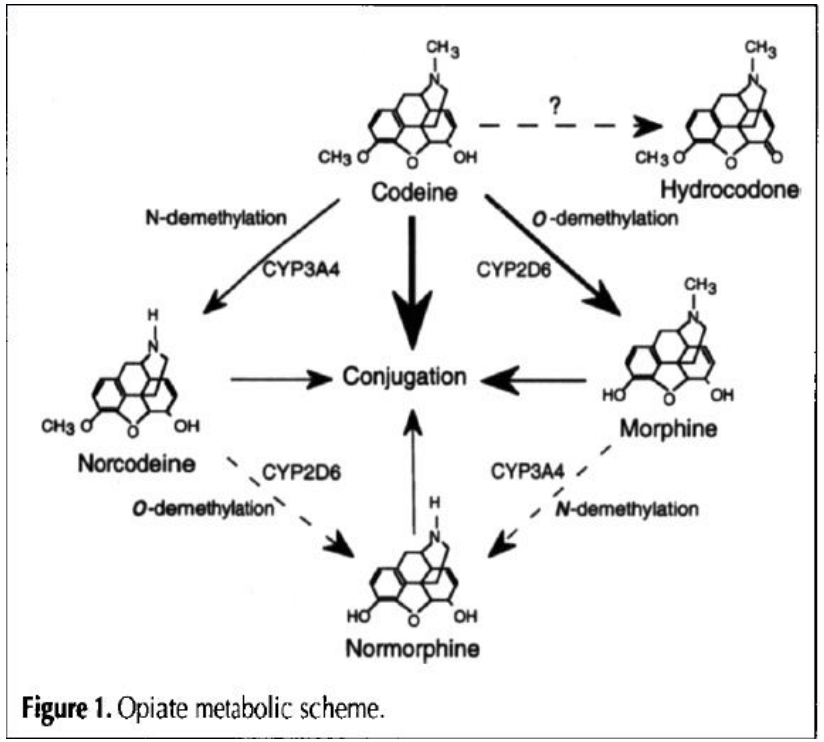
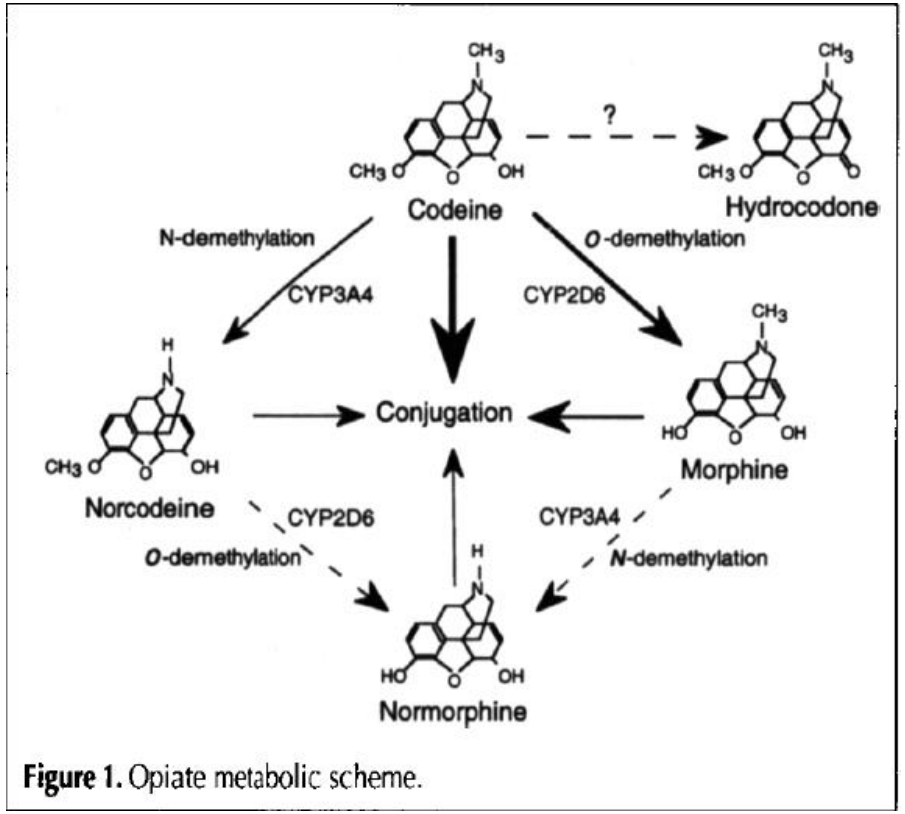


Figure 1. Opiate metabolic scheme.

[6]Oyler JM, Cone EJ, Joseph Jr RE, Huestis MA. Identification of Hydrocodone in Human Urine Following Controlled Codeine Administration. 2000.

Case #1 Conclusion

- Literature: Hydrocodone levels up to 11% of codeine levels
- Talked with medical laboratory scientist to look at the relative concentration of these drugs:
  - Codeine: 263.9 ng/mL
  - Morphine: 146.5 ng/mL
  - Hydrocodone: 41.7 ng/mL (15.8%)
- Question: Patient claims she has had false positive results for hydrocodone before, is this possible?
  - This could be consistent with Tylenol #3 use alone



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Case #2
Can poppy seeds give me these results?

Case Clinical Information

- Newborn female
- Mom showed up to labor and delivery and tested positive for opiates on urine drug screen.
- Physician is concerned for the baby may have been exposed to opioids during the pregnancy and wanted baby to be tested
- Test: Drug detection panel, umbilical cord tissue

Codeine, Cord, Qual	Present ng/g	(Ref Interval: Cutoff 0.5)
Morphine, Cord, Qual	Present ng/g	(Ref Interval: Cutoff 0.5)

- Question: Are these results consistent with poppy seed consumption?

Neonatal abstinence syndrome

- Opioid withdrawal in neonates born to mothers with opioid use disorder
- Need exposure to opioids during pregnancy such that once these drugs are removed (birth), the baby starts to have the opioid withdrawal symptoms:
  - Hyperarousal
  - Sweating
  - Fever
  - Nasal stuffiness/frequent yawning
  - Tachypnea (rapid breathing)
  - Vomiting and loose stools
  - Tremors/jitteriness
- Usually start to see signs and symptoms in the first 24 hours of life, but can be delayed until five days of age.

[7]Jansson, LM. Neonatal Abstinence Syndrome. UpToDate. April 09,2020.

Neonatal abstinence syndrome

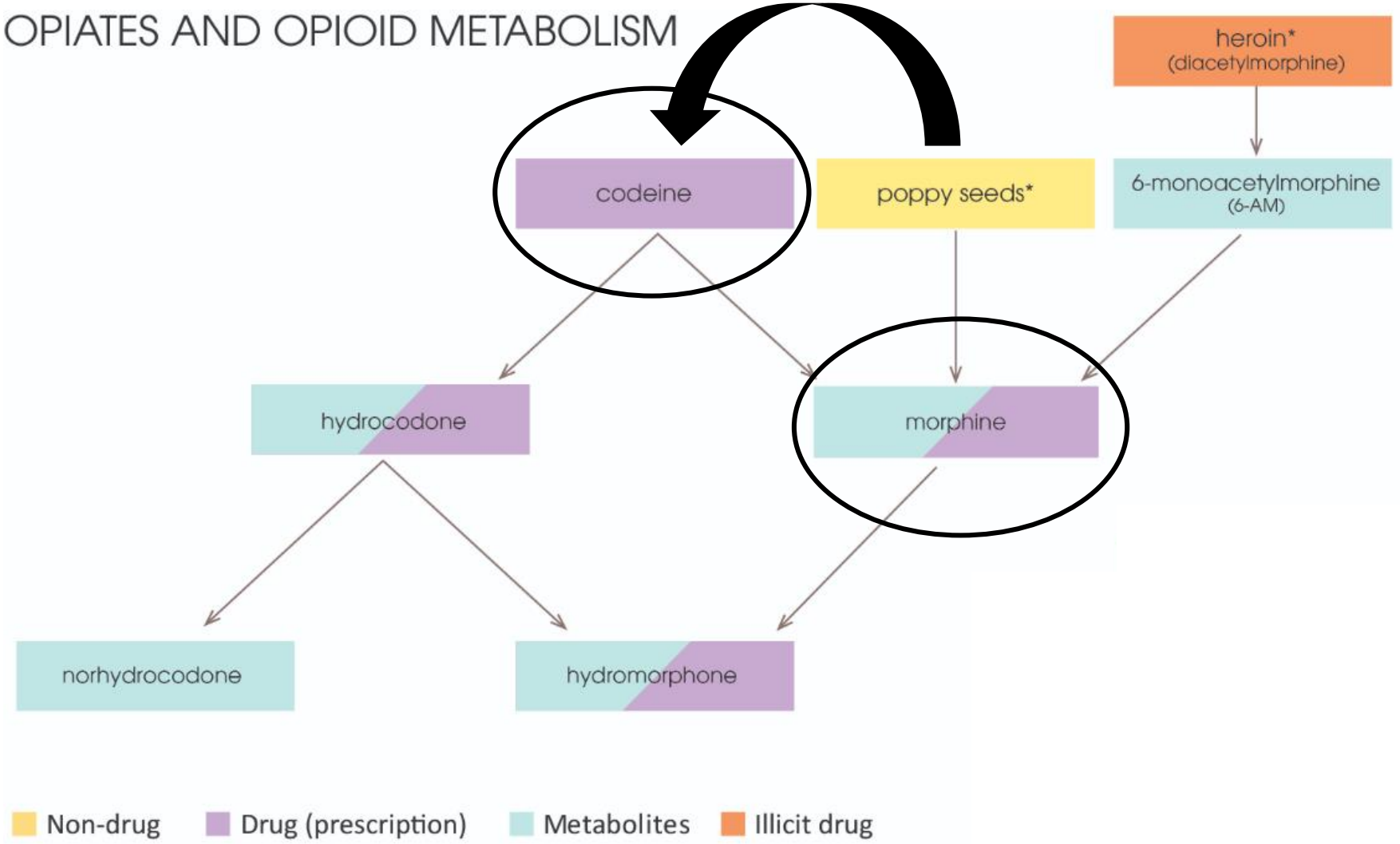
- May need to treat the baby with an opioid for similar reasons we treat opioid addicts with opioid medications
  - Morphine, methadone, buprenorphine
  - Baby cannot be discharged until they are no longer taking these drugs for withdrawal symptoms
- Substance abuse during pregnancy is a crime in 3 states: Tennessee, Alabama, and South Carolina.

[8]Miranda L, Dixon V, and Reyes C. How States Handle Drug Use During Pregnancy. Propublica. 2015.

Case considerations

- Umbilical cord tissue
  - Drugs deposit along the length of the umbilical cord during development
  - Drug detection depends on the quality and completeness of collection and drug use patterns
- Cases of positive opioids in the umbilical cord, it is important to rule out drugs used during delivery (opioids are used for pain with epidurals…)
- What is the clinical picture of the baby **and mother**?
  - When I spoke with the client, neither the baby nor the mother were having opioid withdrawal symptoms despite being inpatients

[9]Newborn Drug Testing - Meconium and Umbilical Cord Tissue. arupconsult.com.



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Case #2 conclusion

- Relative concentrations obtained from the medical laboratory scientist:
  - Morphine: 1.6 ng/g
  - Codeine: 2.1 ng/g
- Question: Are these results consistent with poppy seed consumption?
  - These relatively close and low concentrations along with the clinical picture with no opioid withdrawal could be consistent with poppy seed consumption during the third trimester
  - Probably would need to be pretty consistent poppy seed consumption to keep the levels high enough to be detected in the umbilical cord tissue

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Summary

1. Explain what opioid medications are and their clinical uses
   - Drugs that act on mu-opioid receptors in the peripheral and central nervous system to control acute pain and chronic pain, manage cough through suppression, and control diarrhea episodes.
2. List the potential short- and long-term consequences of opioid use
   - Short: Overdose with sedation and respiratory depression (potential death)
   - Long: Tolerance and hyperalgesia, addiction, and withdrawal (potential death)
3. Discuss the different laboratory tests for opioids and their uses
   - Immunoassays: Quick and cheap, screening test, clinical setting use
   - Mass spectrometry-based assays: Confirmatory testing for use in multiple settings (i.e. clinical, social services, workplace, and forensics)

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