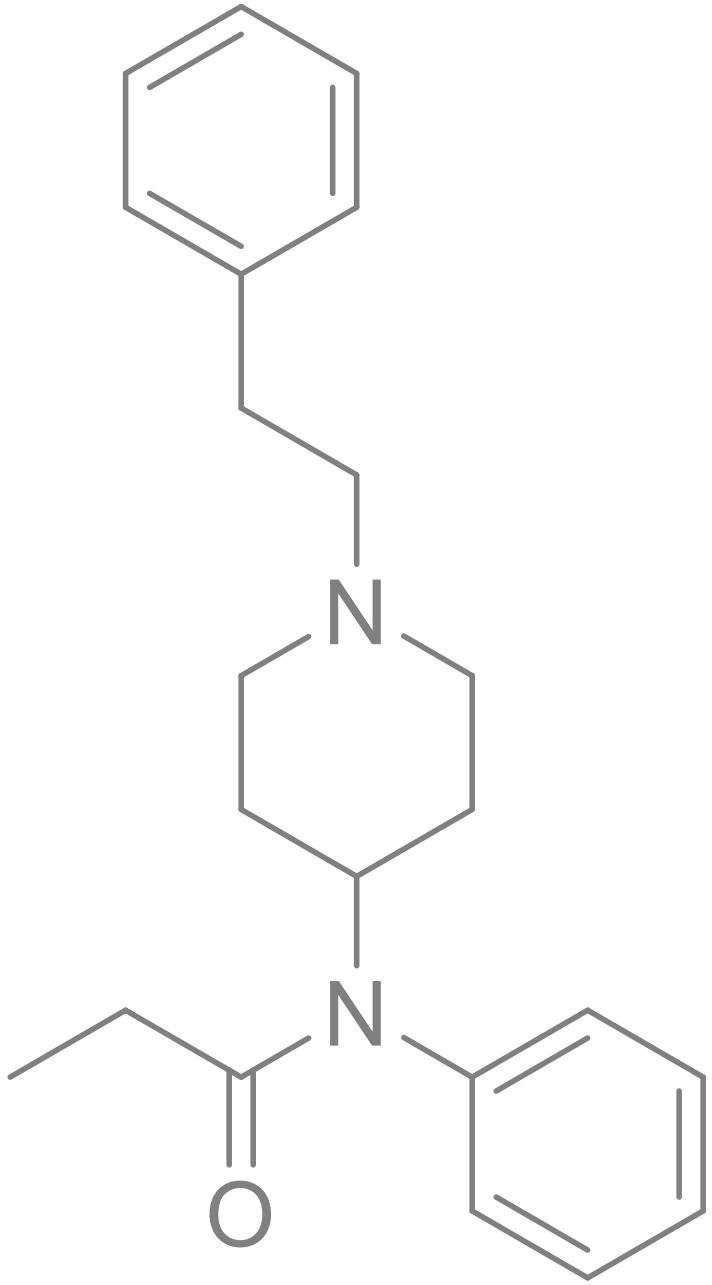


Behind the Scenes of Urine Drug Screens

Claire Snelgrove

BSc, MSc Candidate (Organic Chemistry)

June 30th, 2022

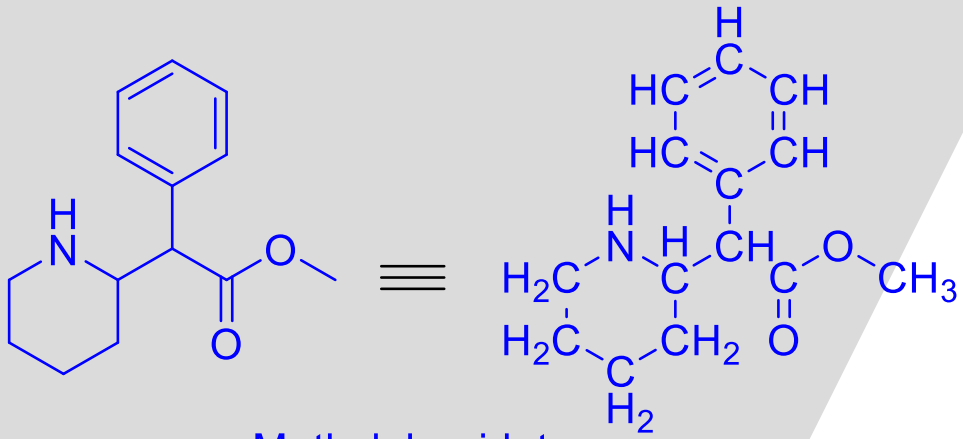
NSS-CoP

Twenty Minute Targets

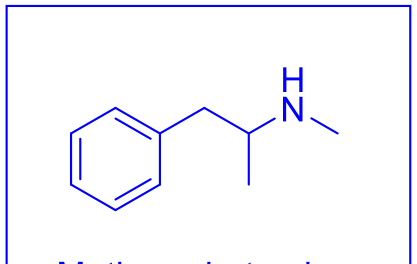
<u>To accomplish:</u>

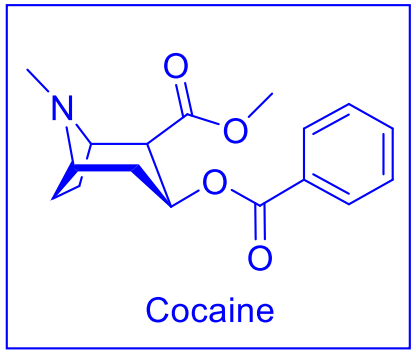
1. Fundamentals of UDS
2. Types of UDS Methods
   - Focus on *how* they work

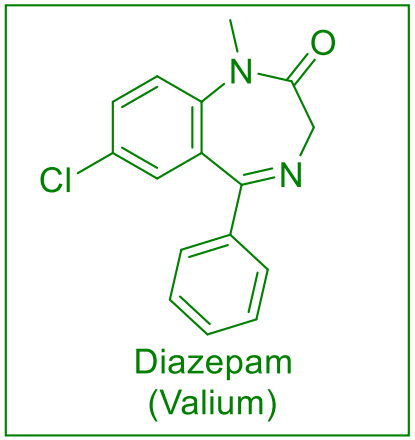
<u>About Structures:</u>

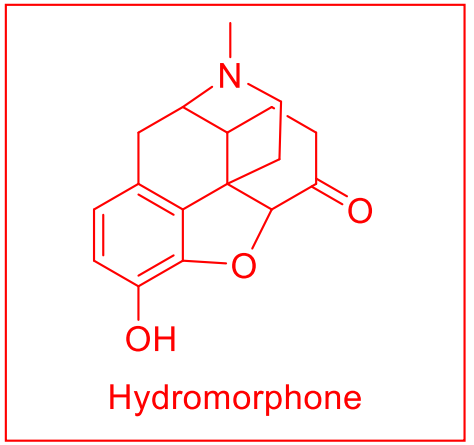


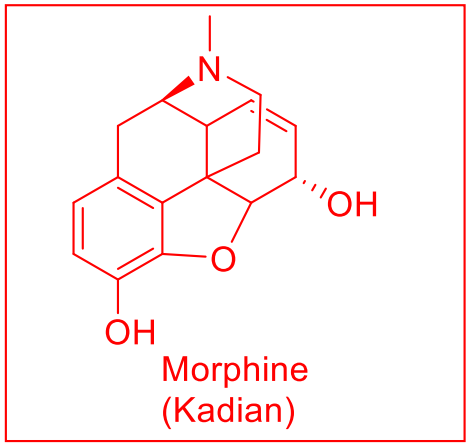
Structures are essentially 'pictures' of a molecule (drug)

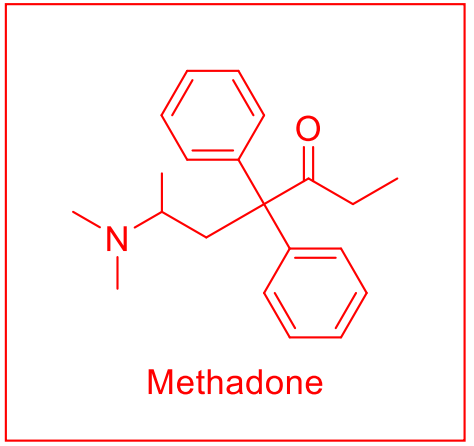












Basics of Urine Drug Screens

- Urine drug screen (UDS) – *a test that detects the presence of certain drugs in a persons urine*

- **Benefits:**
  - Considered quick, easy, and non-invasive to collect
  - Well-established methods and protocols
  - Reasonably long window of detection

- **Limitations:**
  - Specimen integrity (tampering)
  - Variance in detection time
  - Trust implications

- **UDS may be used as part of:**
  - Opioid agonist therapy (OAT)
  - Safer supply programs
  - Workplace drug testing
  - Child surveillance

- UDS may be used punitively, often with harsh consequences

Understanding the Mechanisms Behind UDS

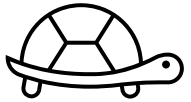
- **Service providers** – preventing misinterpretation of results
- **Substance users** – aiding self-advocacy in the event of an error
- UDS interpretation is not always as straightforward as it seems
  - Overconfidence is common among clinicians
  - Opioid testing and metabolism is a noted area of concern
- Responsibility may fall on the substance user to 'prove' they are not being deceitful
  - Potentially high stakes
  - Stigma and distrust
- *Drug screening results may have severe consequences*
- Learning the basics of how, why, and *when* UDS work is a valuable tool for anyone involved

Int. J. Drug Policy **2019**, 64, 30-33; *J. Opioid Manag.* **2006**, 6, 333-7; *J. Gen. Intern. Med.* **2012**, 27, 1521-7; *Mayo. Clin. Proc.*, **2017,** 92, 774-796; *Subst. Abus.* **2015**, 37, 154-160

Types of Tests

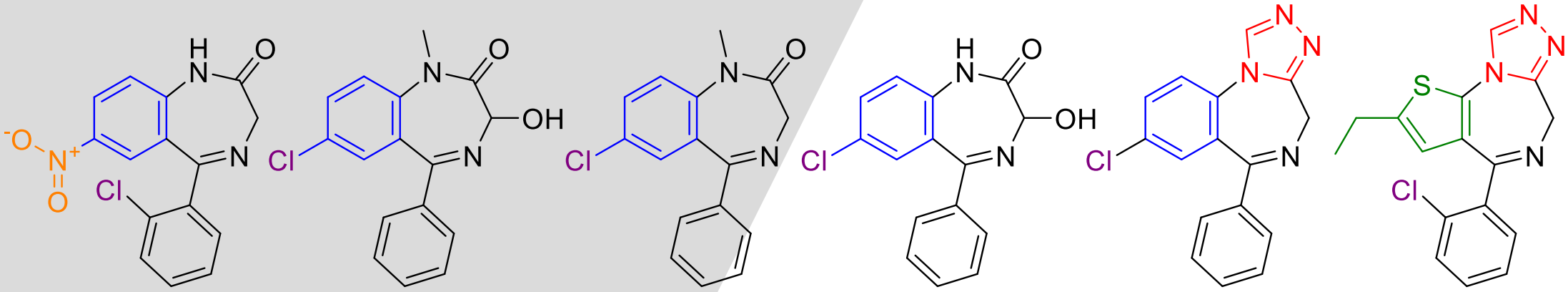
- There are two common testing methods used for UDS, depending on need

Lateral Flow Immunoassay (LFA)	Confirmatory Lab Testing
Quick (~ 5 min)	Slow turnaround
Qualitative	Quantitative
Portable	Requires laboratory equipment
Inexpensive	Expensive
Minimal training	Requires extensive training
Presumptive	Confirmatory
Non-specific	Specific



Lateral Flow Immunoassay (LFA)

- Drug testing strips – two lines = *negative*; one line = *positive*
- Two types:
  - **Competitive assay (small molecule) – drug tests**
  - ‘Sandwich’ assay (large molecule) – COVID (rapid) tests, pregnancy tests
- High variability in sensitivity, specificity, detection cutoffs, between brands
- Calibrated with a specific ‘representative’ drug – ex benzo panel uses oxazepam
- *Below are 6 benzodiazepines – which one caused the positive result seen on the BZD panel to the right? Was it only one? Multiple? Are they all detectable?*



Clonazepam (Klonopin)

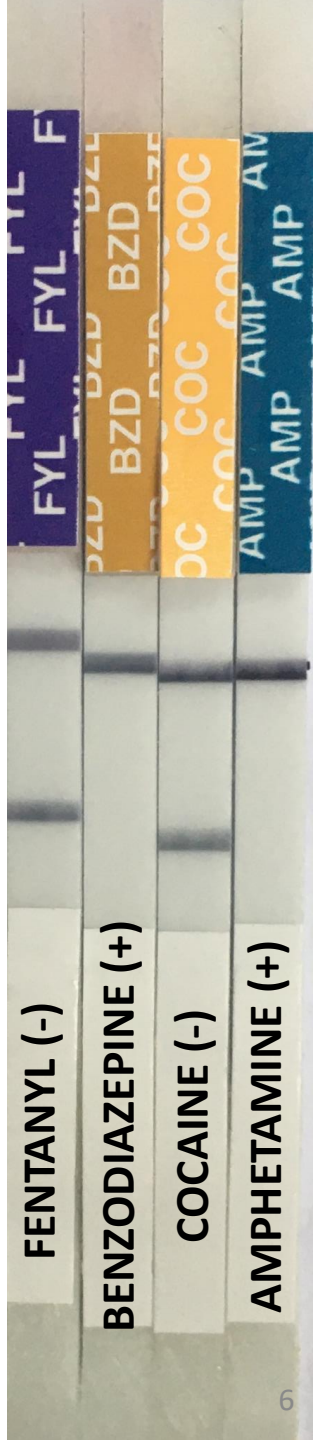
Temazepam

Diazepam (Valium)

Oxazepam *Calibration*

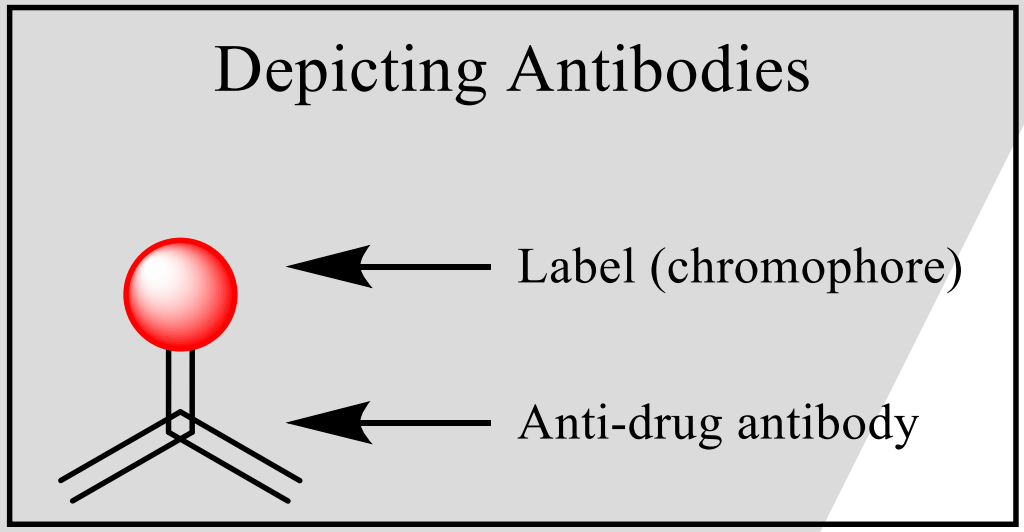
Alprazolam (Xanax)

Etizolam



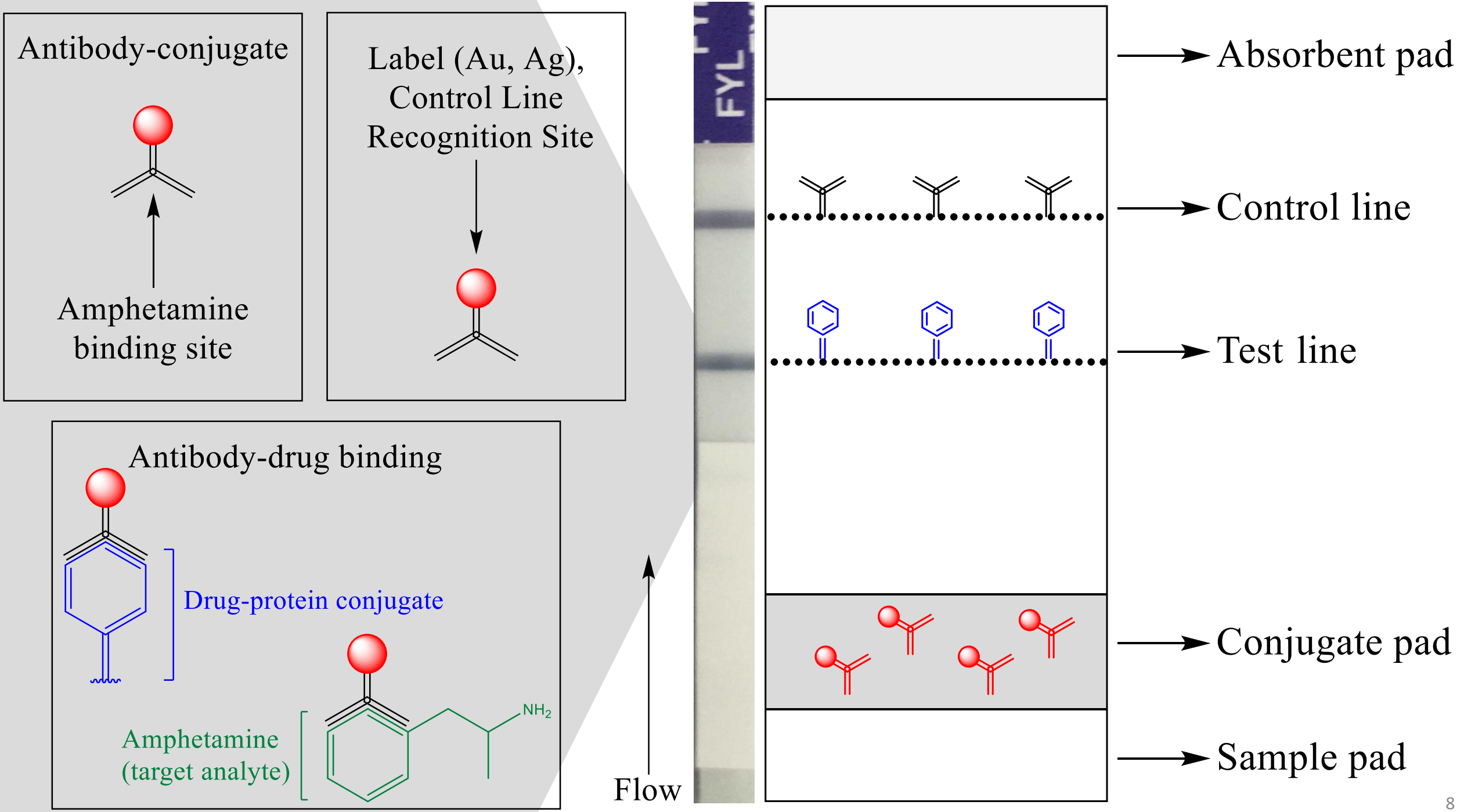
Antibodies in LFA

- Antibodies are the key to LFA function
- Antibodies are large proteins made by the immune system
  - Their job is to identify and bind to a specific antigen
- LFAs exploit this by ‘teaching’ (forcing) antibodies to identify and bind to a specific drug – the target antigen
  - Thus, LFA are calibrated to only *one* ‘representative’ drug

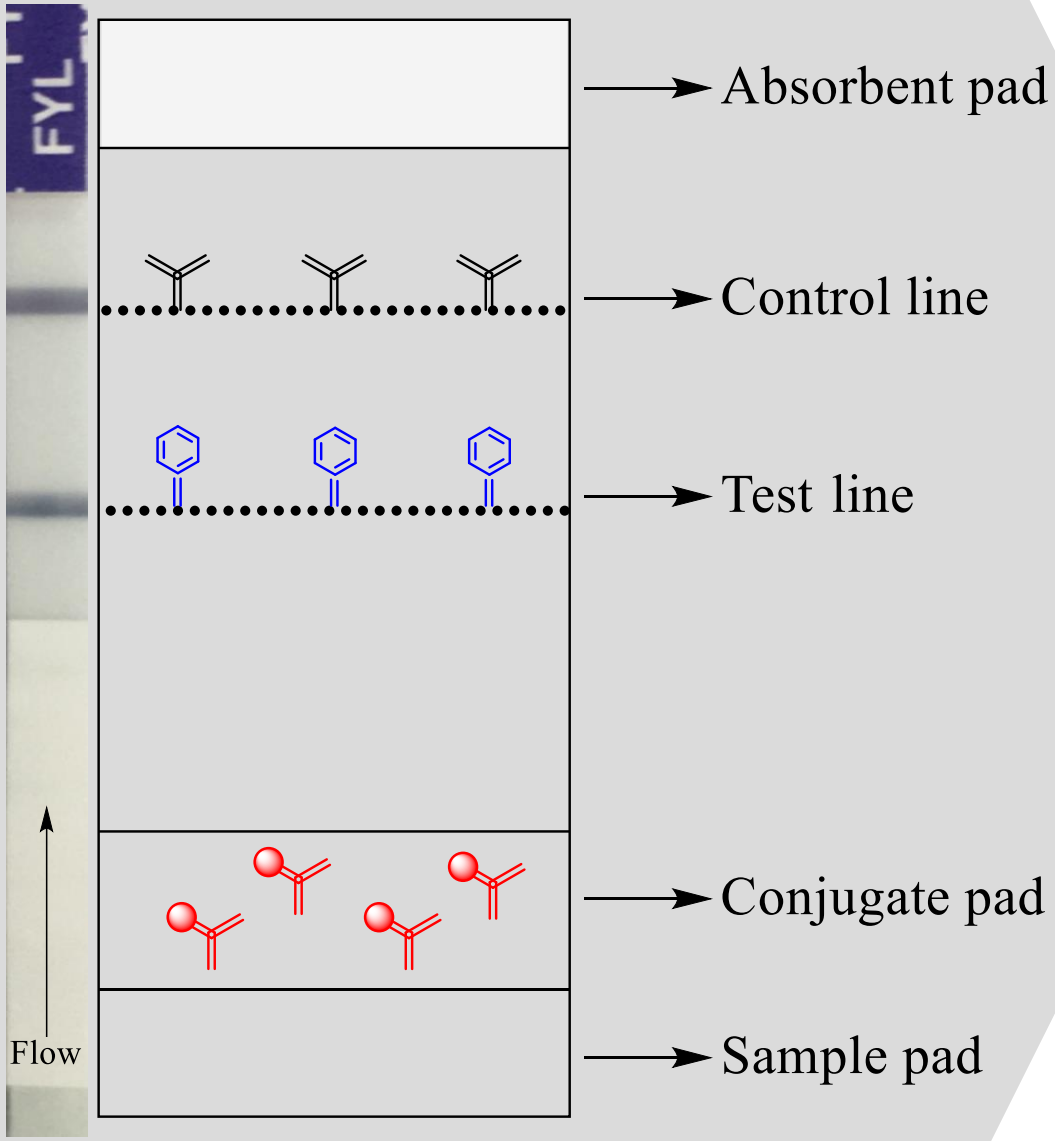


An antibody (depicted as a “Y”) is made visible via conjugation to a chromophore

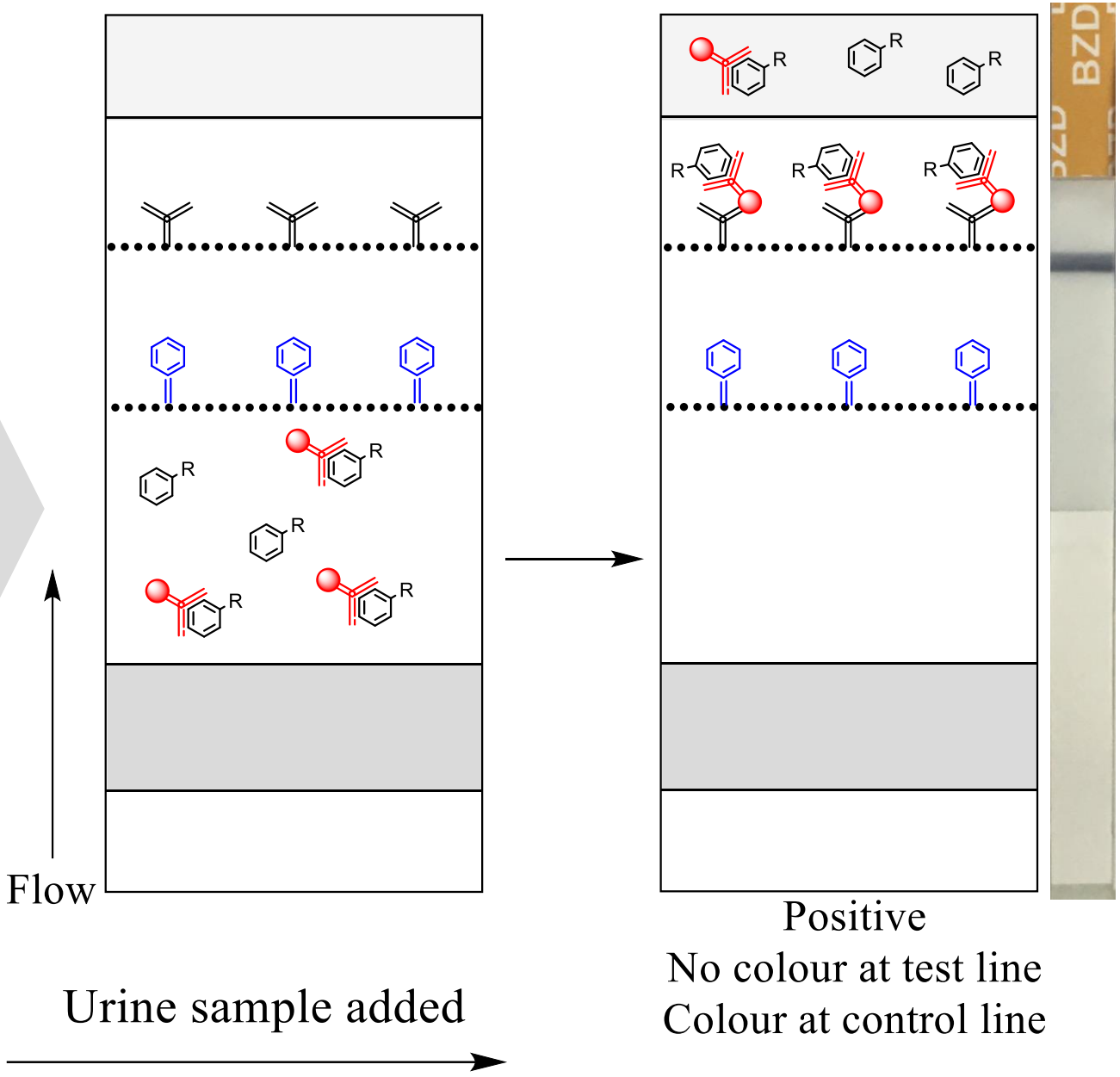
Anti-morphine antibody bound to morphine antigen
J. Mol. Bio. **2004**, *337*, 691-697


Antibody-conjugate
Amphetamine binding site
Label (Au, Ag), Control Line Recognition Site
Antibody-drug binding
Drug-protein conjugate
Amphetamine (target analyte)
NH2
Flow
Absorbent pad
Control line
Test line
Conjugate pad
Sample pad

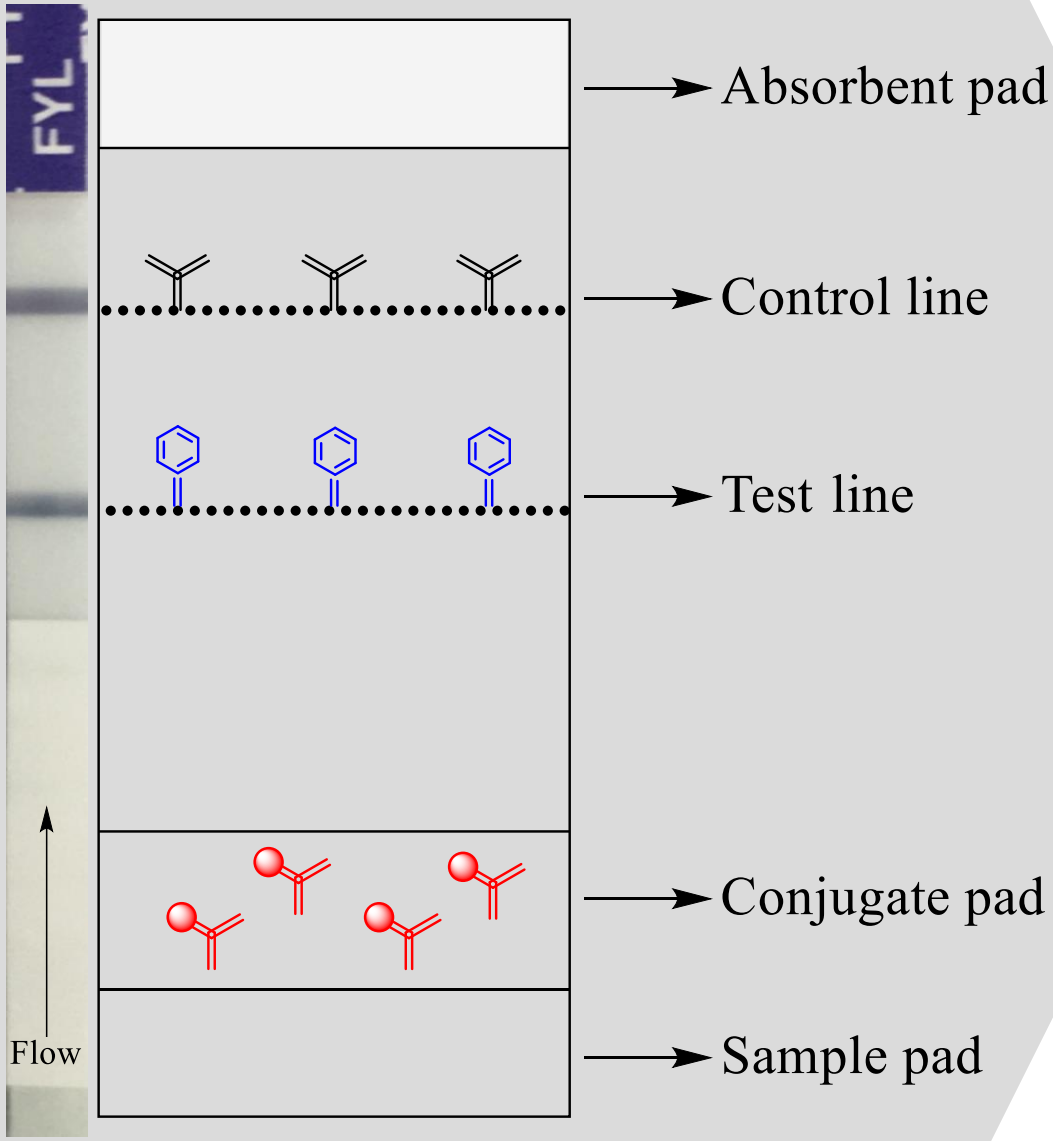
LFA Mechanism



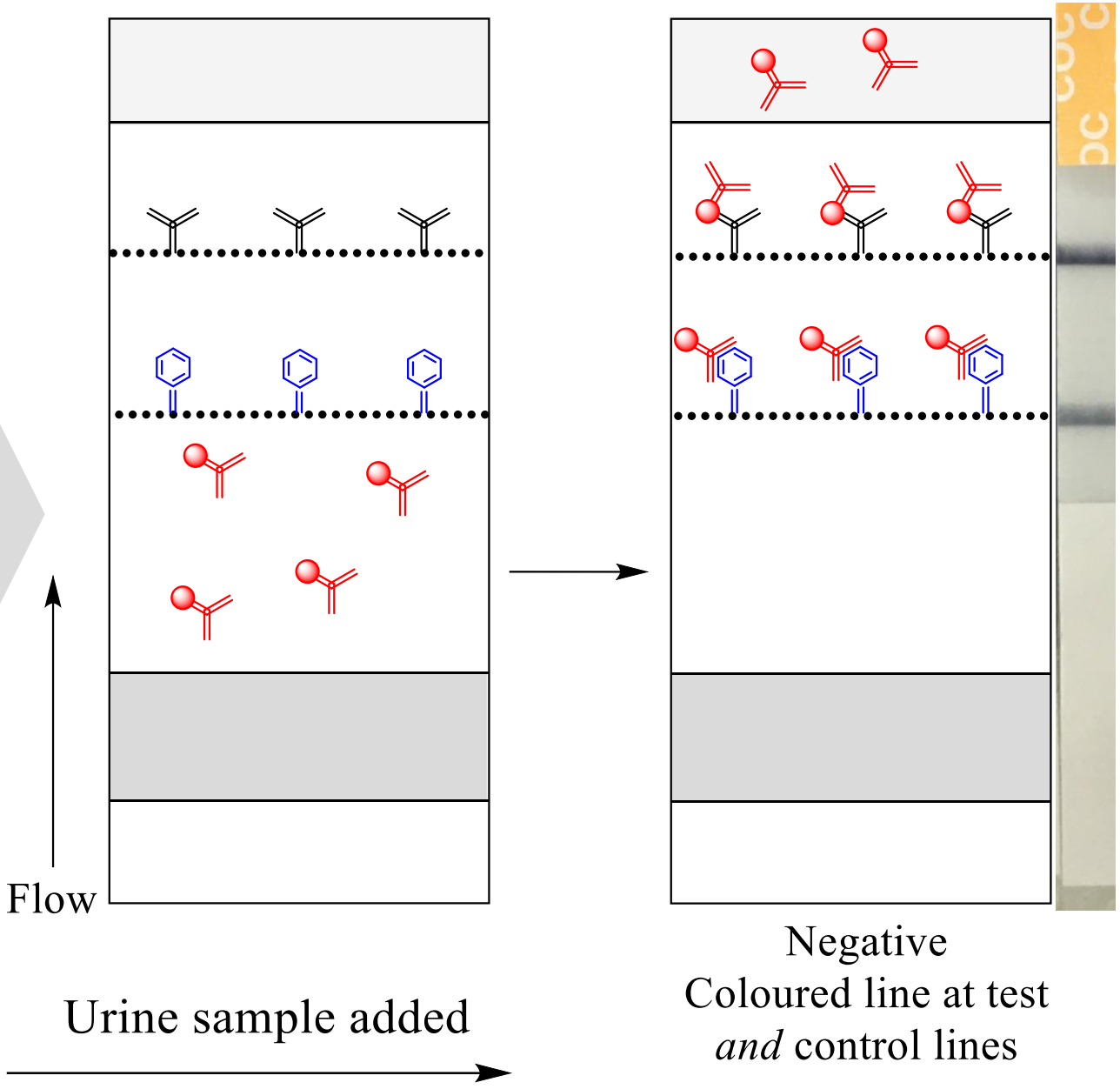
Mechanism of Positive LFA



LFA Mechanism



Mechanism of Negative LFA

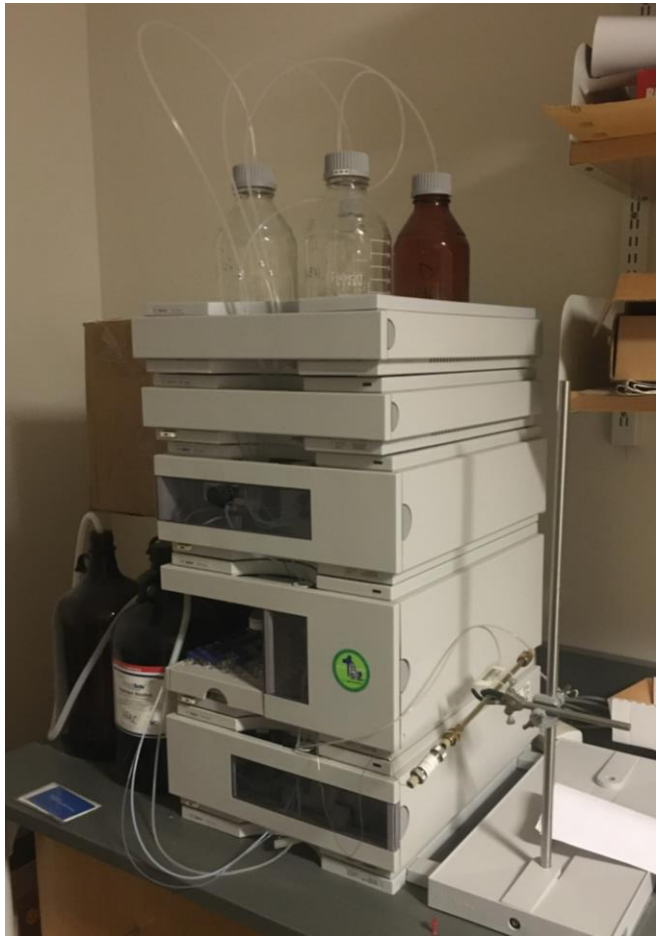


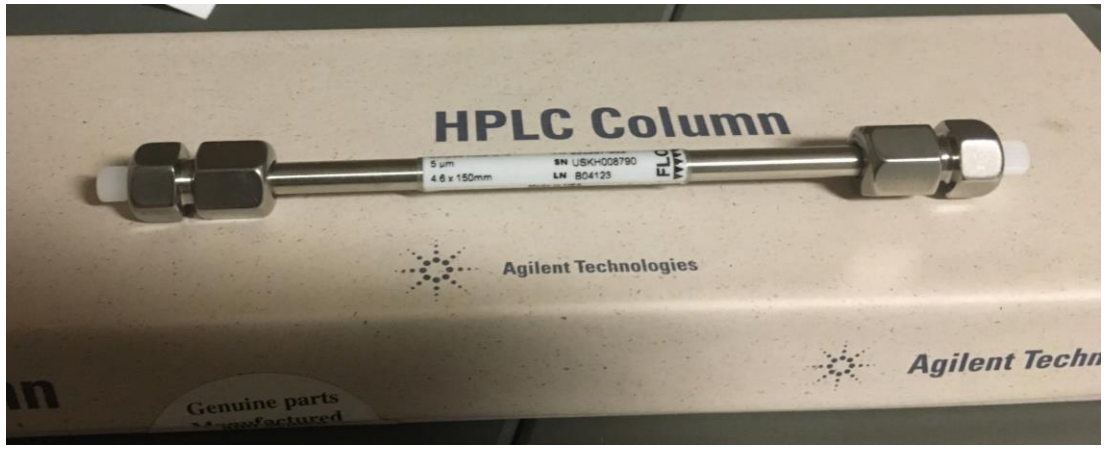
Confirmatory Lab Testing

- The gold standard for drug detection
  - *Gas chromatography-mass spectrometry (GC-MS)*
  - *Liquid chromatography-tandem mass spec (HPLC-MSS/MS)*

- **Benefits:**
  - May be quantitative or semi-quantitative
  - Specific
  - Sensitive
  - Identification of illicit drug composition
  - Provides drug/metabolite ratios

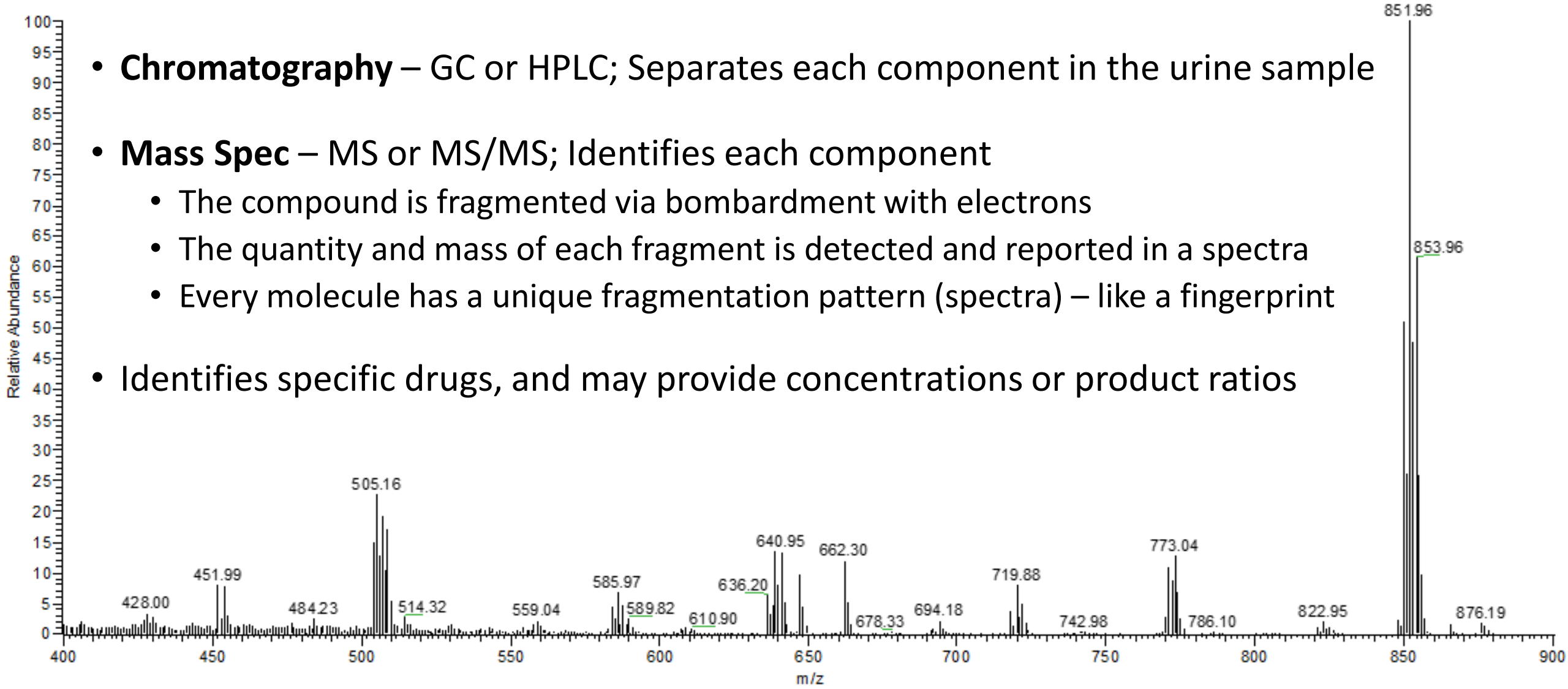
- **Drawbacks:**
  - Requires sample preparation
  - Laboratory equipment and training required
  - Slow turnaround times
  - *Commercial labs only identify 'known' compounds*





How It Works

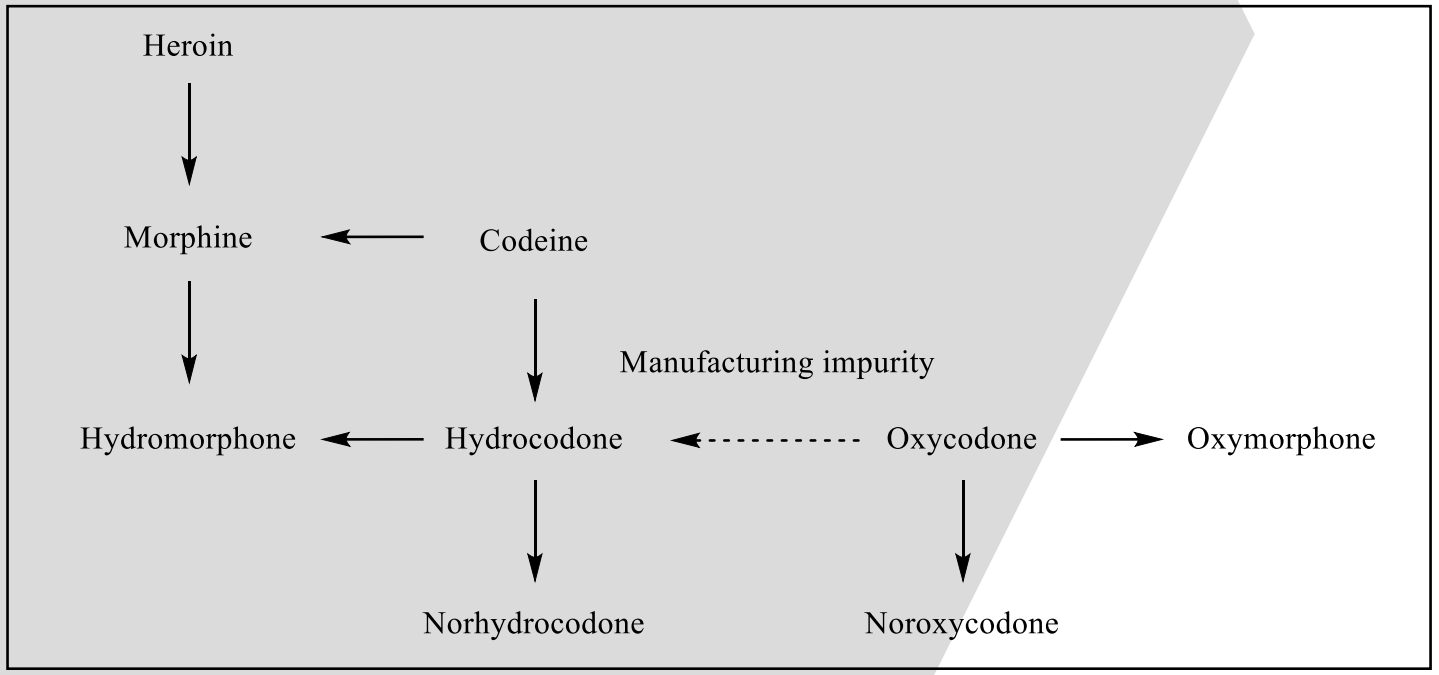
- **Chromatography** – GC or HPLC; Separates each component in the urine sample
- **Mass Spec** – MS or MS/MS; Identifies each component
  - The compound is fragmented via bombardment with electrons
  - The quantity and mass of each fragment is detected and reported in a spectra
  - Every molecule has a unique fragmentation pattern (spectra) – like a fingerprint
- Identifies specific drugs, and may provide concentrations or product ratios



Example of a typical mass spectra (Electron ionization mass spec [EI-MS])

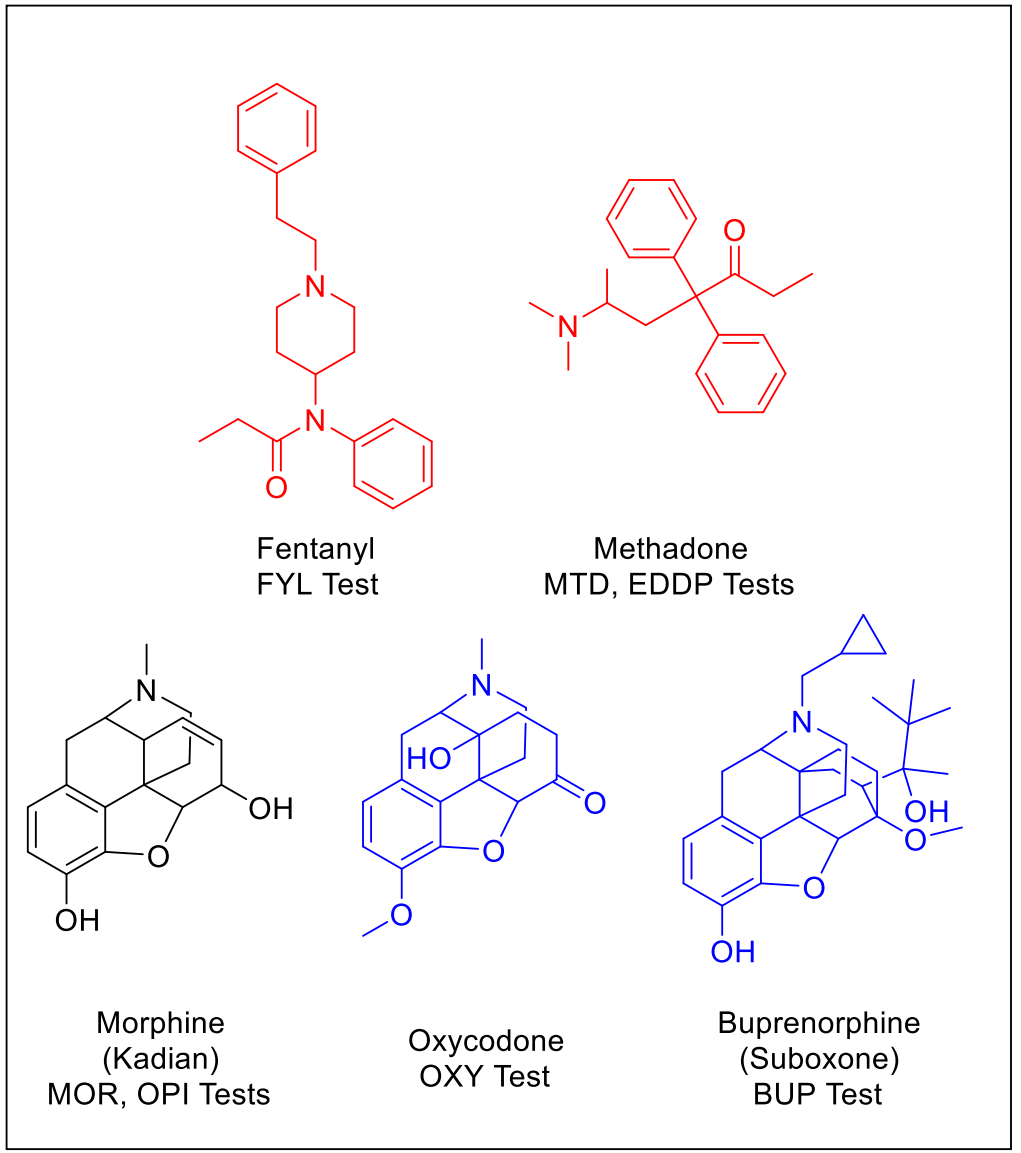
LFA and Opioids

- Opioids are a *very* structurally diverse group of drugs
  - Three 'types' – natural, semi-synthetic, synthetic
- Metabolic crossover and structural dissimilarities can easily lead to confusion or using an incorrect test
- A 'general opiate' test will not detect *all* opioids



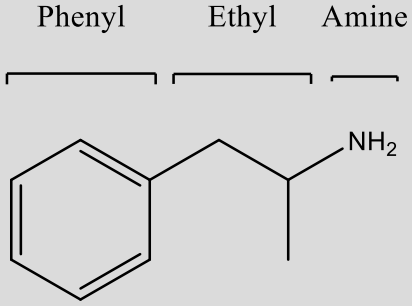
Metabolic crossover of some opiates

Using the wrong test for a specific opioid is a common cause of false-negative results

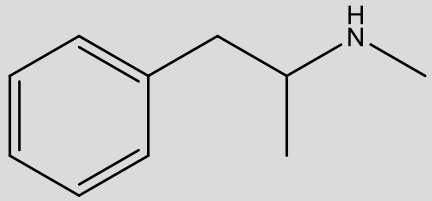


LFA and Amphetamines

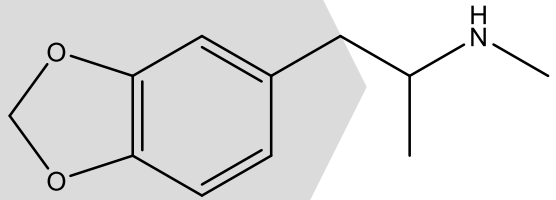
- Cross reactivity is possible with *any* LFA test
  - Occurs when the antibody binds to an incorrect antigen – false positive result
- Amphetamines are *very* small, structurally simple, have minimal structural diversity



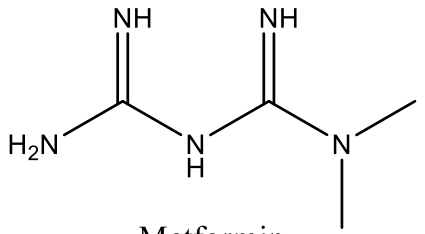
Amphetamine
(Adderall)



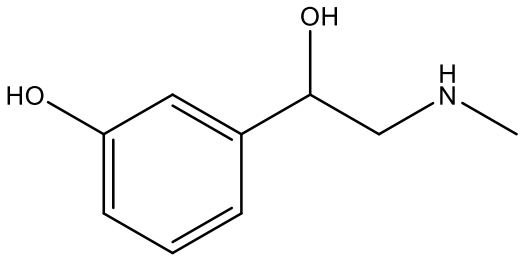
Methamphetamine



MDMA
(Ecstasy)



Metformin



Phenylephrine

- Antibodies rely on recognizing unique structural features possessed by the antigen
- 'Promiscuous binder' – protein (antibody) that may bind to many different compounds
  - Drugs with similar structures may cross-react with amphetamine LFA (ex phenylephrine)
  - Even drugs without similar structures may cross react in some cases (ex metformin)

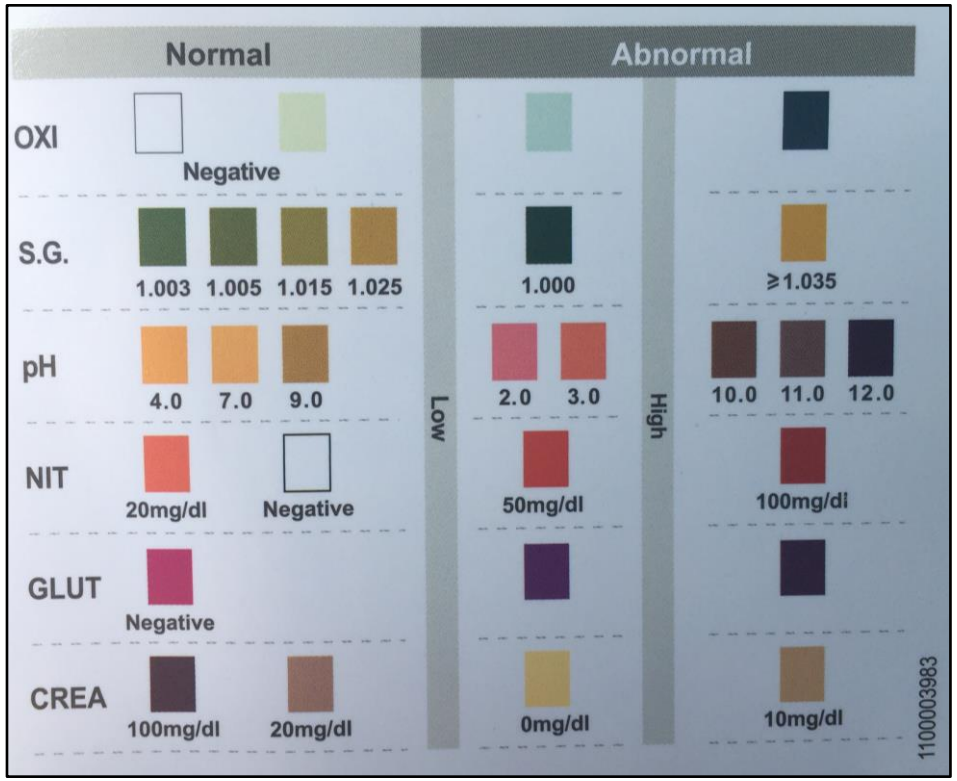
Pharmacotherapy **2012**, 32(5), 98-102.

“Tampering”

- When a sample is intentionally altered in an attempt to obscure or change the observed results
  - Dilution, chemical tampering, synthetic urine, etc

- Chemical tampering prevention:
  - Metabolite detection
  - Additional ‘dipstick’ tests:
  - *Creatinine, temperature, pH, etc*

- Physical tampering prevention:
  - Coloured toilet water
  - Only cold water in sink
  - No bags, coats, etc permitted in restroom
  - Video recording



BTNX tampering cheat sheet

Random Urine Chemistry				
Creatinine Urine				
Creatinine (Urine)	LO	<1.0	2.5-20.0	mmol/L
Test repeated and results confirmed.				

Lab tests are not perfect

UDS – Influencing Factors

- UDS can be extremely useful tools which, in the wrong hands, can have serious repercussions
  - Regular drug testing may not lead to improved clinical outcomes or deter substance use

- **There are *many* factors at play:**
  - Chronic vs single use
  - Metabolic variations between individuals
  - Drug distribution
  - Urinary pH
  - Drug-drug interactions

"Taken together the findings support the view that UDS may, in effect, be deterring people who are at high risk for abuse (as indicated by a positive test for illicit substances) from further engagement with the clinic."

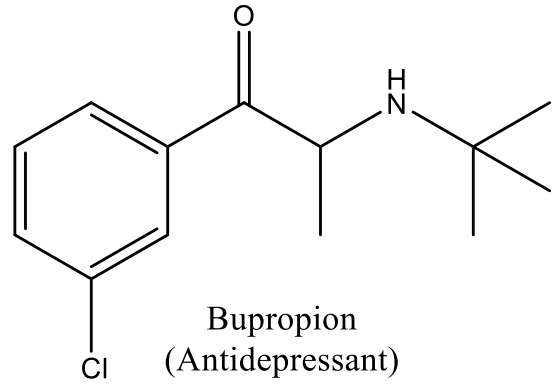
- Anyone using UDS should be aware of the possible limitations, proper interpretation, and limitations of the method they use
  - LFA tests are *not* confirmatory
  - Lab tests are not all-encompassing

- In the constantly changing toxic drug supply, even lab tests may not identify every substance

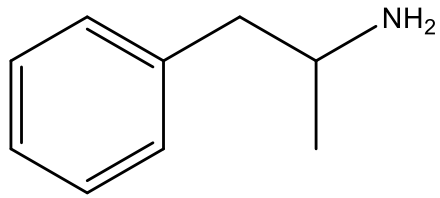
J. Pharm. Pract. **2016**, *29(5),* 516-526.; *Anesth. Analg.*, **2017**, *125(6)*, 2094-2104.

Reliance on LFA Only – Potential Harms

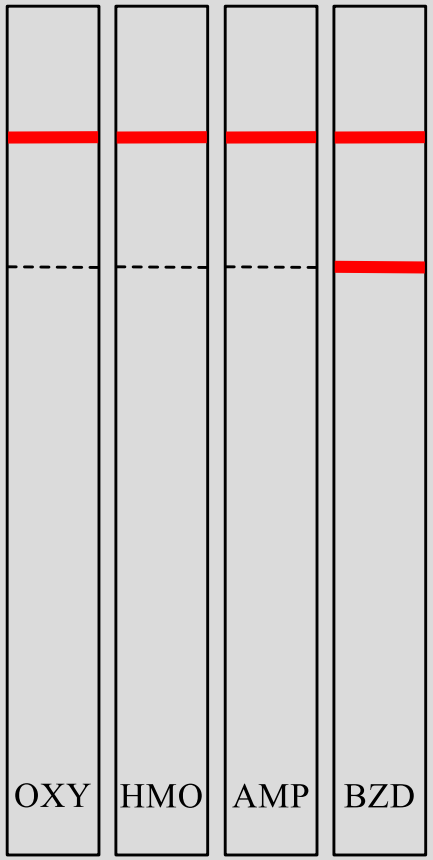
- Safer supply client currently prescribed oxycodone, had previously abused illicit opioids and stimulants
- Upon LFA testing, test is positive for oxycodone, hydromorphone, and amphetamine
- What happened? Did they relapse?



Bupropion
(Antidepressant)



Amphetamine
(Adderall)

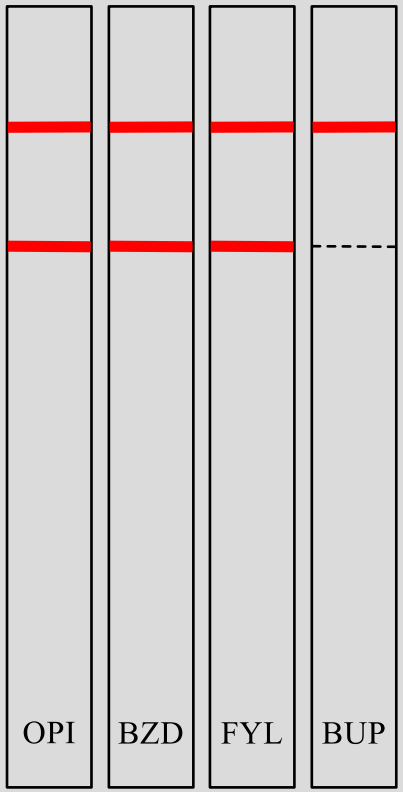


- **Lab testing shows:**
  - *Oxy – expected product/metabolite + ratios*
  - *No heroin, 6-MAM, morphine, codeine*
  - *Hydromorphone – trace*
  - *No stimulants (amp, meth)*

- Lab test indicates no evidence of non-prescribed opioid abuse;
- Manufacturing impurities in prescribed oxycodone may lead to trace hydrocodone
  - False-positive (impurities)
- New antidepressant prescription from primary care physician cross-reacted with the LFA
  - False-positive (cross-reactivity)
- This individual could face serious consequences if results are not interpreted properly

Lost Opportunities for Intervention

- OAT patient prescribed buprenorphine (Suboxone)
- Has been on a stable dose with no reported drug use for several months
- Most recent LFA gives expected results
- Likely given their prescription and sent home



- **Lab test:**
  - *Buprenorphine DETECTED*
  - *Norbuprenorphine (bupe metabolite) NOT DETECTED*
  - *HO-Alprazolam (Xanax metabolite) DETECTED*
  - *HO-Etizolam (Etizolam metabolite) DETECTED*
  - *Fentanyl and metabolites DETECTED*

Analyte	Lab Reporting Cutoff (ng/mL)
Etizolam	2, 5*
Flualprazolam	5, 15*
Flubromazolam	1, 1*
Carfentanil	1, 1*
Sufentanil	5
Acetylfentanyl	5, 50*
Alfentanyl	5

** Indicates metabolite*

Detection cutoffs of some benzodiazepines and opioids unlikely to be detected by LFA

- Lab test indicates potential tampering, due to lack of buprenorphine metabolite in urine sample
- Benzodiazepines – including several 'designer drugs' – and fentalogues present
- Taking pressed Xanax? Taking 'fentadope'? Single relapse or chronic?
- May not have reported drug use due to fear of punishment, judgement, stigma
- *This person is at a high risk of overdose and death, and may benefit from help*
- May be unknowingly dependent on benzos – may need benzo taper; may require treatment adjustment

Final Thoughts

- Additional factors – metabolism, lifestyle, other medications, etc, can and will impact drug testing results

- Drug test results can have severe consequences
  - Ensure the proper test is used
  - Learn about possible sources of error
  - If in doubt, consult a lab

- The impact of UDS on substance users themselves is not well-studied
  - *Hearing directly from those whose lives are impacted by these tests is crucial*

- UDS can be a useful tool for monitoring a patient or client, HOWEVER: