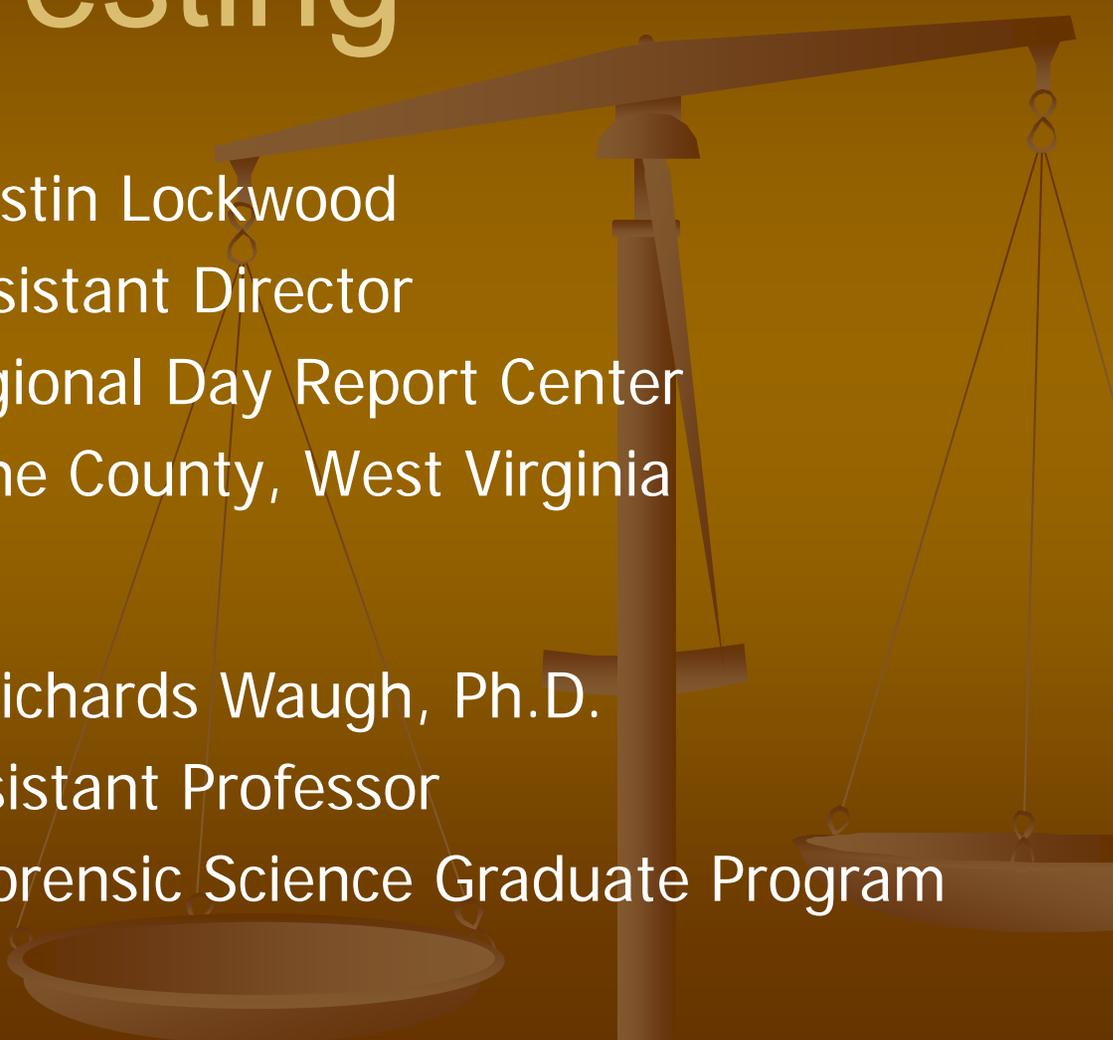


The Nitty-Gritty of Drug Testing



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The iCup 13 Panel Drug test for the following drugs:

THC (marijuana, pot, grass, weed, hash, Mary Jane, dope)

Cocaine (coke, crack, blow, nose candy, snowball, tornado)

Opiates (heroin, morphine, opium, smack, thunder, hell dust)

Amphetamines (Dexedrine, speed, uppers)

Methamphetamines (meth, crank, ice, chalk, black beauties, crystal meth)

Buprenorphine (Suboxone, Subutex, Temgesic)

Methadone (Dolophine, Methadose, Phytsetone)

Oxycodone (Percocet, Percodan, Oxycontin, Tylox, oc, ox, oxy)

Barbiturates (barbs, downers, Amytal, Nembutal, Seconal, Tuinal)

Benzodiazepines (Valium, Xanax, Librium, Ativan, Halcion, Diazepam)

Propoxyphene (Darvocet, Darvon)

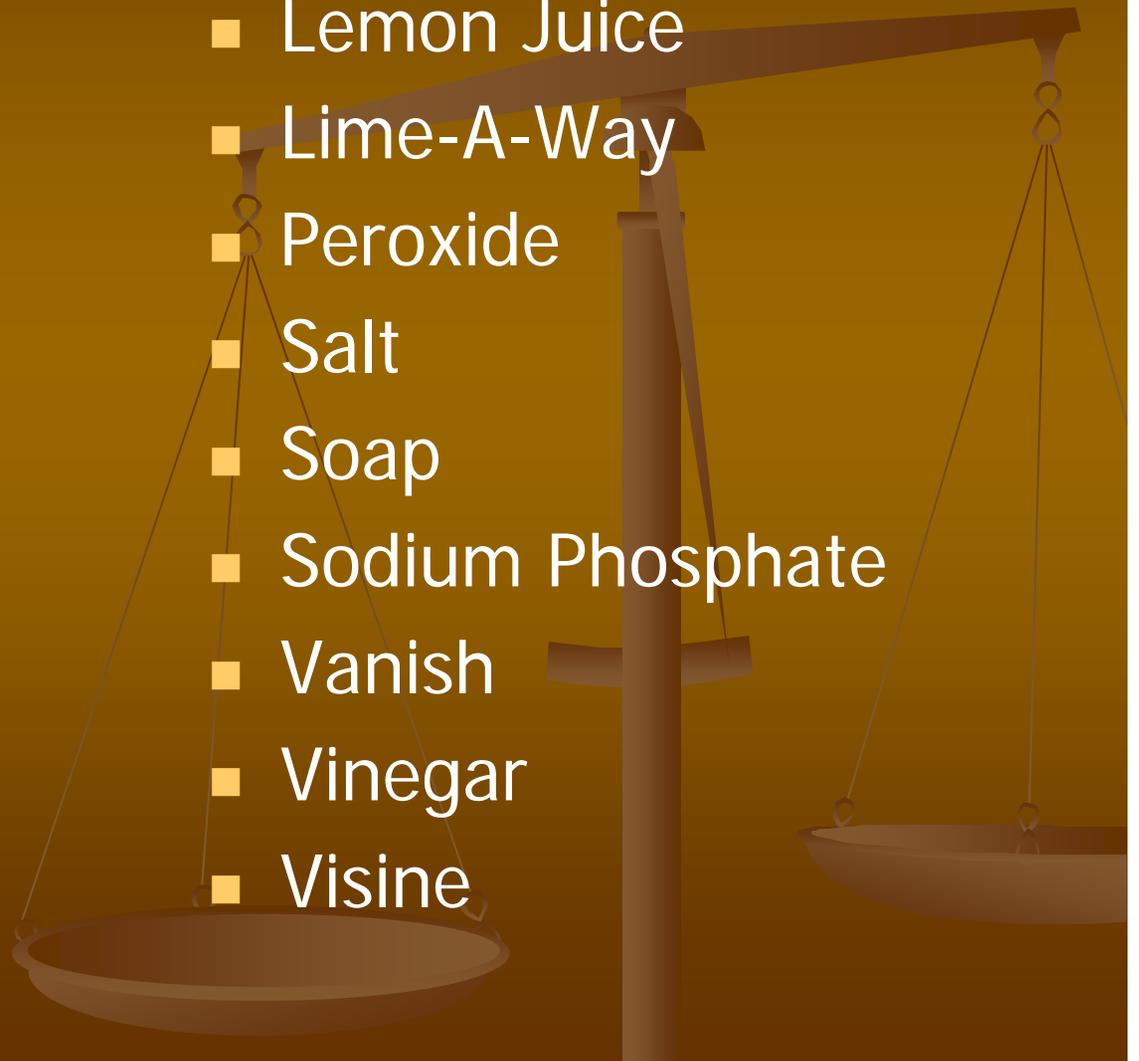
Phencyclidine (PCP, angel dust)

Tricyclic Antidepressants (Nortriptyline)

13 Panel iCup Drug Testing Levels and Detection Periods		
Drug	Detection Period	Cutoff Level
Cocaine COC	2-4 Days	300 ng/mL
Marijuana THC	15-30 Days	50 ng/mL
Opiates OPI	2-4 Days	2000 ng/mL
Amphetamines AMP	2-4 Days	1000 ng/mL
Methamphetamines mAMP	3-5 Days	1000 ng/mL
Phencyclidine PCP	7-14 Days	25 ng/mL
Benzodiazepines BZO	3-7 Days	300 ng/mL
Barbiturates BAR	4-7 Days	300 ng/mL
Methadone MTD	3-5 Days	300 ng/mL
Tricyclic Antidepressants TCA		1,000 ng/mL
Oxycodone	2-4 Days	100 ng/mL
Propoxyphene	1-2 Days	300 ng/mL
Buprenorphine BUP (suboxone, subutex)	2-3 Days	10 ng/mL

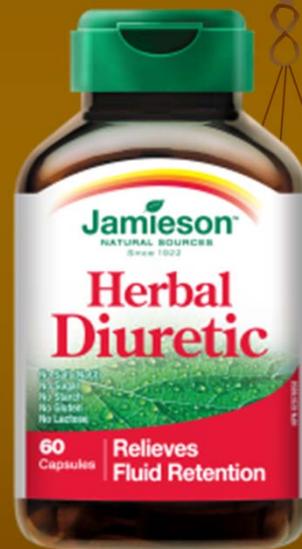
Substances Used as Adulterants

- Alcohol
- Ammonia
- Bleach
- Ascorbic acid
- Bleach
- Blood
- Detergent
- Drano
- Golden Seal Root
- Lemon Juice
- Lime-A-Way
- Peroxide
- Salt
- Soap
- Sodium Phosphate
- Vanish
- Vinegar
- Visine



Diluents

- *in vivo* – ingestion of diuretics, drink copious amounts of water, eat or drink certain foodstuffs

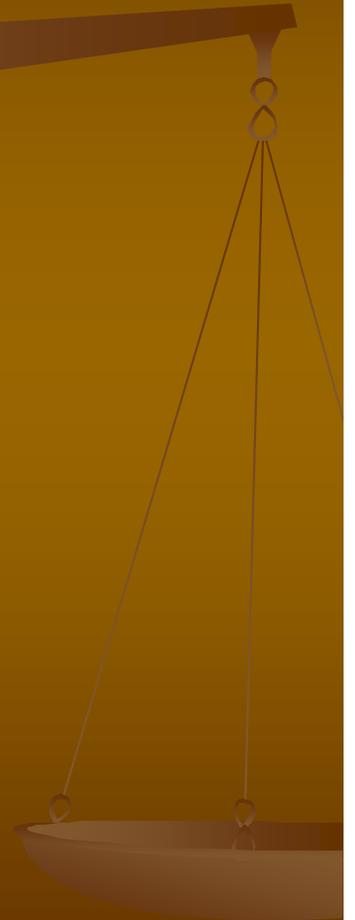
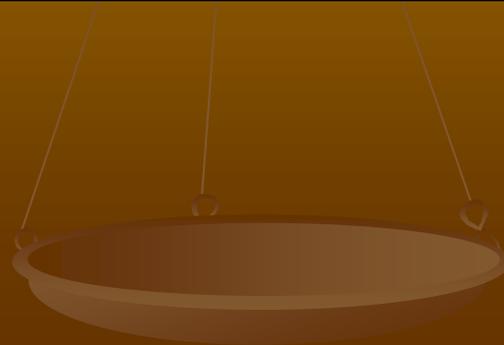
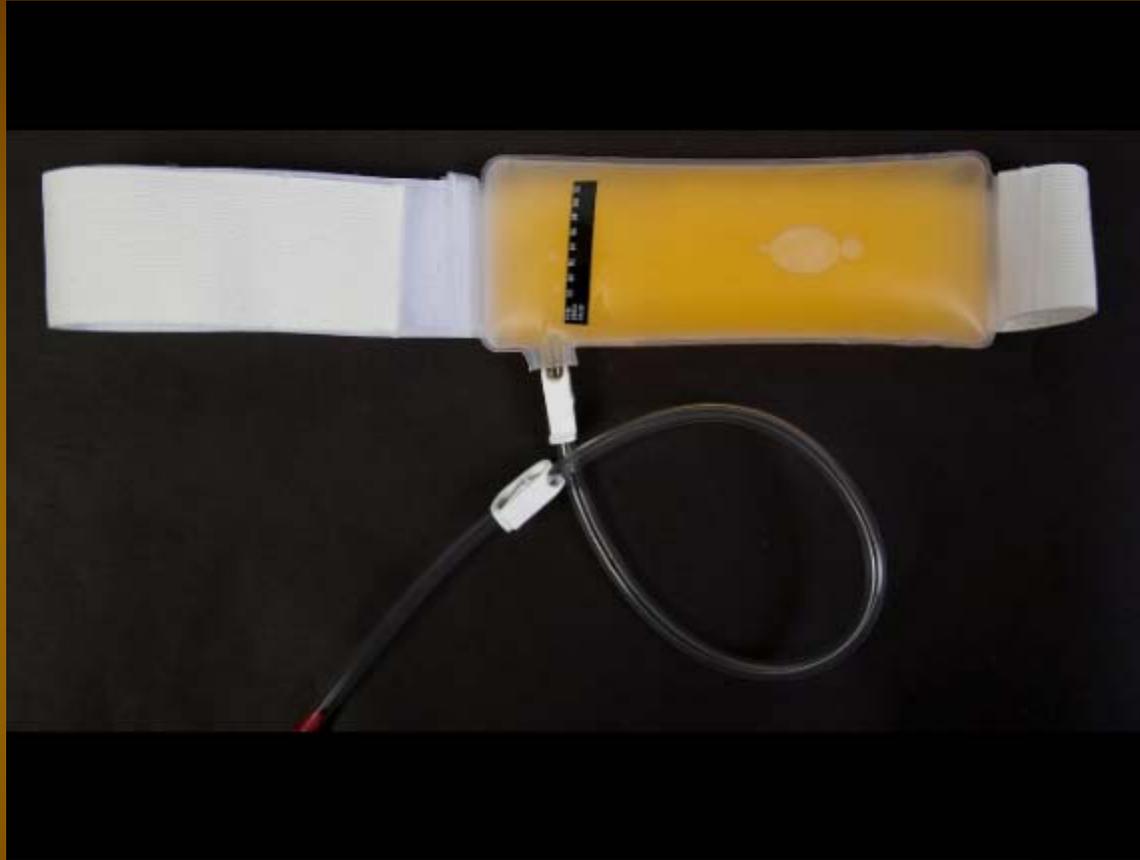


- *in vitro* – add liquid to the sample



Methods of adulterations







- Top – left
 - Soapy urine
- Top – right
 - Urine with Drano
- Bottom
 - Key for adulteration test strip

TEST AND READING TIME	ABNORMAL (LOW)	NORMAL			ABNORMAL (HIGH)
Creatinine 45 seconds	 Negative 10	 20 0	 50 100 mg/dl		
Nitrite 45 seconds	 Negative	 0	 0.1-0.2 0.5-1.0		 ≥15 mg/dl
Glutaraldehyde 45 seconds		 Negative			 Positive
pH Immediate	 2 3	 4	 5 6 9		 ≥10
Specific Gravity 45 seconds	 1.000	 1.005	 1.010 1.020		 ≥1.030
Oxidants 60 seconds		 Negative			 Positive

Urine Collection Procedure





Step One:
Client empties pockets



Step Two:

Employee prepares using protective gloves



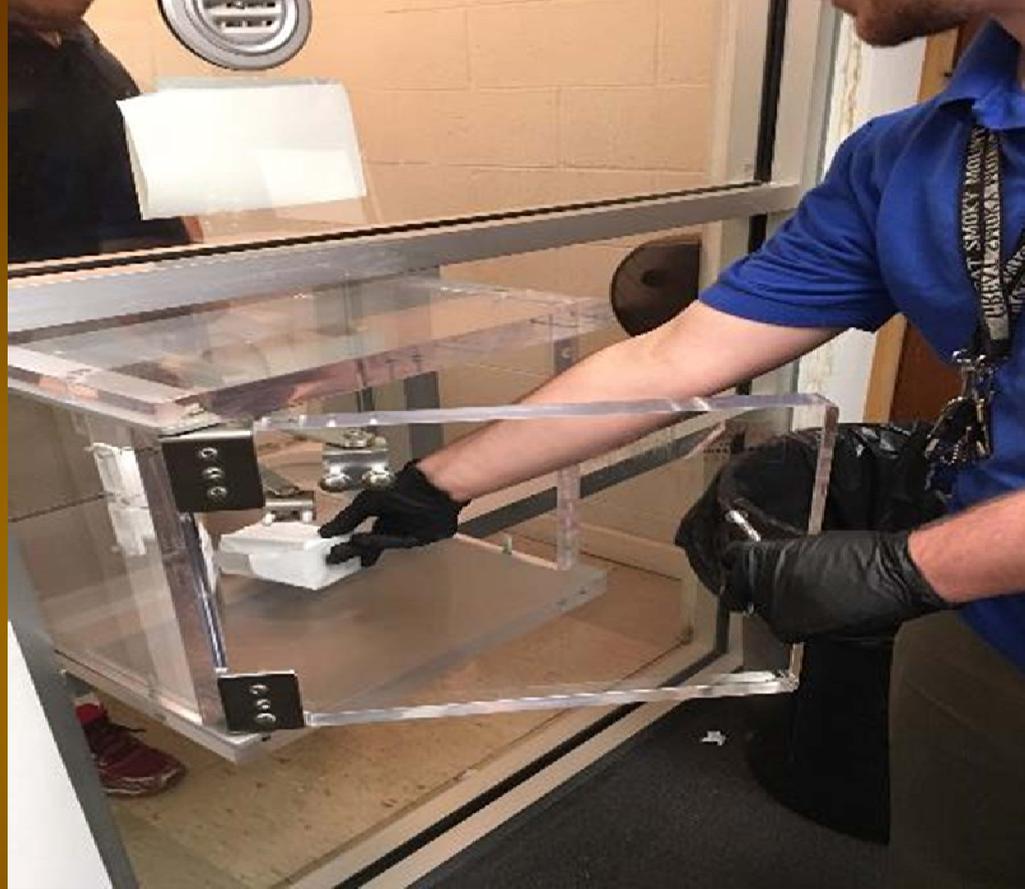


Step Three:
Client washes hands

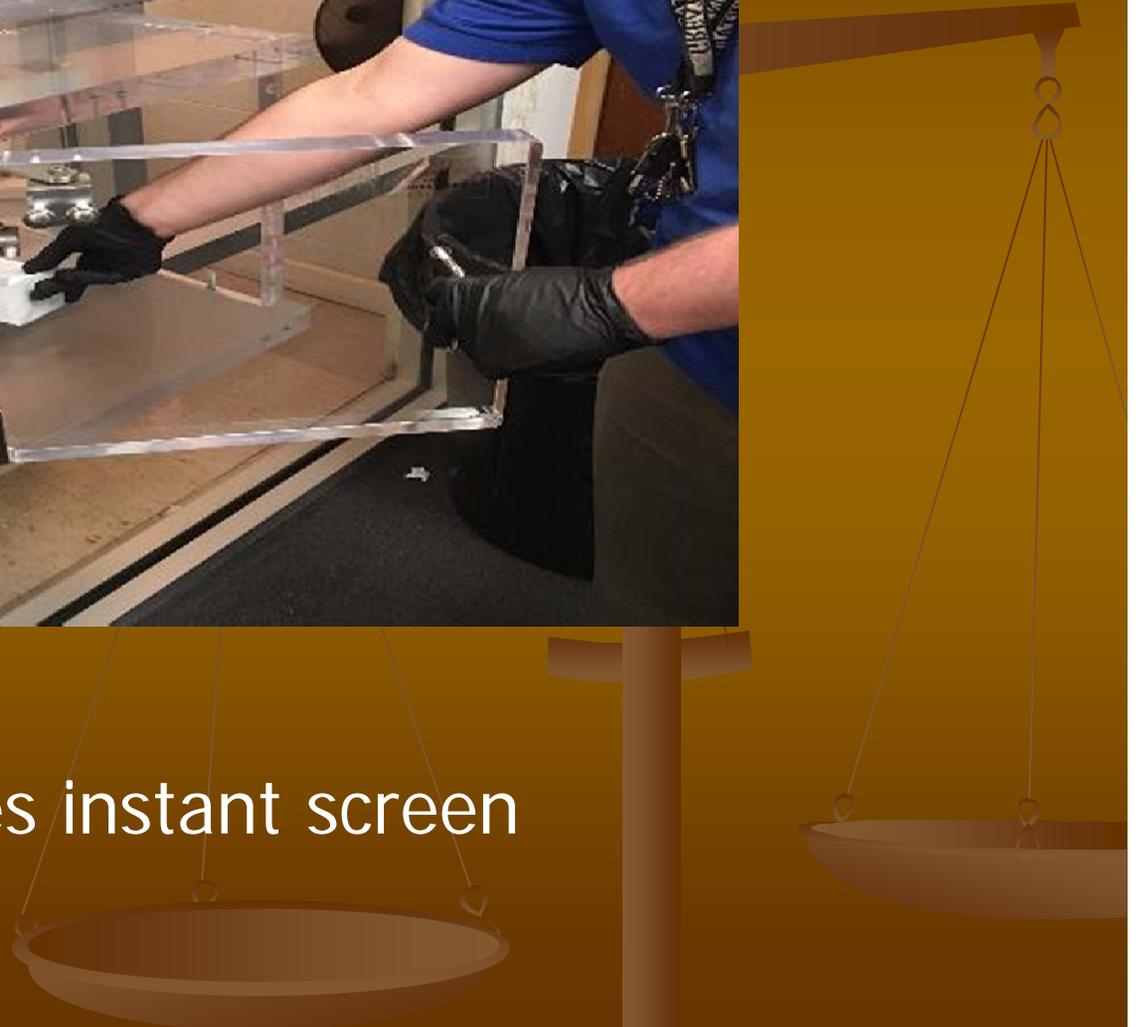


Step Four:
Collector puts on rubber gloves



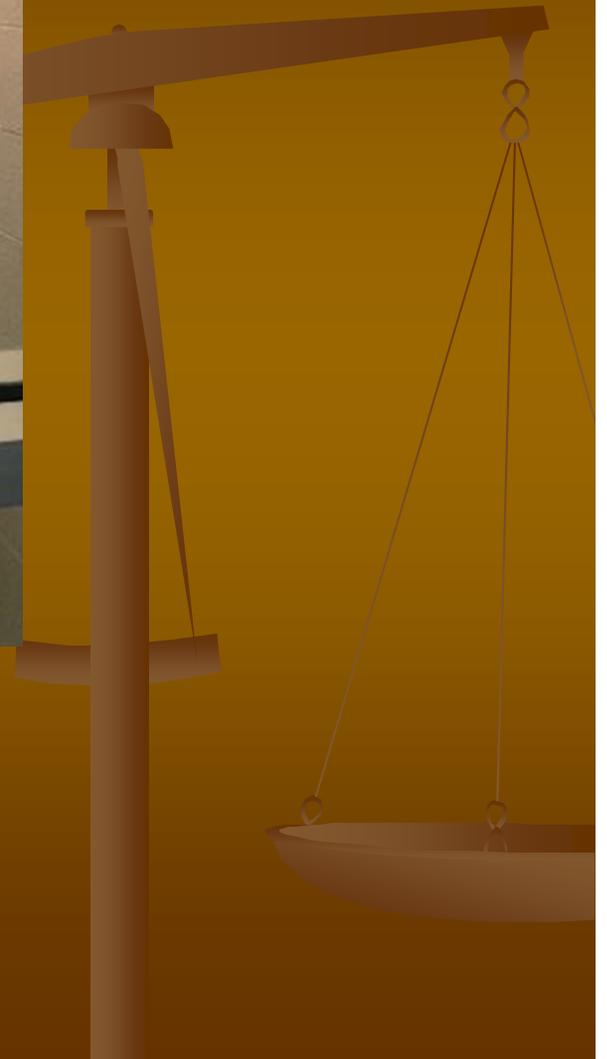


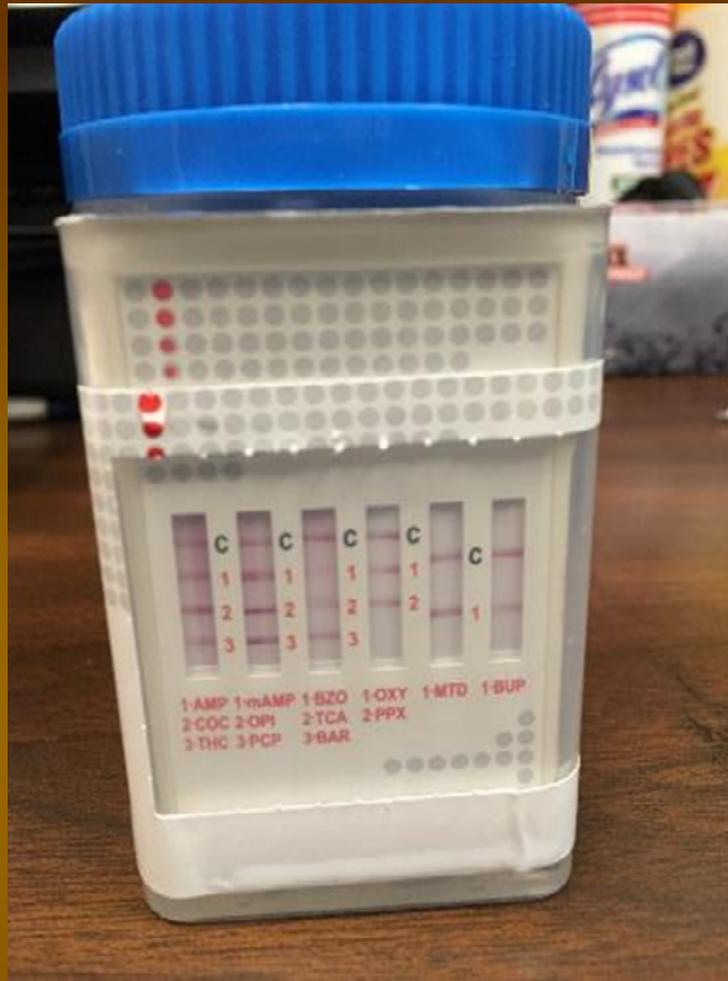
Step Five:
Client passes instant screen





Step Six:
Client provides sample



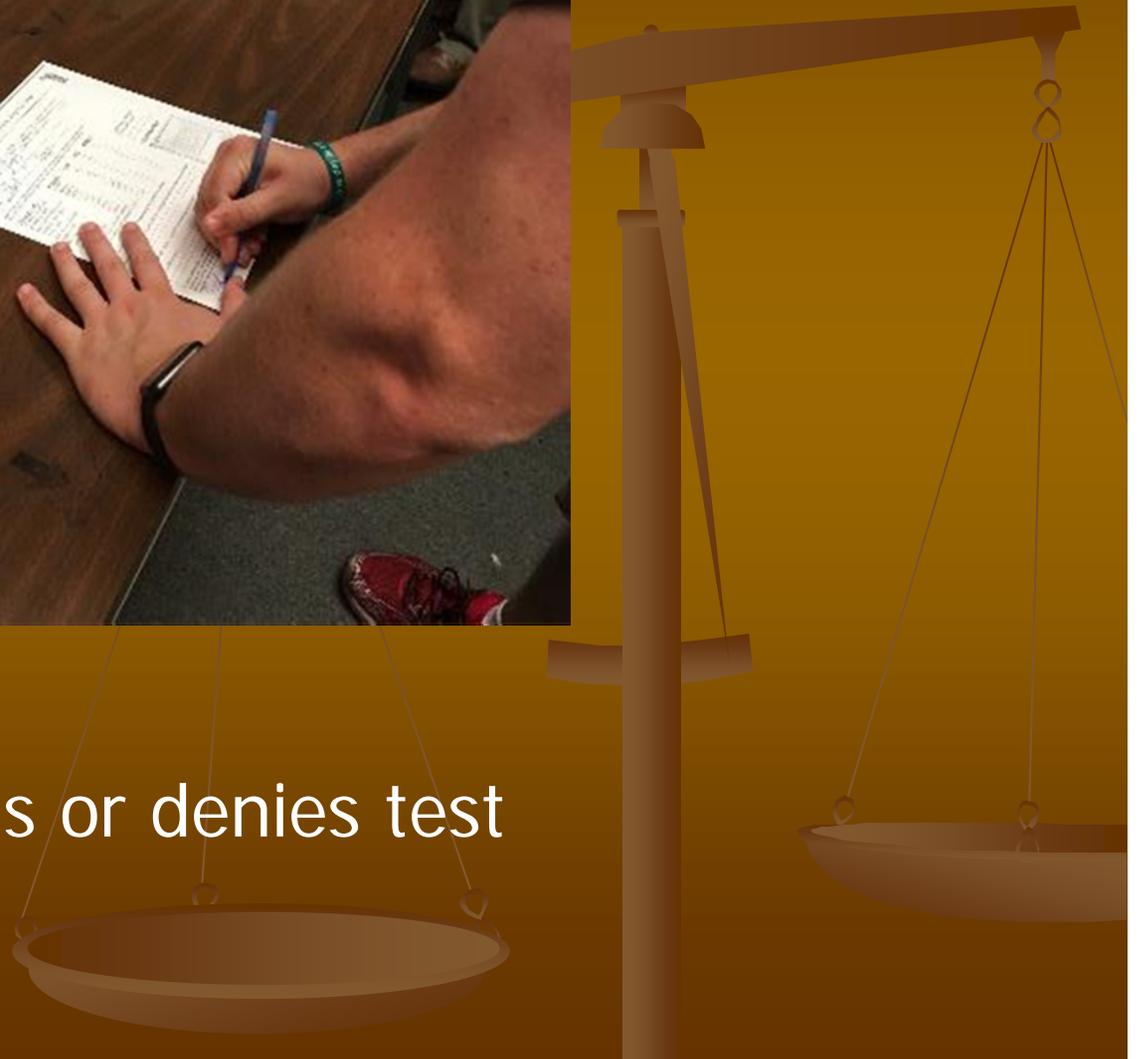


Step Seven:

Provide sample, read results



Step Eight:
Client admits or denies test
results

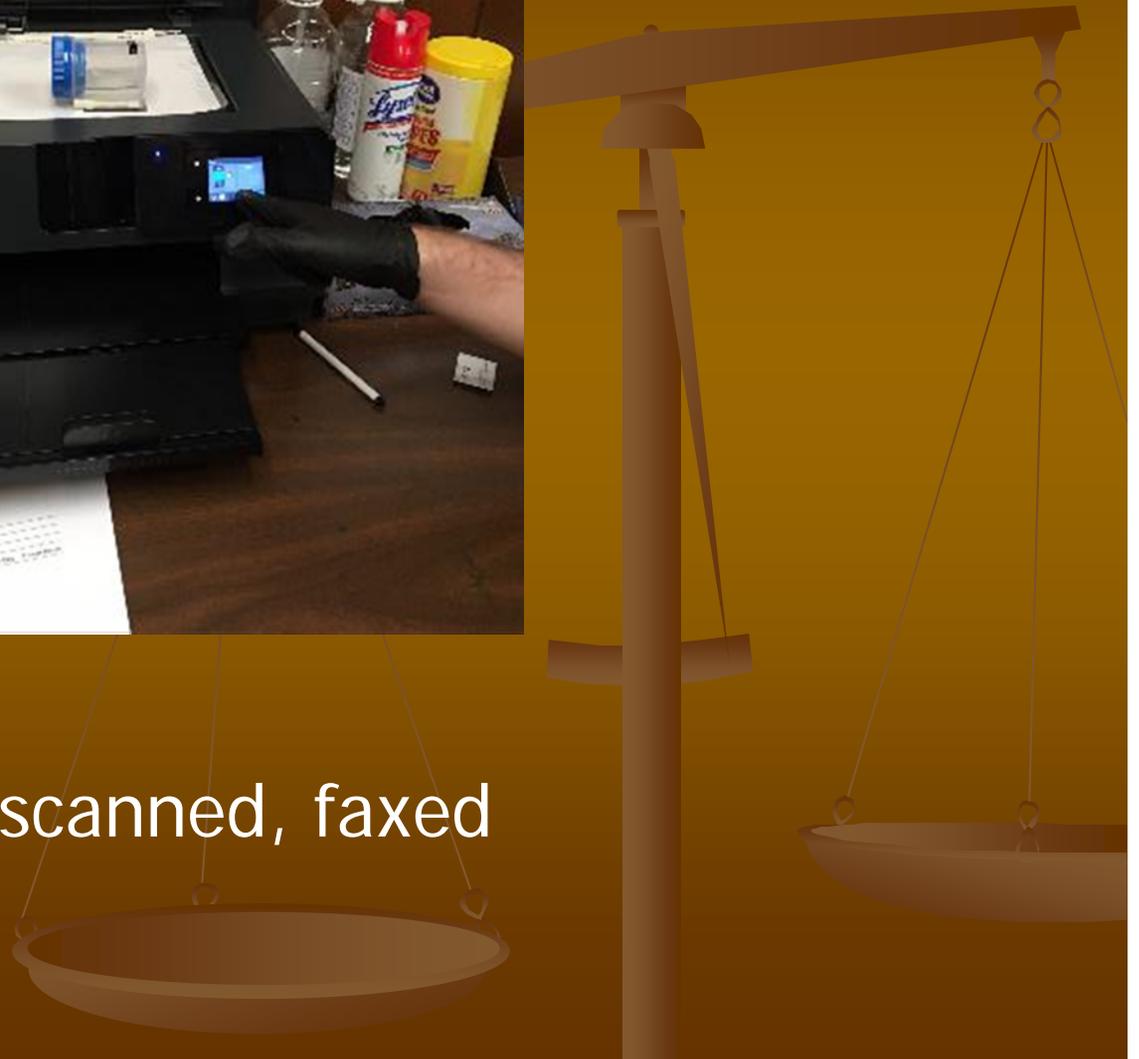






Step Nine:

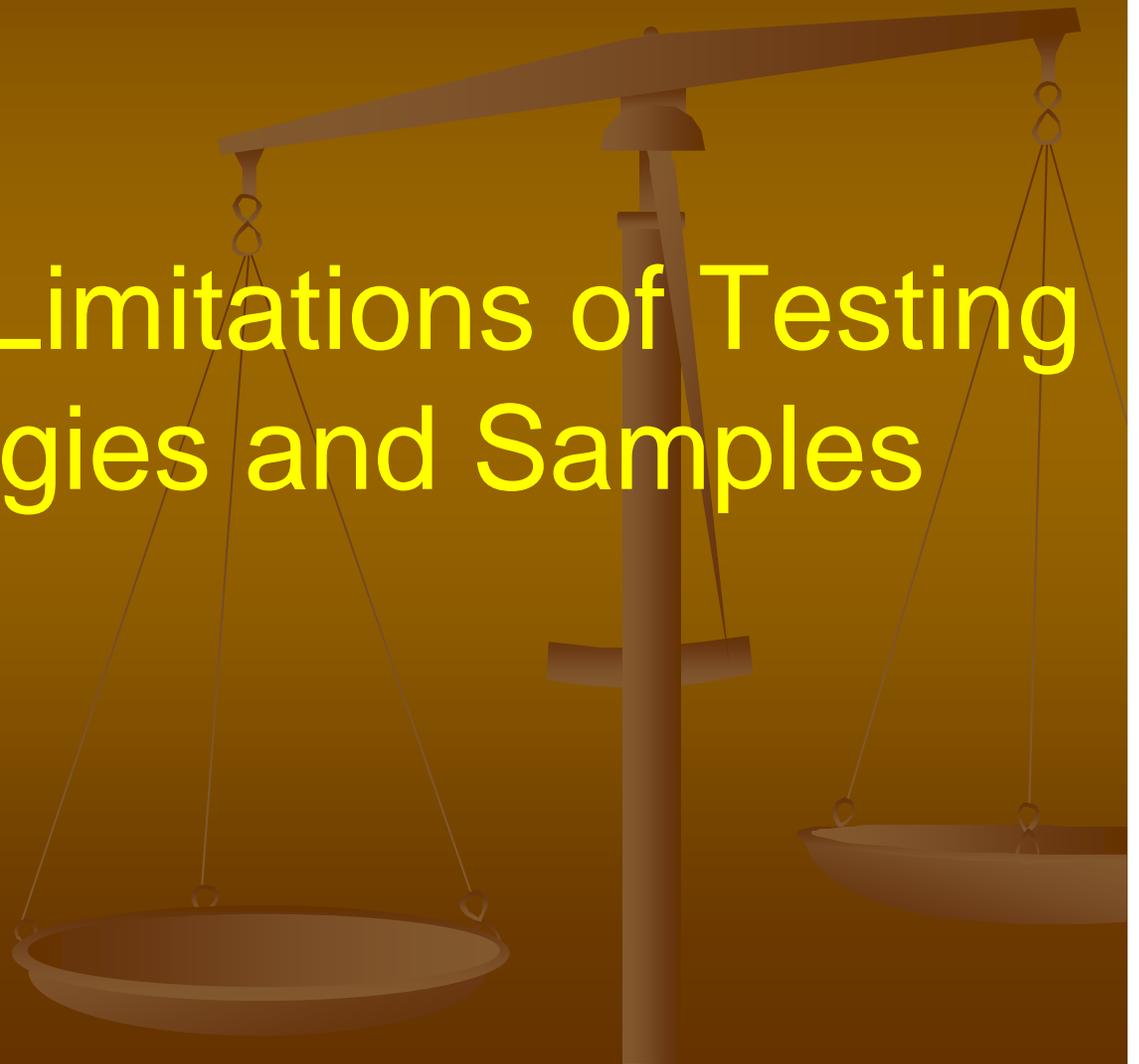
Results are scanned, faxed



Questions for Discussion?

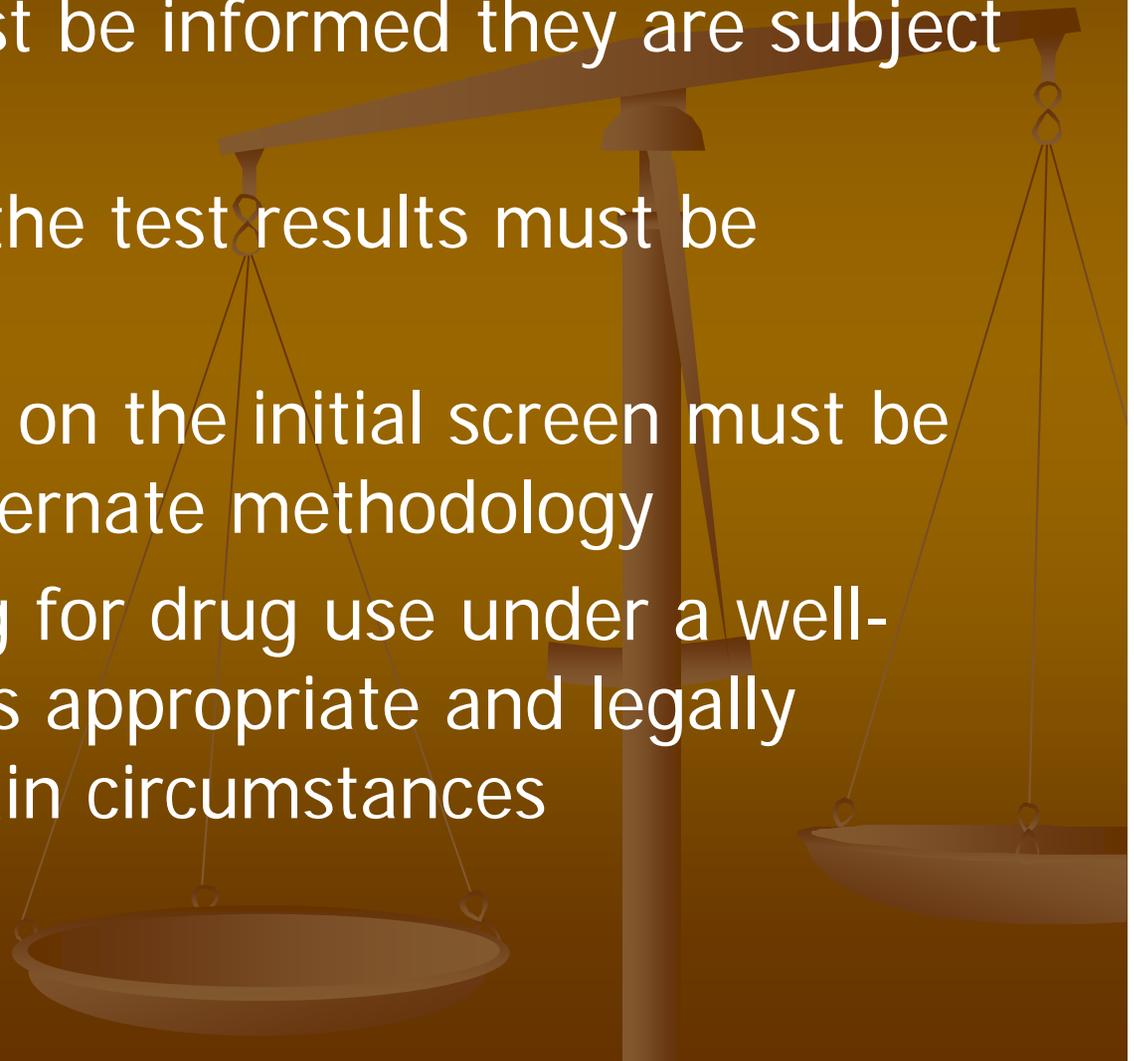


Benefits and Limitations of Testing Methodologies and Samples



Testing Conditions

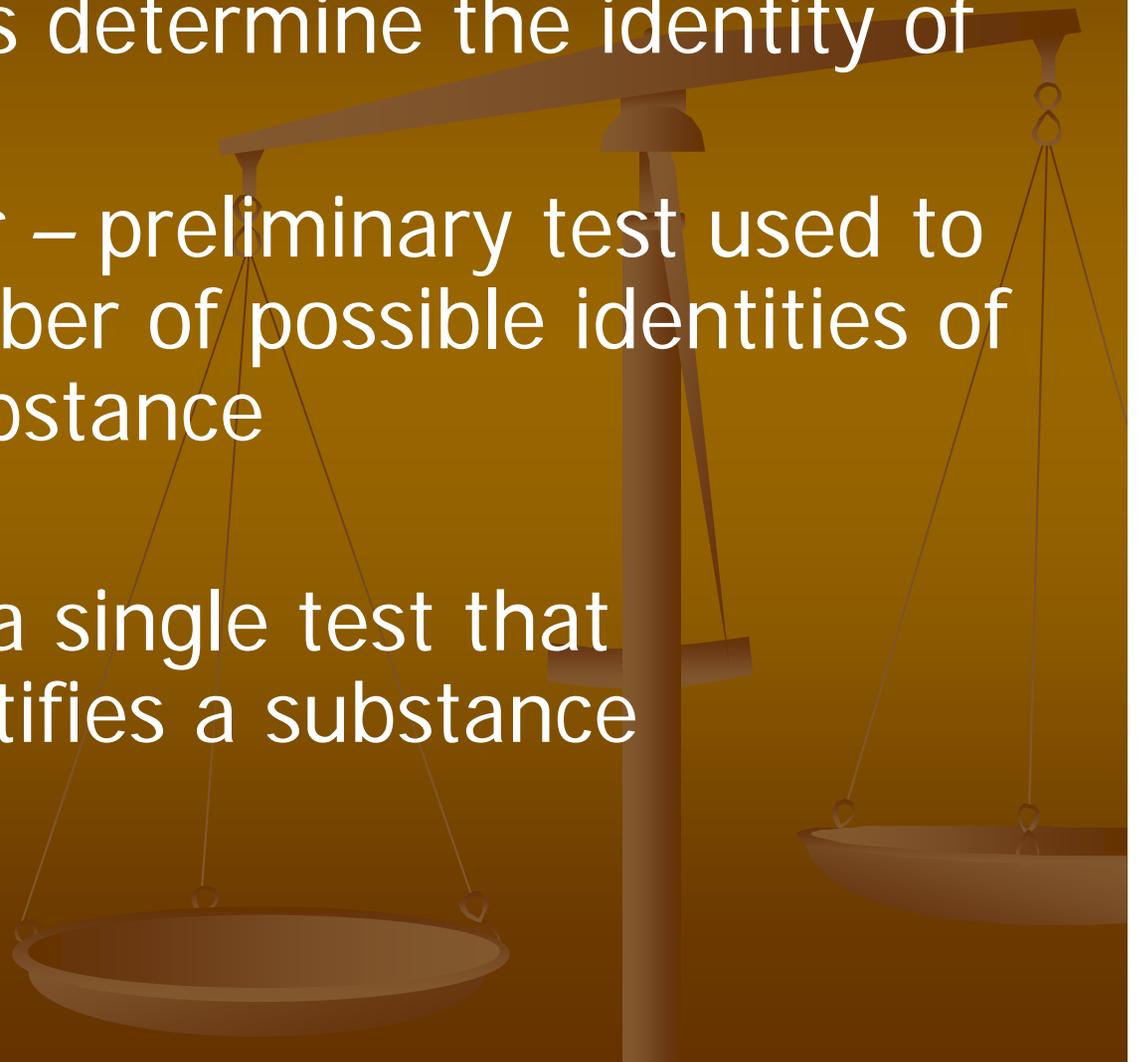
- All individuals must be informed they are subject to testing
- Confidentiality of the test results must be assured
- All positive results on the initial screen must be confirmed with alternate methodology
- Random screening for drug use under a well-defined program is appropriate and legally defensible in certain circumstances



Screening and Confirmation

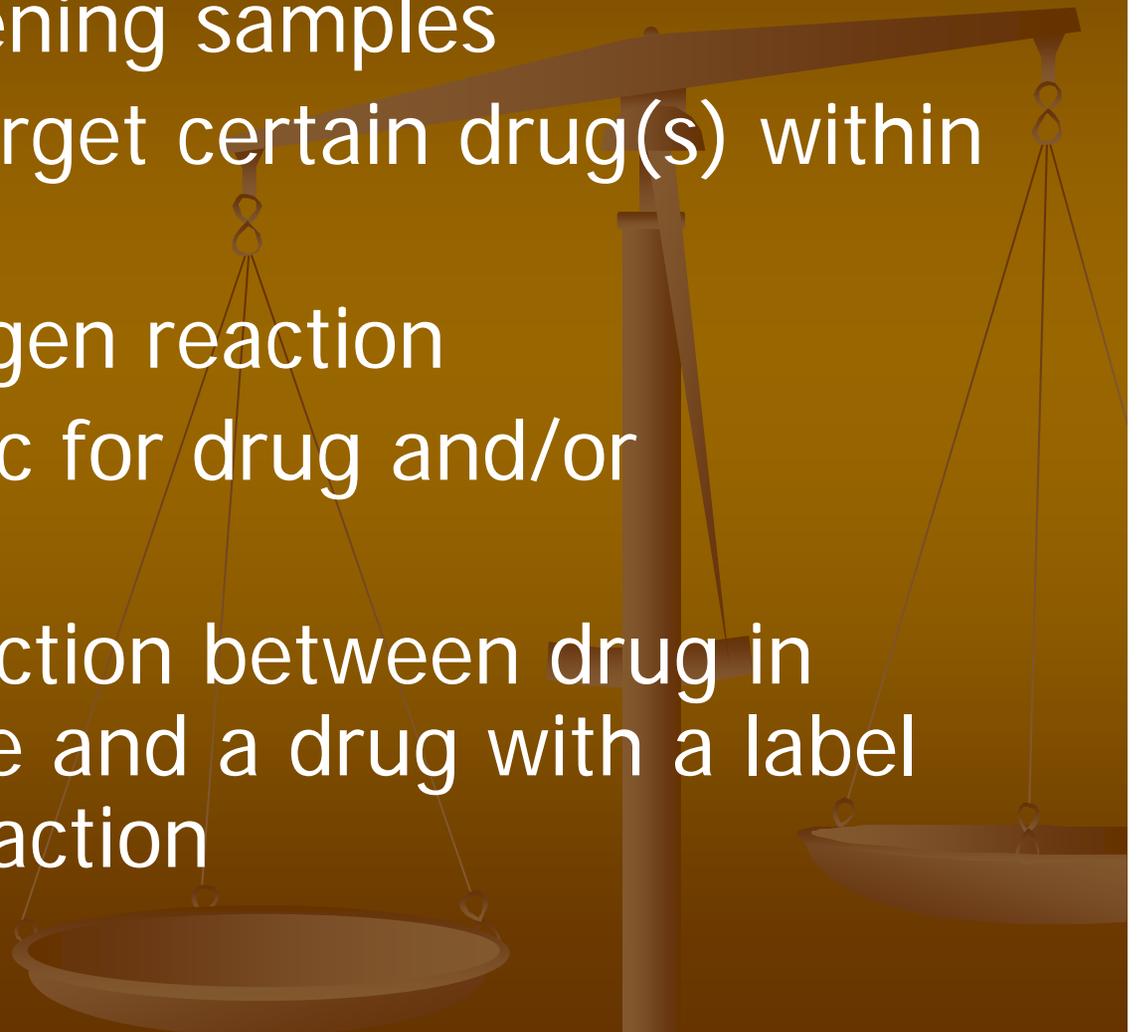
Forensic Scientists determine the identity of drugs using...

- *Screening Tests* – preliminary test used to reduce the number of possible identities of an unknown substance
 - Immunoassay
- *Confirmation* – a single test that specifically identifies a substance
 - GC/MS



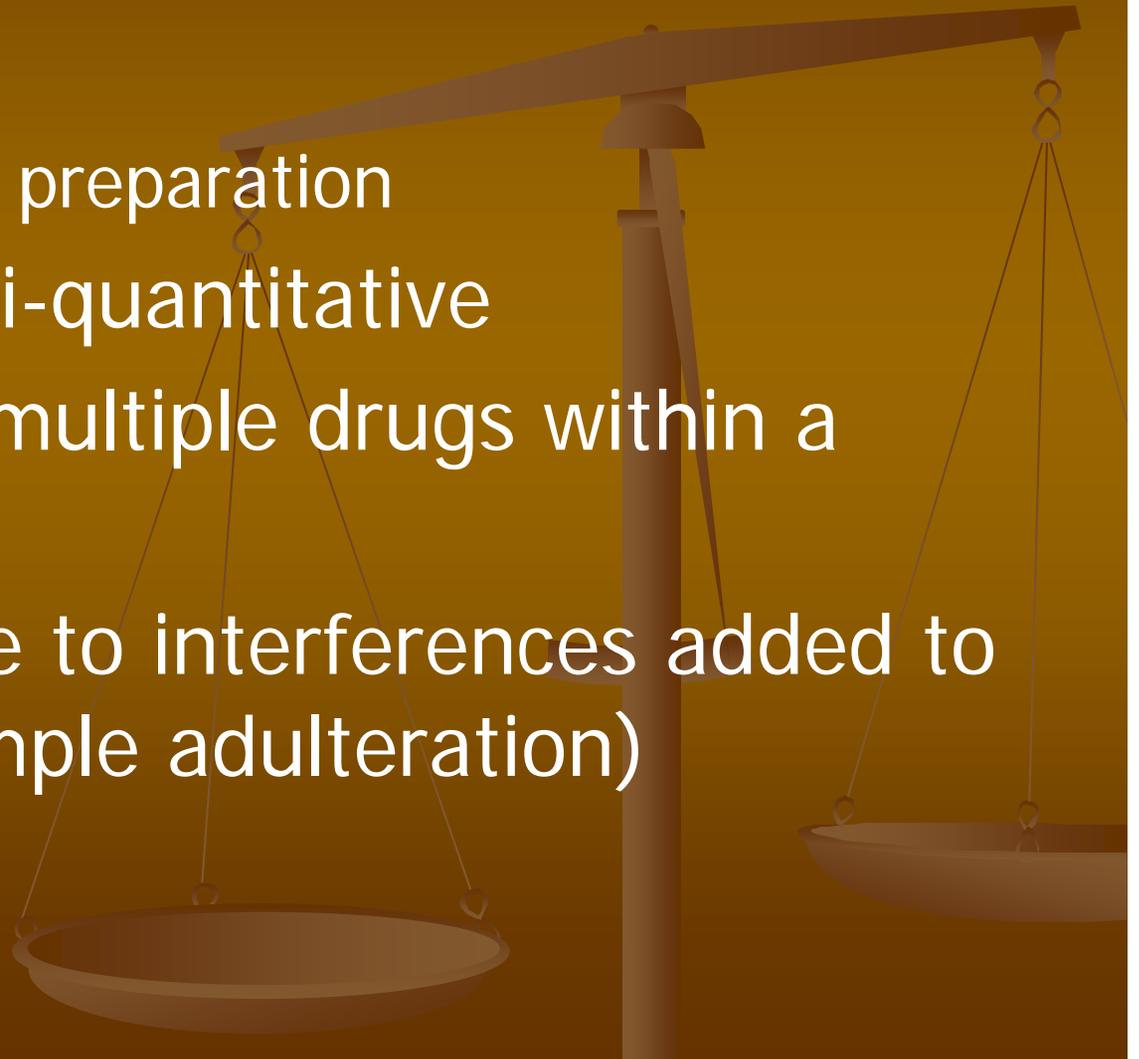
What is an Immunoassay?

- Method of screening samples
- Developed to target certain drug(s) within each class
- Antibody – Antigen reaction
- Antibody specific for drug and/or metabolites
- Competitive reaction between drug in collected sample and a drug with a label added to the reaction



General Immunoassay Information

- Drug(s) do not need to be extracted from the sample
 - Minimal sample preparation
- Results are semi-quantitative
- May react with multiple drugs within a class of drugs
- May be sensitive to interferences added to the sample (sample adulteration)

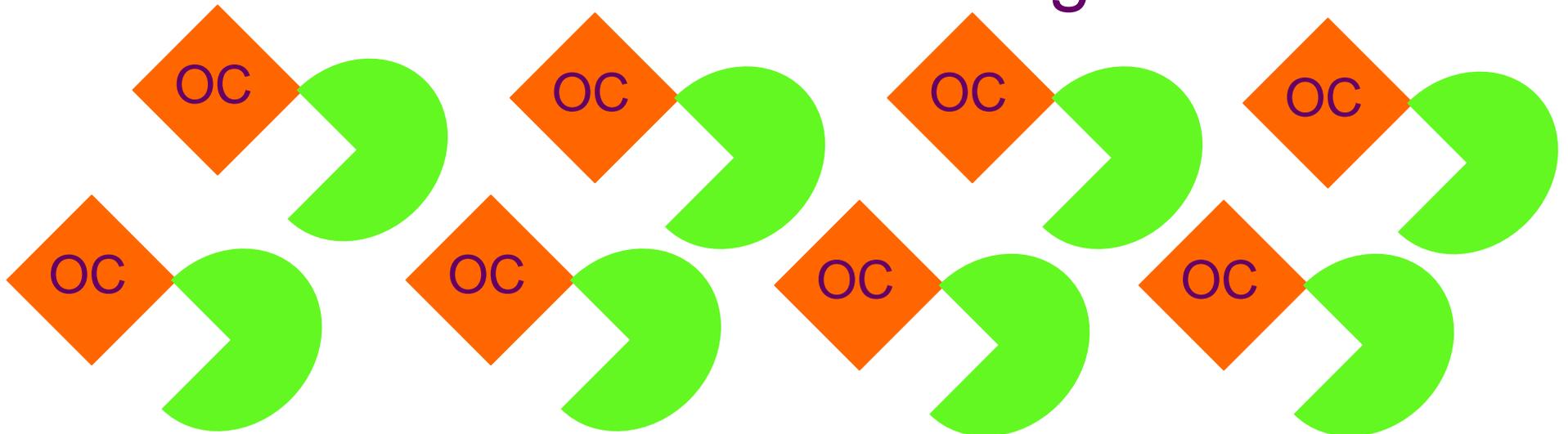


Parts of an immunoassay for Oxycodone

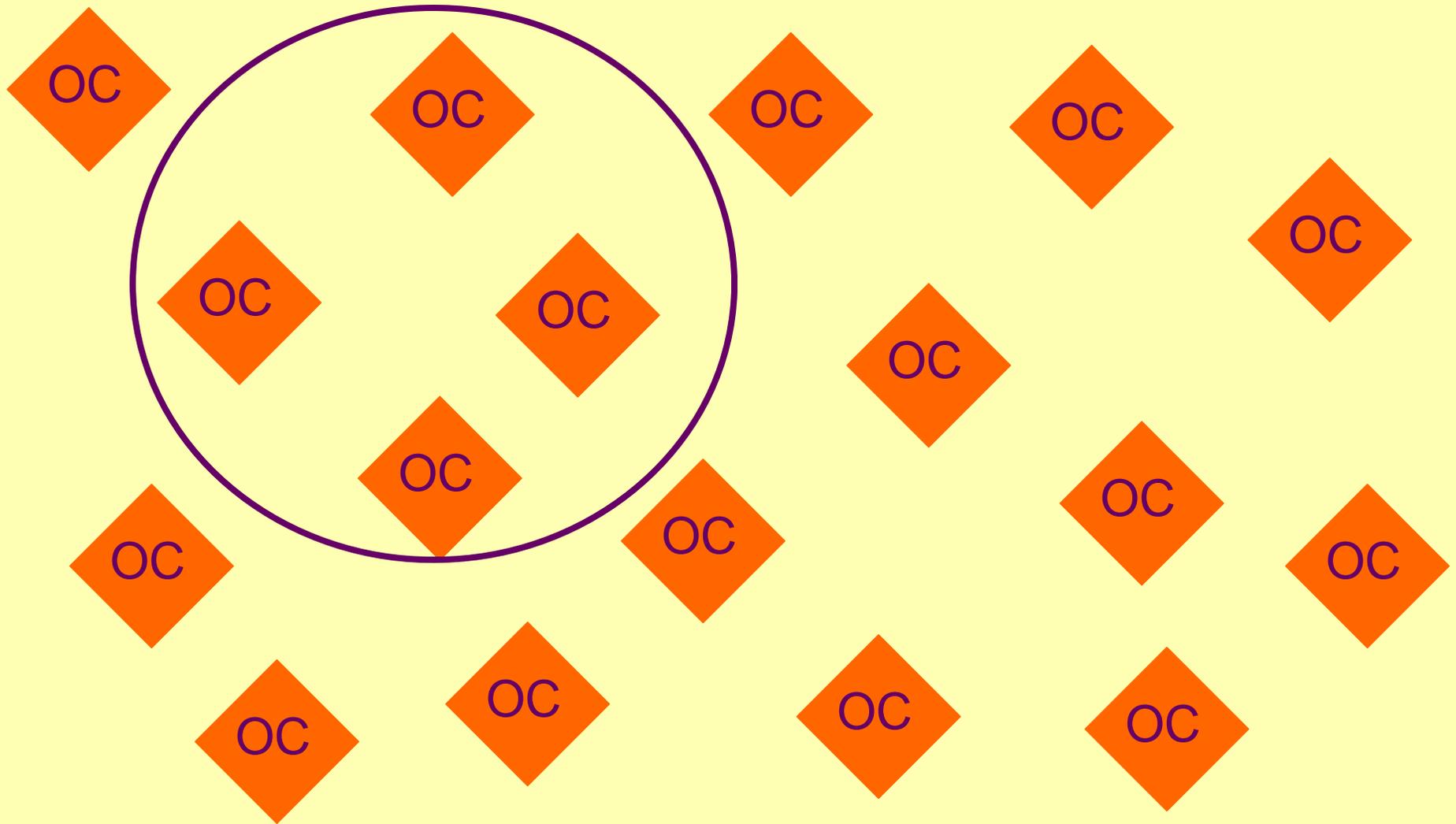
Antibodies that recognize Oxycodone



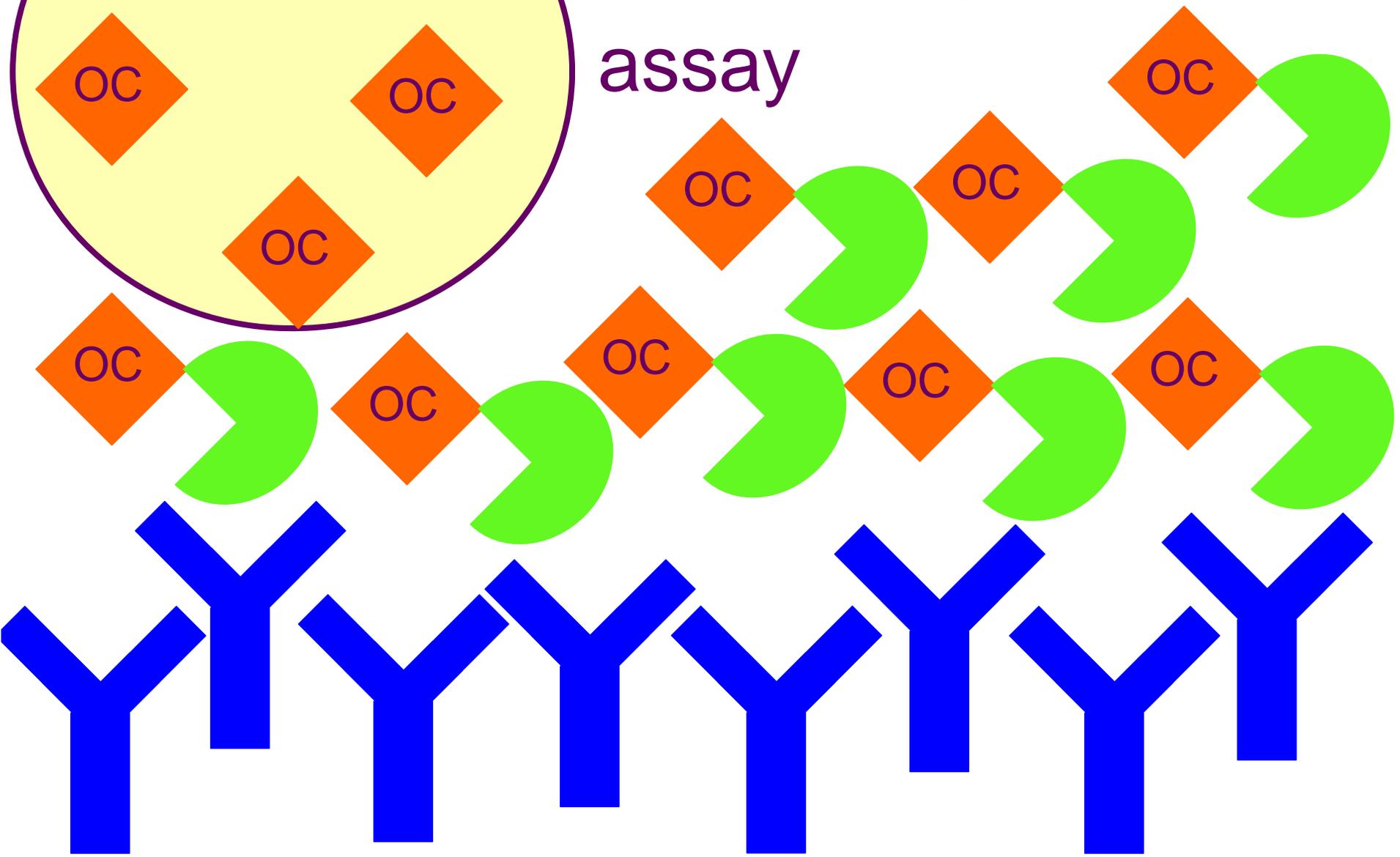
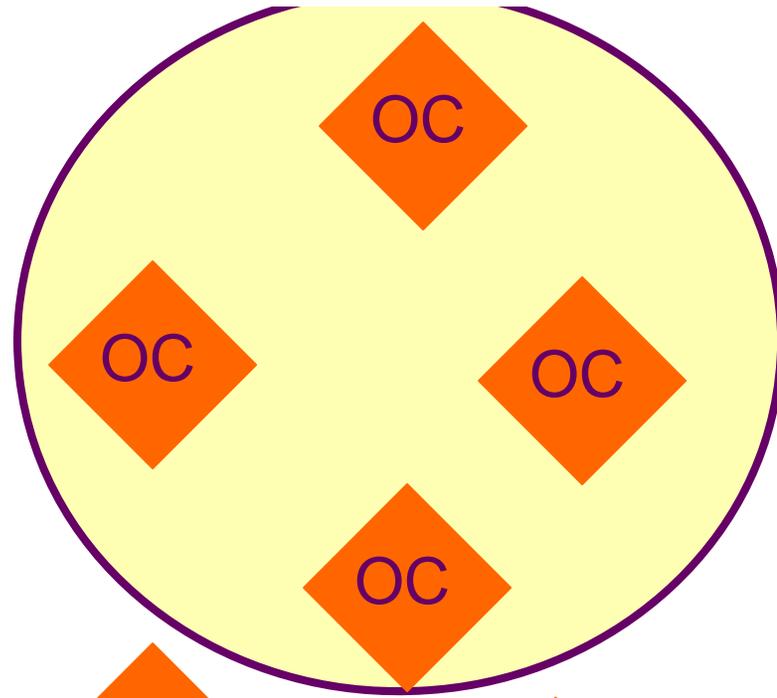
Oxycodone molecules bound to an enzyme that makes a color change occur



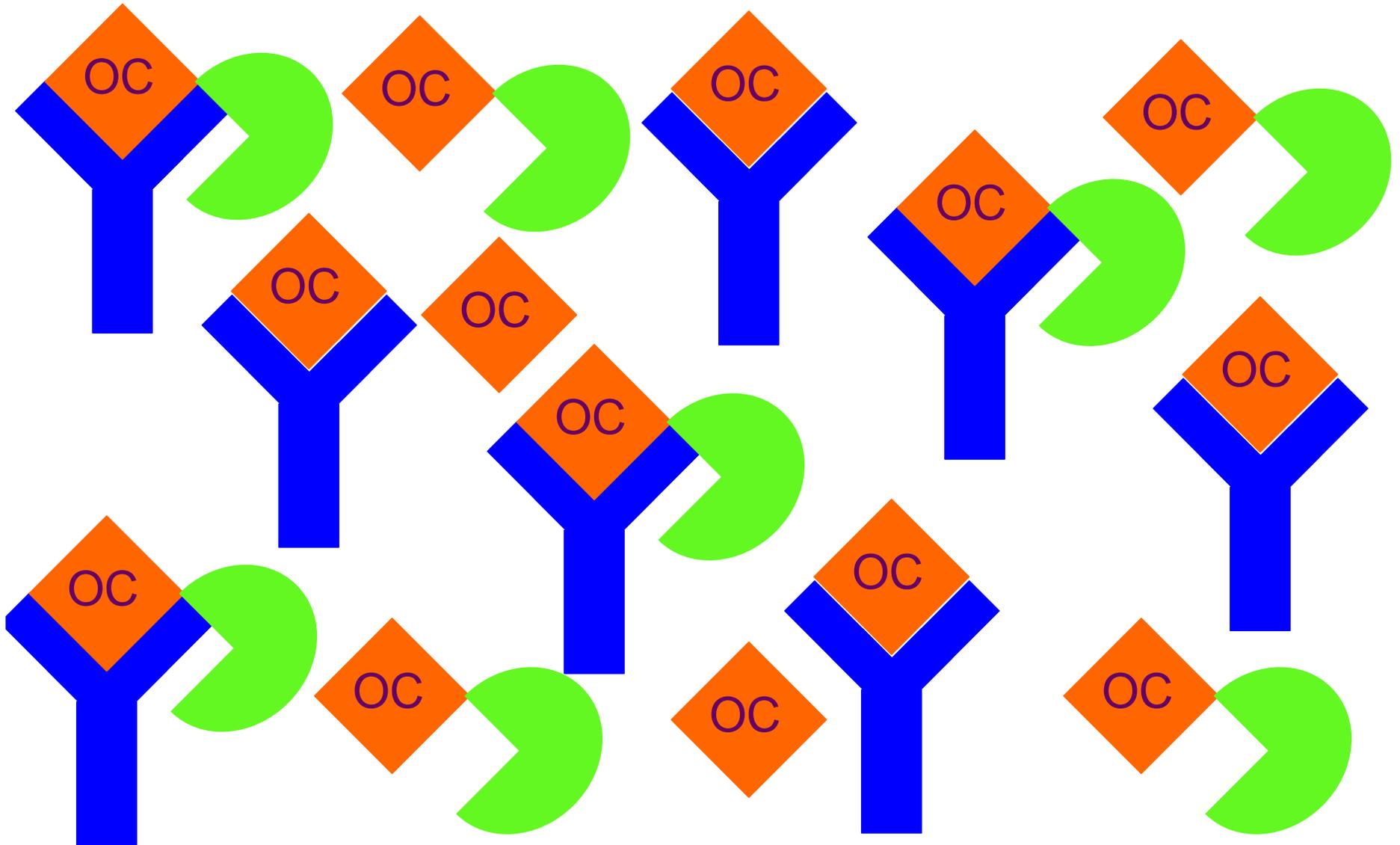
Molecules of Oxycodone in Urine to be sampled



Add a small sample of urine to the immunoassay

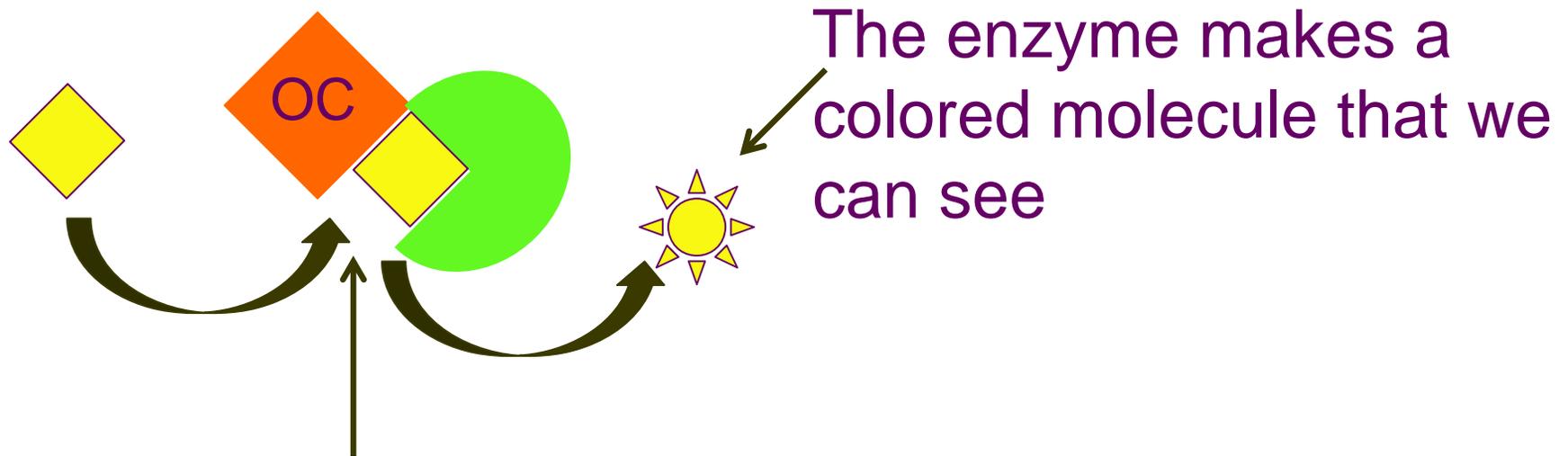


Incubate sample for a bit.



Added to the mixture...

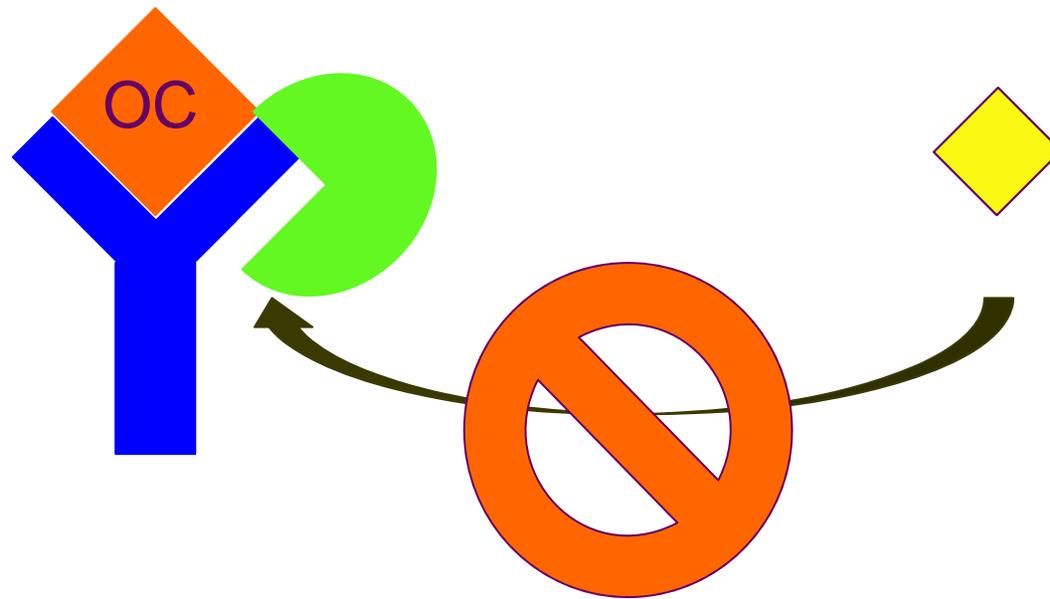
 = Molecule that “fits” into enzyme



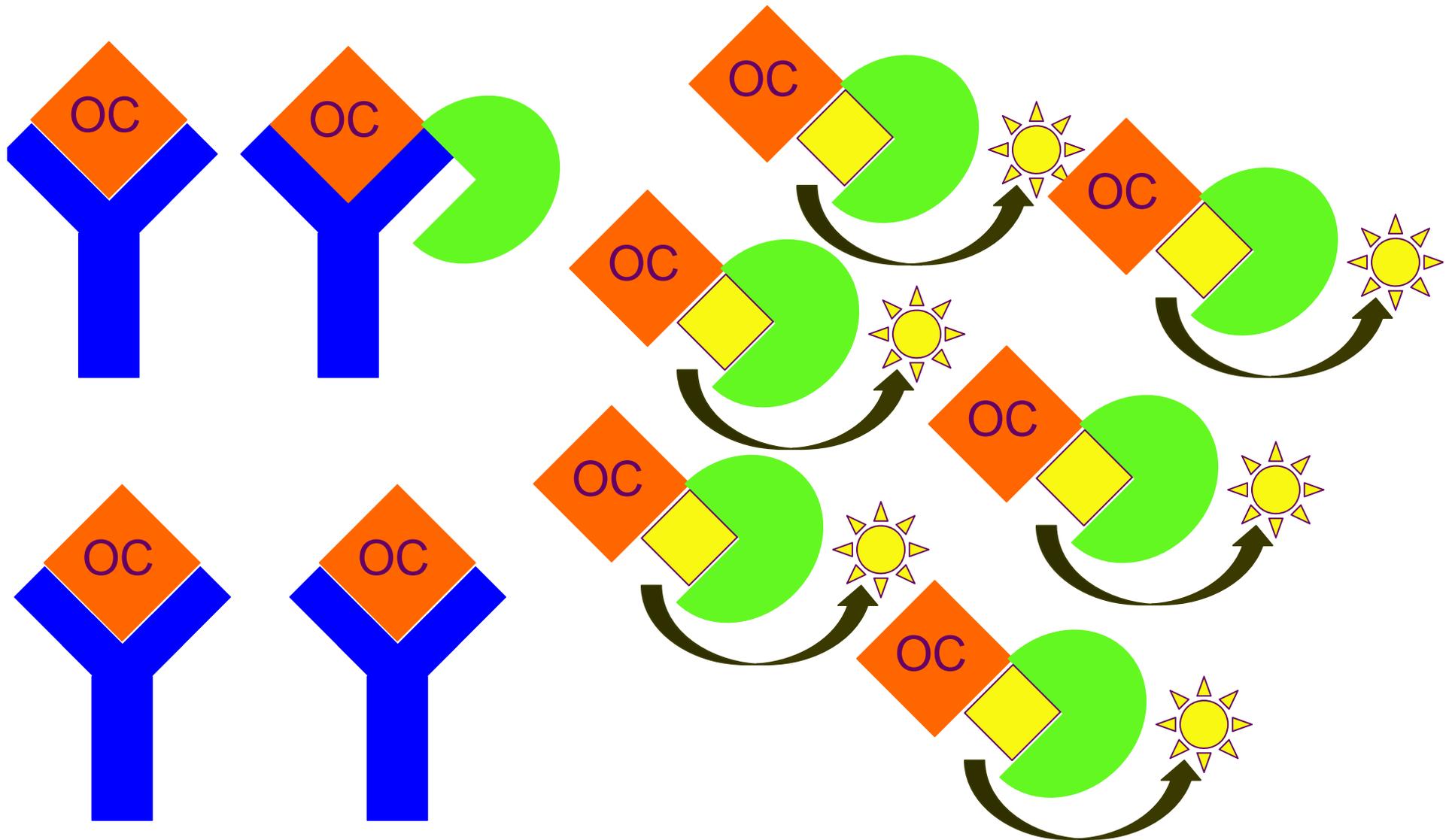
The enzyme makes a colored molecule that we can see

Will be taken up by the enzyme

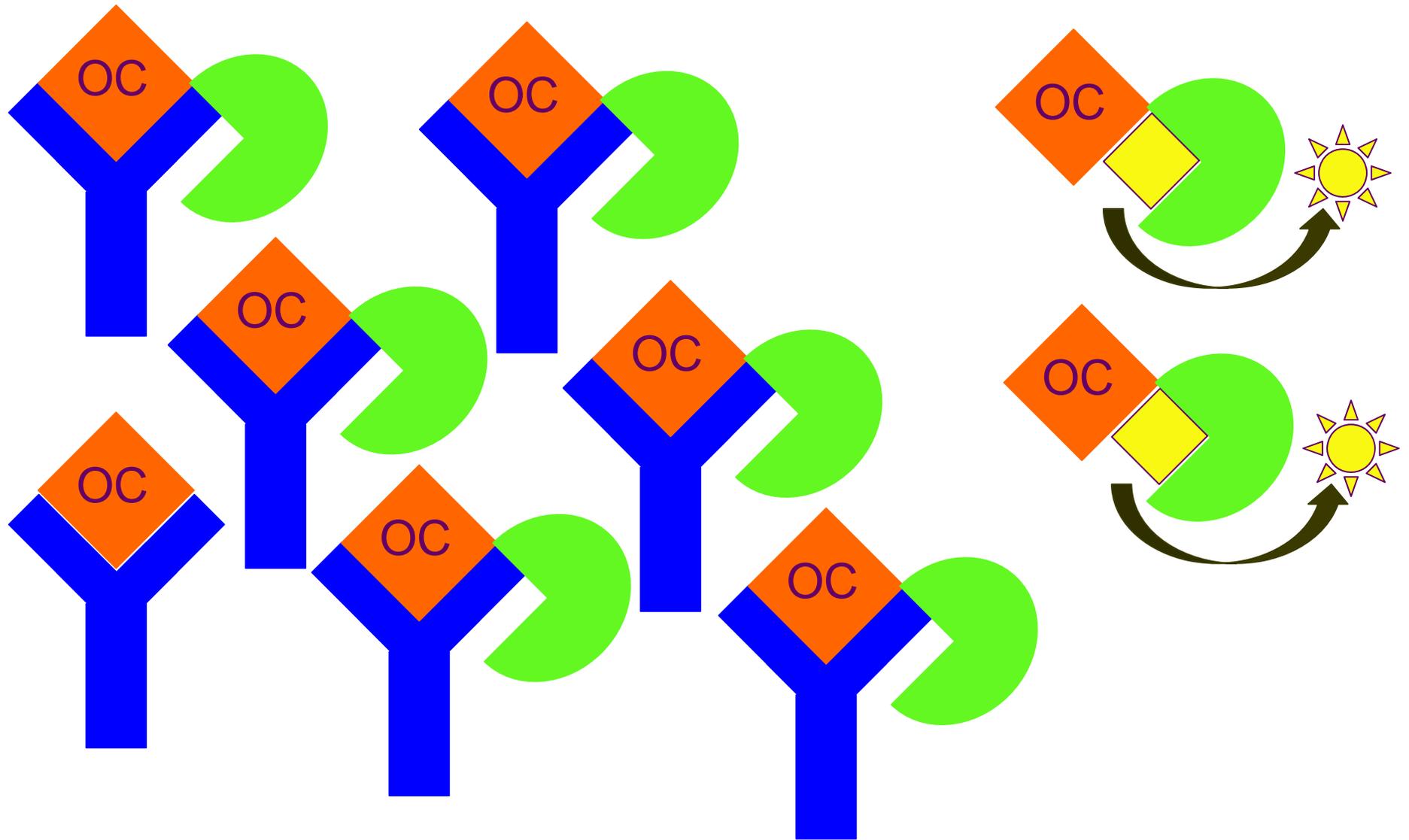
Color change cannot happen if enzyme is blocked by antibody



Lots of Oxycodone in Urine....



Not Much Oxycodone in Urine....



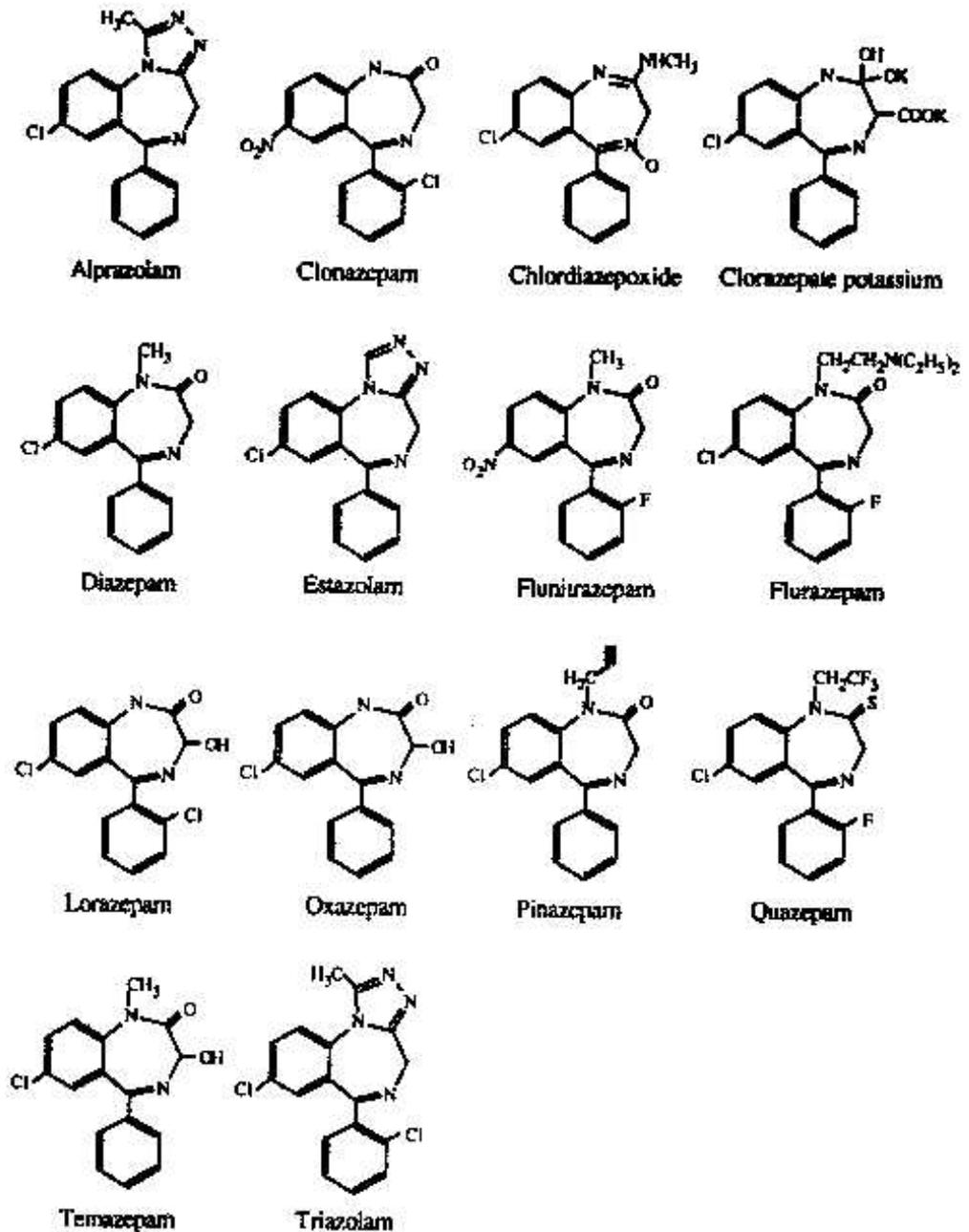
Target Analytes

Drug/Drug Class	Target Analyte
Amphetamines*	Amphetamine/Methamp.
Cannabinoids*	Carboxy-THC
Cocaine*	Benzoylecgonine
Opiates*	Morphine
Phencyclidine (PCP)*	PCP
Benzodiazapines	Nordiazepam/oxazepam
Barbiturates	Secobarbital
LSD	LSD
Methadone	Methadone
Propoxyphene	Propoxyphene

* represents drugs that are mandated by the Federal Government for "Sensitive Occupations"

Referred to as "NIDA 5" or "DHHS 5"

Immunoassays Can Work for a Class of Drugs



Structure 1. Structures of selected benzodiazepines.

Positive Immunoassay Results Must be Confirmed

- Cut-off levels differ between DHHS and DOD

	HHS	DoD	(ng/mL)
Amphetamines	1000	500	
Barbiturates	*	200	
Cannabinoids	50	50	
Cocaine metabolite	300	150	
PCP	25	25	
Opiates	300	2000	
LSD	*	0.5	

* Drug class not included in this program

- Confirmation of Positives (above cut-off)
 - Isolation of drugs/metabolites by extraction
 - Identification: specific drug of abuse
 - Quantitation: amount detected

A typical immunoassay instrument and results



S.No : 16 TYPE :Urine
(2008-10-23 10:46:17)
ID [3301]
NAME[08-3301 urine]

ITEM	RESULT	NR	M S	RC#
AMPH	0			7553
BARB	0			7554
BENZ	2359	High		7555
COCM	0			7556
MTDN	0			7557
OPIT	0			7558
OXYC	0			7559
PPX	0			7560
THC50	78	High		7561
BUPRE	0.8			7562
TTCA*	0.0			7563

S.No : 6 TYPE :Serum
(2008-10-23 09:33:47)
ID [3191]
NAME[3191 hosp serum a]

ITEM	RESULT	NR	M S	RC#
AMPH	0			7467
BARB	206	High		7468
BENZ	589	High		7469
COCM	0			7470
MTDN	0			7471
OPIT	789	High		7472
OXYC	0			7473
PPX	0			7474
BUPRE	0.0			7475
TTCA*	0.0			7476

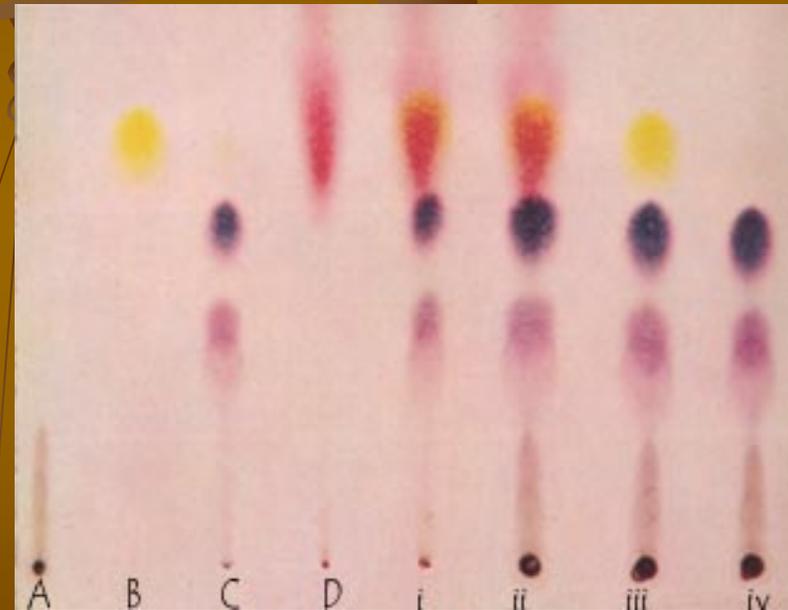
Confirmation by GC-MS

- GC - Gas chromatography
- MS - Mass spectrometry

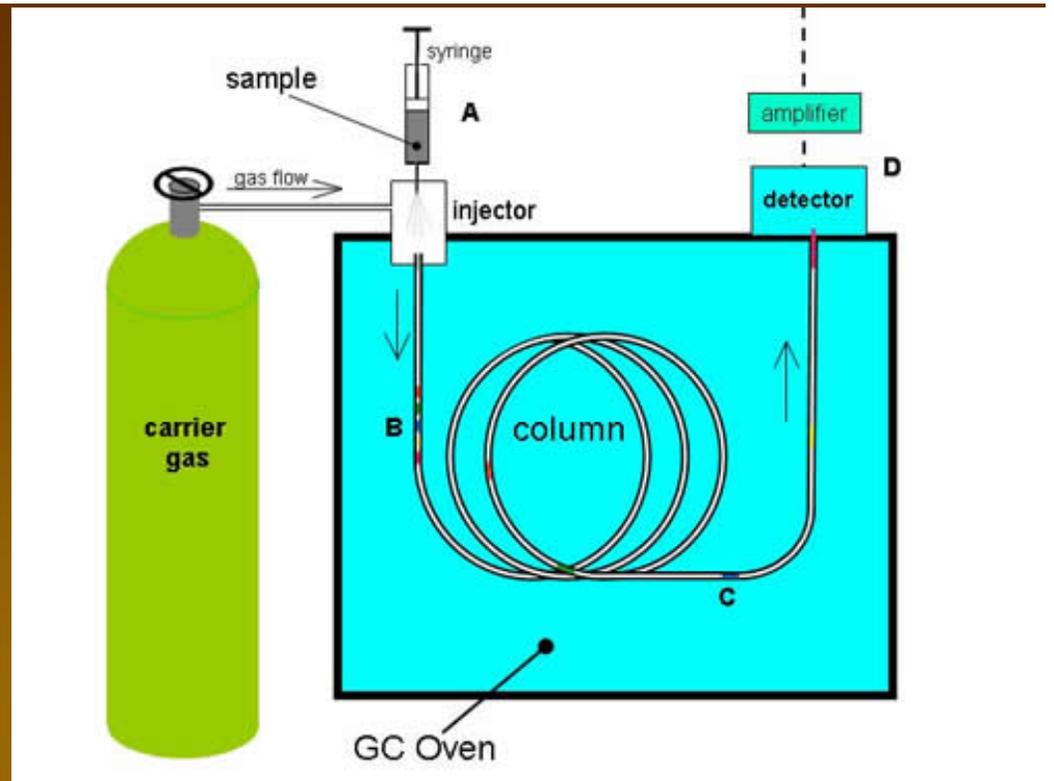


Chromatography

- An analytical technique used to separate the components of a mixture for the purpose of identification and quantification.
- Useful to forensic scientists because drugs often come in the form of a mixtures

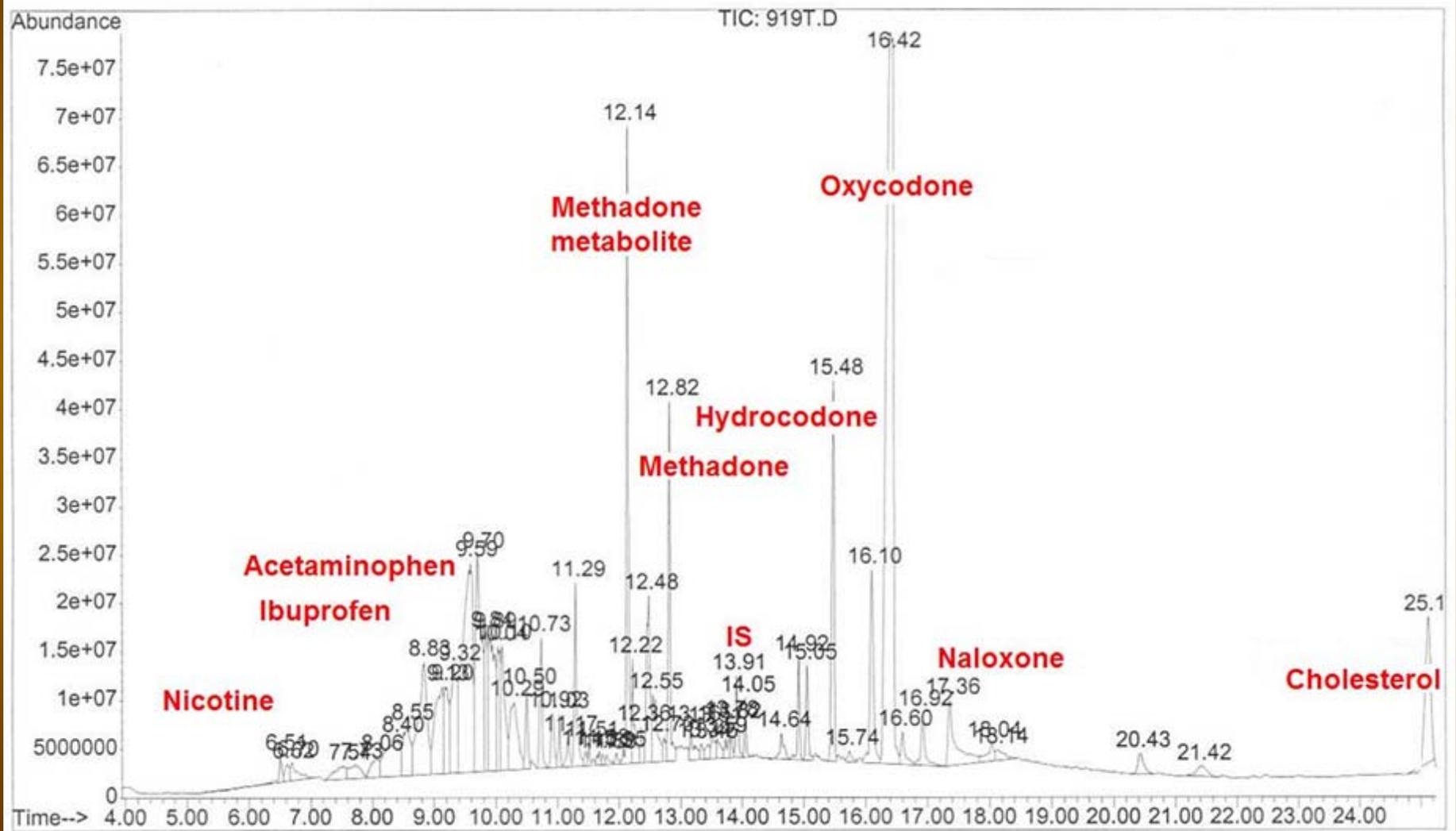


What a GC Looks Like Inside



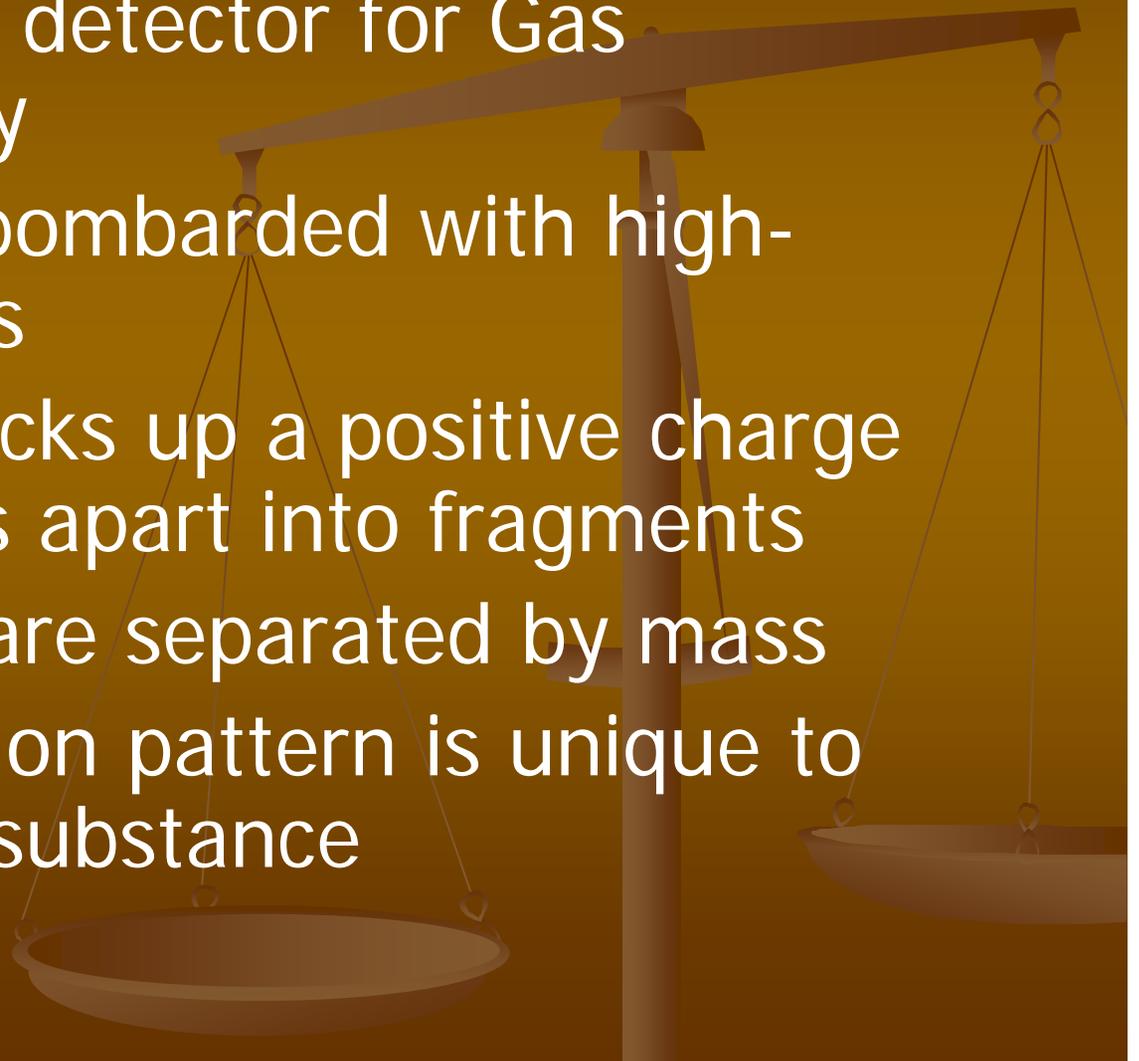
- Separates molecules based on
 - Size
 - Chemical properties
- Information provided
 - Retention time – how long the drug molecules stay in the instrument

What GC Data looks like

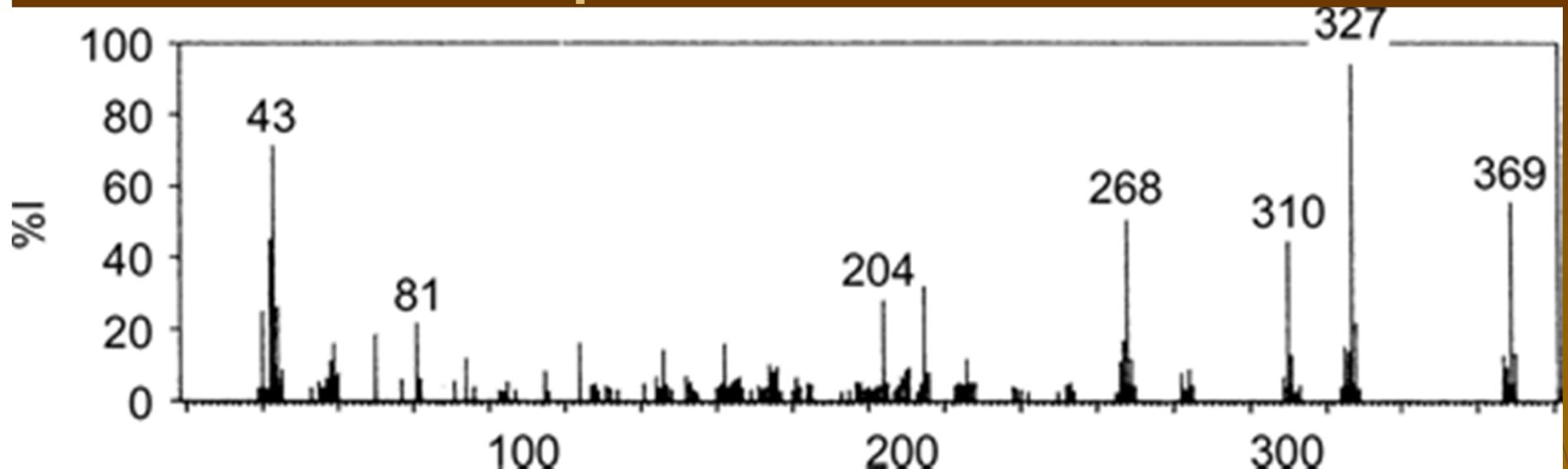


Mass Spectrometry

- Often used as a detector for Gas Chromatography
- A substance is bombarded with high-energy electrons
- The molecule picks up a positive charge and then breaks apart into fragments
- The fragments are separated by mass
- The fragmentation pattern is unique to each individual substance

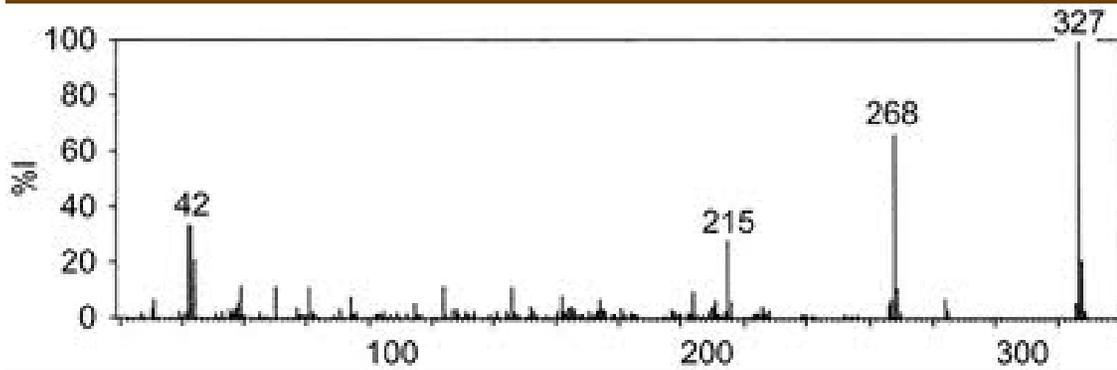


Mass spectrum of Heroin

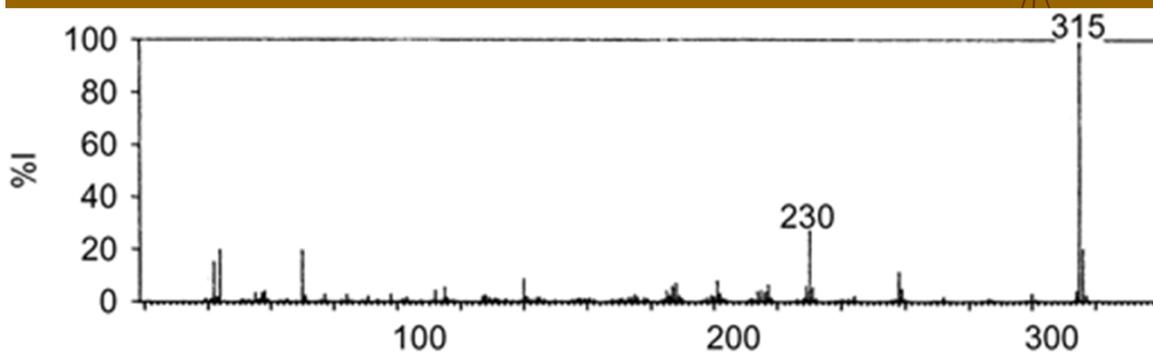


- Each line on the graph represents pieces of the heroin molecules that came out of the GC
- What forensic scientists look for
 - Are all the correct pieces present?
 - Are they there in the relative amounts expected?

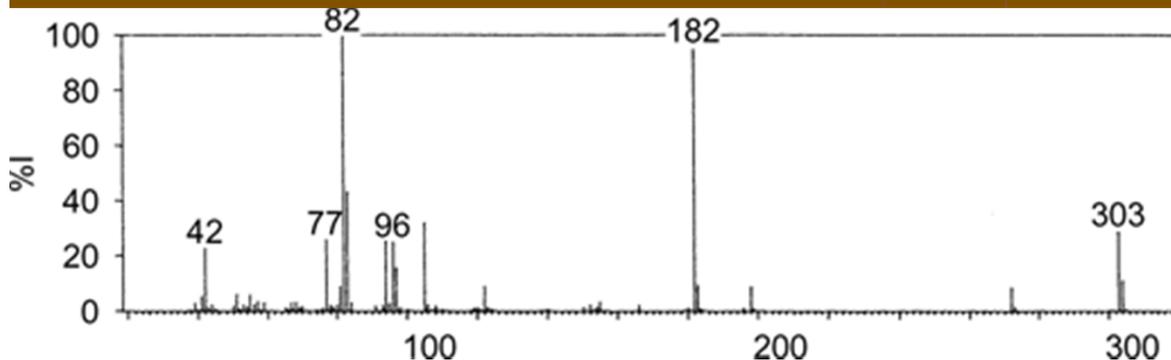
Mass Spectra of a few Drugs of Abuse



■ 6-monoacetylmorphine



■ Oxycodone

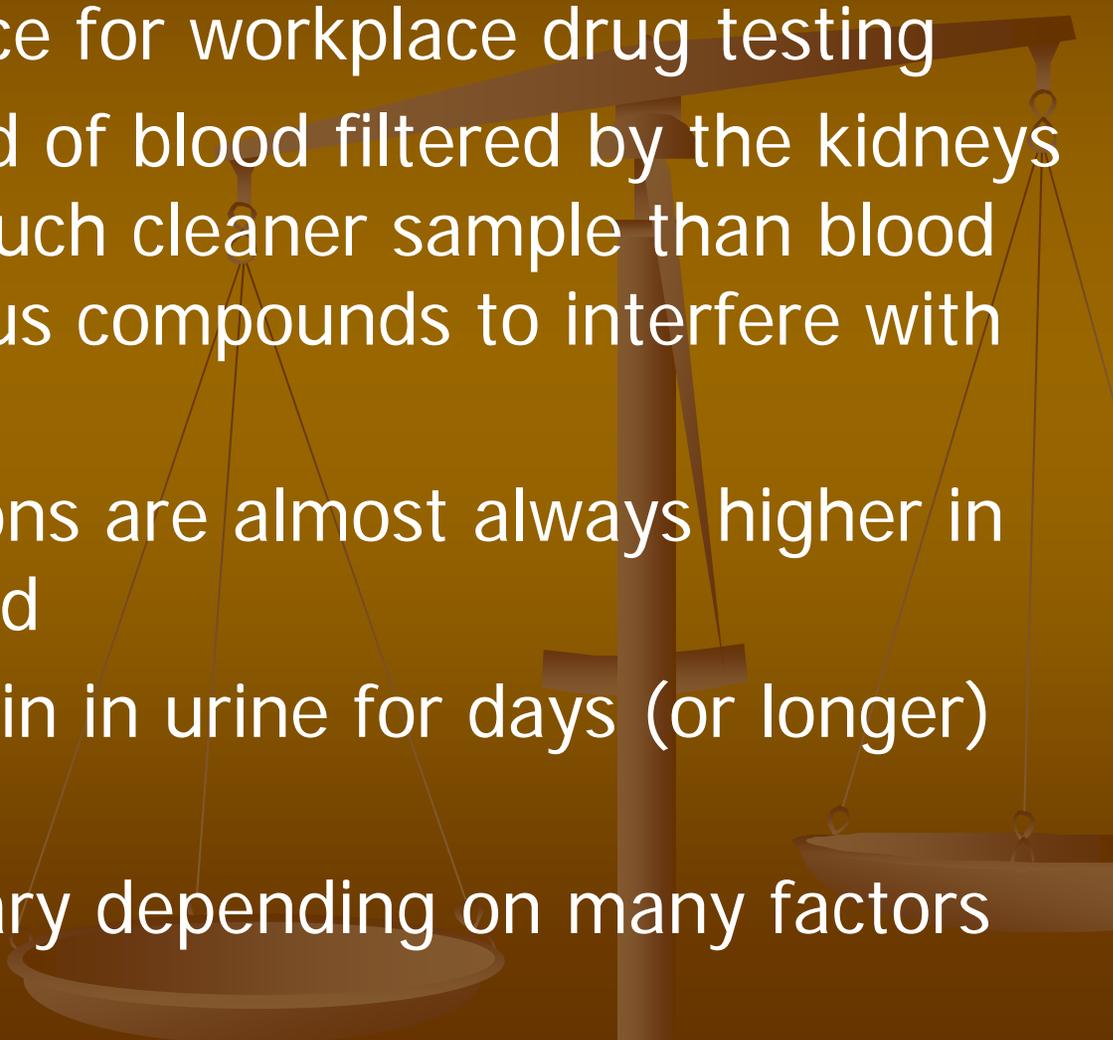


■ Cocaine

Samples used for Forensic Drug Testing



Urine



- Specimen of choice for workplace drug testing
- Urine is composed of blood filtered by the kidneys and presents a much cleaner sample than blood (fewer endogenous compounds to interfere with detection)
- Drug concentrations are almost always higher in urine than in blood
- Some drugs remain in urine for days (or longer) after use
- Concentrations vary depending on many factors

Urine

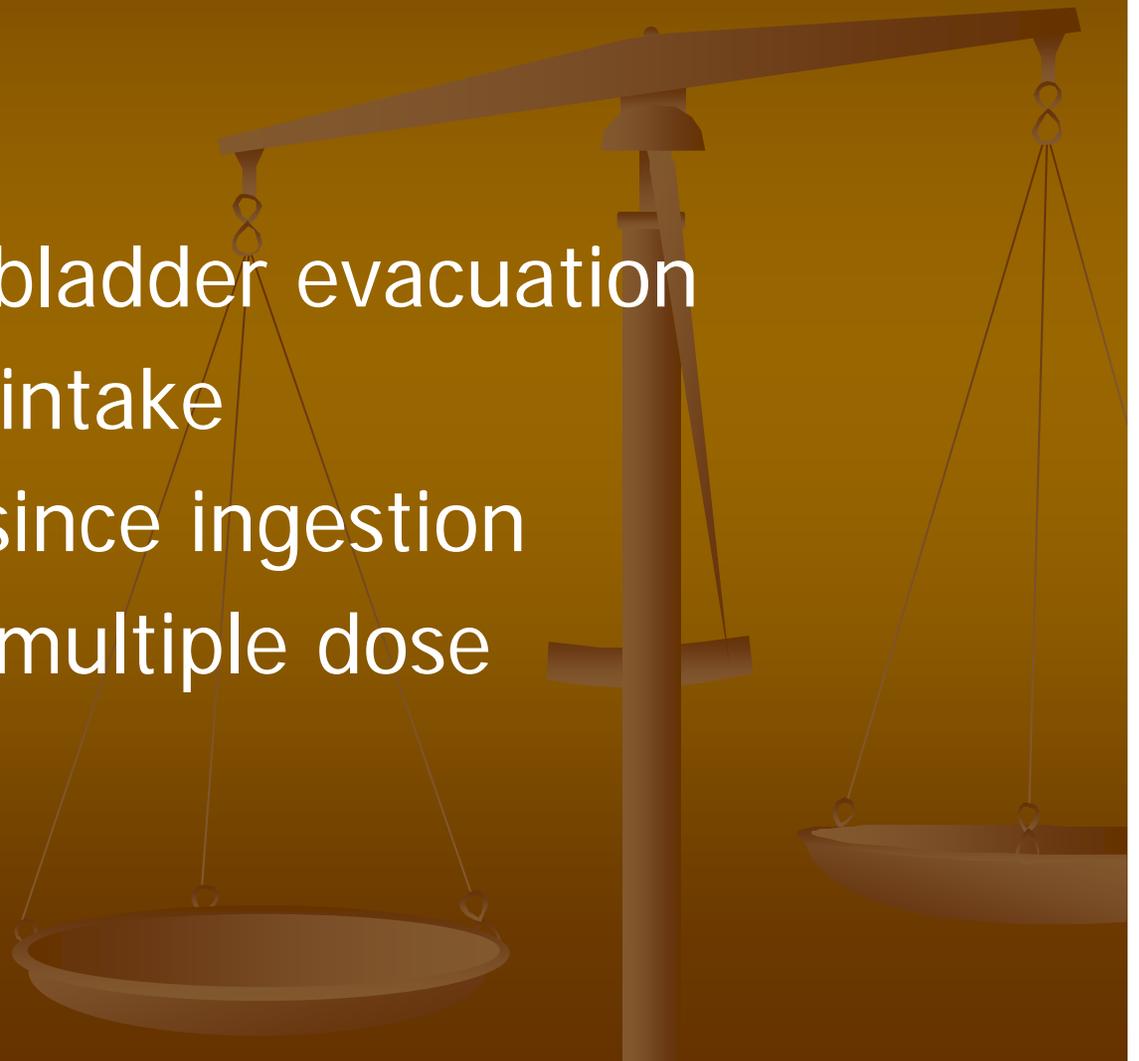
- Helpful in finding drugs that are difficult to detect in blood
- Drug quantitations in urine are almost always meaningless
- Urine drug concentrations should **NOT** be used to imply a level of impairment
- The presence of a drug in the urine indicates what drug(s) to look for and quantitate in the blood

**URINE
SPECIMEN**



Factors Complicating Urine Drug Concentration Interpretation

- Urine flow
- Urine pH
- Time since last bladder evacuation
- Volume of fluid intake
- Dose and time since ingestion
- Single dose vs. multiple dose
- Kidney function
- Liver function



Detection Times of Common Drugs of Abuse in Urine

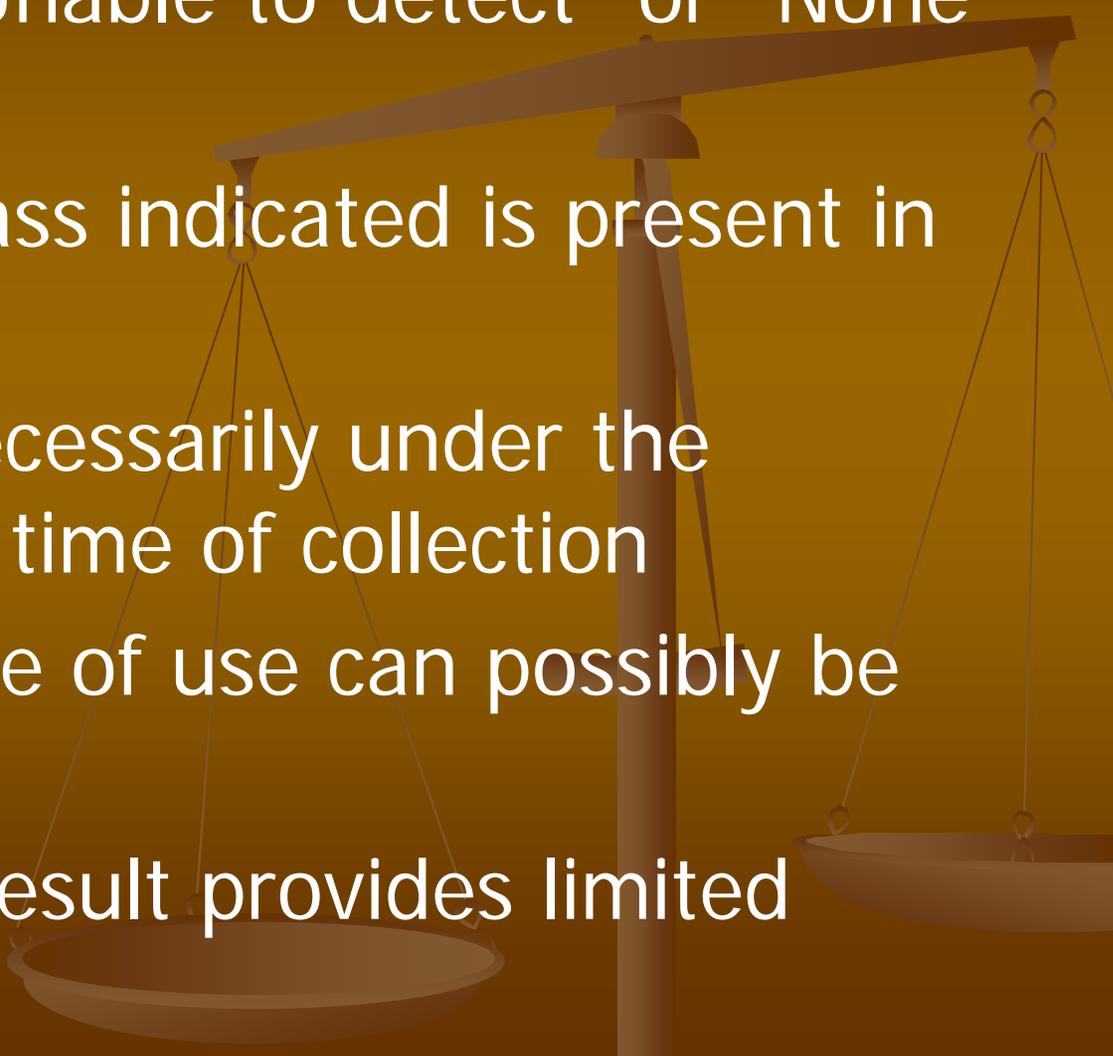
Approximate Detection Times Of Some Common Drugs Of Abuse In Urine

T3

Drugs	Duration of Detection in Urine
Alcohol	up to 1 day
Amphetamines (including MDMA, MDA)	1-2 days
Barbiturates	1-3 days
Benzodiazepines	Up to 21 days
Cannabinoids	Up to 60 days*
Cocaine	1-3 days
Methadone	1-3 days
Opiates (including codeine and morphine)	1-3 days
Propoxyphene	1-3 days

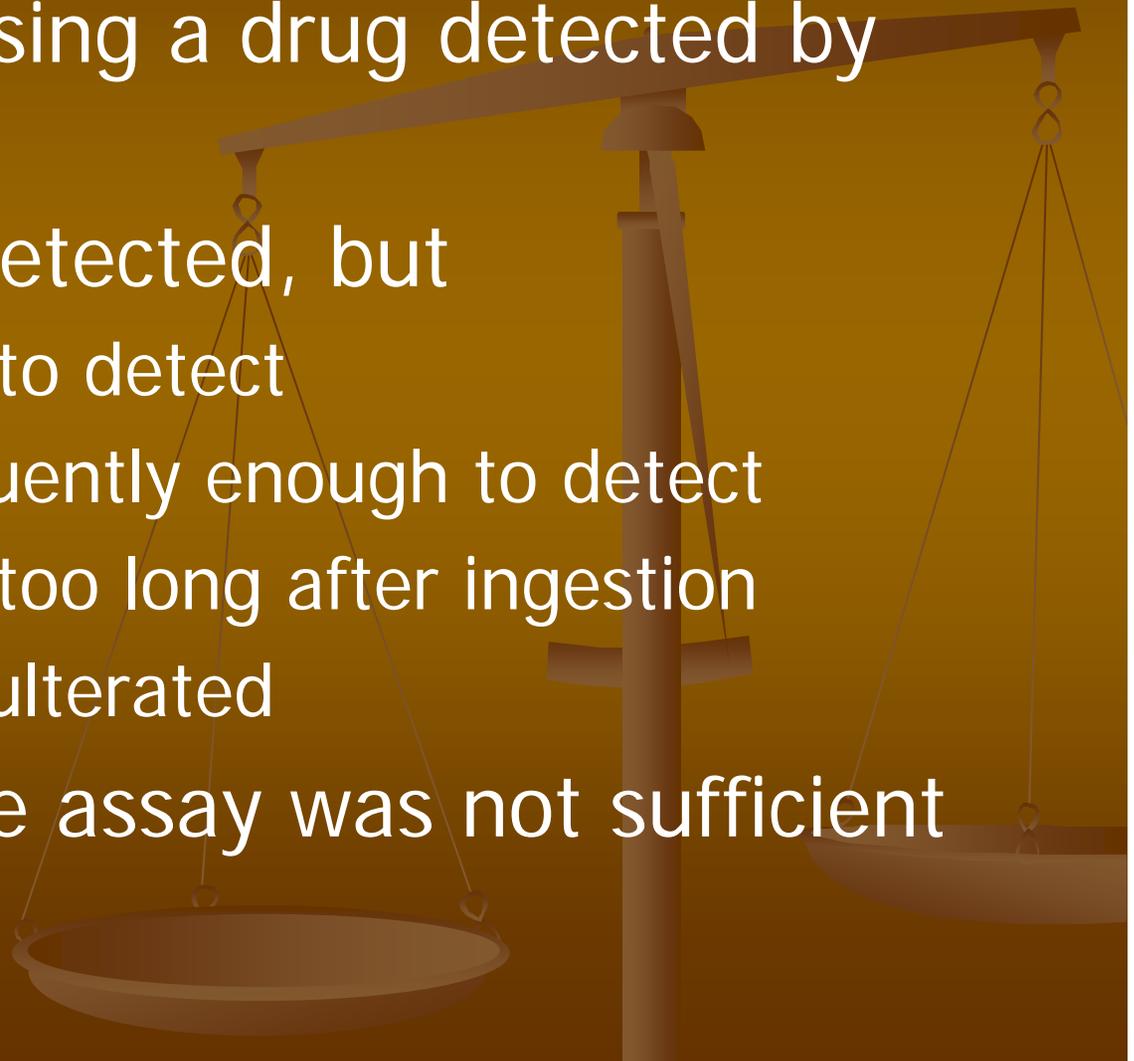
**At 50 ng/mL cutoff concentration*

Positive Result Interpretation

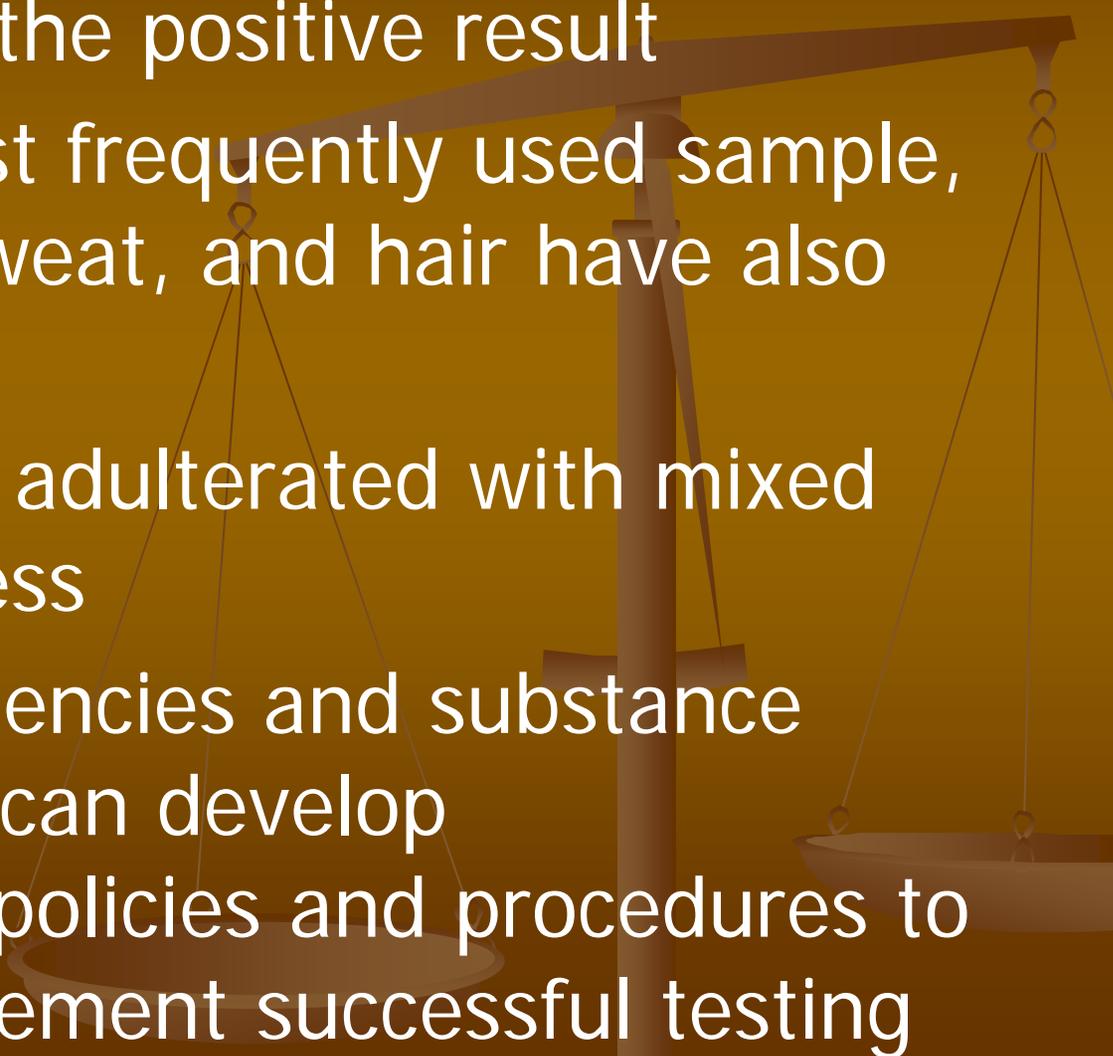
- Usually reads “Unable to detect” or “None detected”
 - Drug or drug class indicated is present in the urine
 - Person is not necessarily under the influence at the time of collection
 - Some time frame of use can possibly be provided
 - Single positive result provides limited information
- 

Negative Result Interpretation

- Subject is not using a drug detected by test
- Taking a drug detected, but
 - Dose too small to detect
 - Not taking frequently enough to detect
 - Urine collected too long after ingestion
 - Sample was adulterated
- Sensitivity of the assay was not sufficient



Summary:

- Testing involves a screening step and the confirmation of the positive result
 - Urine is the most frequently used sample, but oral fluid, sweat, and hair have also been used
 - Samples can be adulterated with mixed results for success
 - Child welfare agencies and substance abuse agencies can develop comprehensive policies and procedures to design and implement successful testing
- 

QUESTIONS???

