Immunoassays as an Initial Test in Drug Testing

John M. Mitchell, Ph.D.
Center for Forensic Sciences
RTI International

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Presentation Goals

- To enhance understanding of immunoassay tests used for the detection of drugs
- To enhance understanding of results reported from immunoassay testing

Drug Testing Systems

- One Test Systems
  - Use immunoassay (IA) to detect the presence of drugs and
  - Are most often used for medical purposes in clinical and rehabilitation settings
- Two Test Systems
  - Commonly use immunoassay as an initial test followed by a more specific confirmatory test using a different technology and
  - Are used for results requiring a high level of certainty such as in forensic workplace testing

What is an Immunoassay?

- An *immunoassay* is a biochemical test that measures the concentration of a substance in a liquid (a portion of a biological specimen) using the reaction of an antibody or antibodies to its antigen (drug)

What are Antibodies and Antigens?

- Antibodies are a type of protein produced by the immune system in response to foreign substances (antigens)
- Antibodies bind to the antigen responsible for their production

Immunoassay Principles I

Antibodies:

- Usually harvested from sheep or rabbits
- Usually IgG (represented as a “Y”)
- Developed against classes of drugs
- Recognize antigen (drug) by its shape

Immunoassay Principles II

- Use Tagged Drug Targets
  - Indicator (Tag) is bound to the target drug
  - “Tag” may be
    - An enzyme
    - A fluorophore
    - A particle
- Detection is based on competitive binding
  - Antibodies bind with Drug in Sample OR with Tagged Drug Targets

Immunoassay principles

- Sample WITH drug in it
- Add an antibody
- Incubate

Immunoassay Principles III

- Antibody binds drug in the sample
- Add tagged drug target
- Little or no antibody binding of the tagged compound
- Little or no change in signal

Immunoassay Principles IV

- Sample WITHOUT drug in it
- Add an antibody
- Incubate
- Add tagged drug target

Immunoassay Principles

- Antibody binds with the tagged compound
- Binding causes change in signal produced

Testing with Immunoassays

- Immunoassays are used to screen donor specimens for the possible presence of a drug or a class of drugs
- These tests may be conducted on-site as part of the collection process or in a laboratory or other facility
- On-site testing is normally conducted with a Point of Collection Test (POCT) device

Testing with Immunoassays

- Most testing is conducted in laboratories which have validated procedures and validated analytical instrumentation
- Immunoassays allow some laboratories to test more than 10,000 donor specimens a day

Immunoassay Specificity

- Specificity is the affinity of an immunoassay for the target drug
- Specificity is measured by cross reactivity:
  - the response exhibited when an immunoassay reacts with a compound other than the target drug
- Specificity limits the conclusions that can be drawn from immunoassay results

Low Specificity

- An immunoassay with low specificity will react with many antigens (drugs) with similar structure
- Example: One Amphetamine immunoassay with a cutoff of 300 ng/mL of D-Amphetamine gives a positive result with:
  - D,L-Amphetamine (300 ng/mL), Phentermine (400 ng/mL), Tranylcypromine (500 ng/mL), Methamphetamine (1,000 ng/mL), Ephedrine (1,000 ng/mL), or Phenylpropanolamine (1,000 ng/mL)
  - Therefore, a positive result from this immunoassay test would not prove use of amphetamine

Heroin Metabolism

- Heroin is metabolized in the body to 6-acetylmorphine, which is further metabolized to morphine
- Morphine is converted to morphine-glucuronide which increases water solubility facilitating the removal of morphine from the body
- “Total Morphine” includes the morphine and morphine glucuronides found in the urine

Heroin

↓

6-Acetylmorphine

↓

Morphine (free)

↓

Morphine glucuronide

High Specificity

- A highly specific immunoassay will not react with similar antigens (drugs) and therefore may not detect their presence in a donor's specimen
- Example: One immunoassay for 6-acetylmorphine (6-AM, heroin metabolite) with a cutoff of 10 ng/mL will provide, in the absence of 6-AM, a positive result only when the urine specimen contains 9,000 ng/mL of free morphine (free morphine = ~10 percent of total morphine)
  - As a result, morphine use may not be detected in urine specimens with less than 90,000-100,000 ng/mL of Total Morphine

Variable Specificity

- Specificity varies with manufacturer of the immunoassay reagent
- This is exemplified in NLCP PT program data

Immunoassays for Opiates

Opiate Immunoassay Cross Reactivity with Hydrocodone

(Cutoff = 2,000 ng/mL Morphine)

Assay	Reported Cross-Reactivity (ng/mL = positive)	Hydrocodone (ng/mL)		
		1,500 (Percent Positive)	2,000 (Percent Positive)	4,000 (Percent Positive)
EMIT EIA	1,545	12	100	100
CEDIA	2,000	0	0	100
DRI EIA	7,813	0	0	0
KIMS	7,166	0	0	0

Opiate Immunoassay Cross Reactivity with Hydromorphone

(Cutoff = 2,000 ng/mL Morphine)

Assay	Reported Cross-Reactivity (ng/mL = positive)	Hydromorphone (ng/mL)			
		2,000 (Percent Positive)	4,000 (Percent Positive)	6,000 (Percent Positive)	10,000 (Percent Positive)
EMIT EIA	5,349	0	55	100	100
CEDIA	2,000	14	100	100	100
DRI EIA	11,000	0	0	11	0
KIMS	10,800	0	0	0	60

Opiate Immunoassay Cross Reactivity with Synthetic Opioids

(Cutoff = 300 ng/mL Morphine)

Assay	Reported Cross Reactivity (ng/mL = Positive)			
	Hydro-codone	**Hydro-morphone**	**Oxy-codone**	**Oxy-morphone**
EMIT EIA	247	498	1,500	9,300
CEDIA	625	525	9,500	16,000
DRI EIA	1,700	4,000	16,000	40,000
KIMS	1,086	1,425	>75,000	Not Available

Opiate Immunoassay Cross Reactivity with Morphine-3-glucuronide

(Cutoff = 2,000 ng/mL Morphine)

Assay	Reported Cross-Reactivity (ng/mL = positive)	PT Sample Composition (ng/mL)		
		Mor-3-G = 2,500 Free Mor = 500 (Percent Positive)	Mor-3-G = 3,000 Free Mor = 500 (Percent Positive)	Mor-3-G = 3,600 (Percent Positive)
EMIT EIA	6,167	94	100	47
CEDIA	2,000	100	100	100
DRI EIA	2,500	100	100	100
KIMS	3,785	57	100	100

Opiate Immunoassay Cross Reactivity with Oxycodone

(Cutoff = 2,000 ng/mL Morphine)

Assay	Reported Cross-Reactivity (ng/mL = positive)	Oxycodone (ng/mL)	
		75,000 (Percent Positive)	100,000 (Percent Positive)
EMIT EIA	47,774	81	86
CEDIA	64,000	100	100
DRI EIA	100,000	0	83
KIMS	>670,000	0	0

Specificity of Other Immunoassays

DRI Oxycodone Assay Cross-Reactivity (Cutoff = 100 ng/mL)

Compound	Reported Cross Reactivity (ng/mL= Positive)	Cross Reactivity (Percent)
Oxymorphone	103	103
6-acetylmorphine	50,000	<0.2
Hydrocodone	75,000	<0.13
Morphine	350,000	<0.04
Codeine	500,000	<0.02
Mor-3-Glu	900,000	<0.01

Benzodiazepines

- Prescription use is high
  - Alprazolam, Lorazepam, Clonazepam in top 25 of number of generic prescription
- Diversion is significant
  - Alprazolam, Lorazepam, Clonazepam in top 25 of number of crime lab drug seizures
- At least 4 immunoassays are available

Benzodiazepine Immunoassay Cross Reactivity

(Cutoff = 200 ng/mL Benzodiazepine)

Assay	Reported Cross Reactivity (ng/mL = Positive)			
	Alprazolam	Clonazepam	Diazepam	Lorazepam
EMIT EIA	65	260	44	600
CEDIA	138	188	110	208
DRI EIA	25	3,000	40	4,000
KIMS	240	540	200	674

Other Available Immunoassays

- Currently Available
  - Propoxyphene
  - Methadone
  - Ecstasy (MDMA)
- Future
  - Buprenorphine

Summary I

- A positive immunoassay test is only a "*presumptive*" positive test
- The drugs detected by an immunoassay are limited by specificity and the cutoff
- Both specificity and the cutoff may vary with the manufacturer of the immunoassay reagent

Summary II

- Even with two test procedures (initial and confirmatory testing), the number of positives is limited to the specificity and sensitivity of the immunoassay
- All data concerning drug prevalence, particularly in the case of opioids, must be interpreted with an understanding of the immunoassay used in the testing

Summary III

- Immunoassays currently used in workplace drug testing to initially test for opiates do not appear to be adequate for detecting many of the abused synthetic opioids
- There are immunoassays available or in development that will allow testing for some of the more commonly abused pharmaceuticals