ANTIDOTES IN DEPTH 2019
CLINICAL TOXICOLOGY, SUBSTANCES OF ABUSE AND CHEMICAL EMERGENCIES

Limiti delle determinazioni tossicologiche con metodi immunochimici

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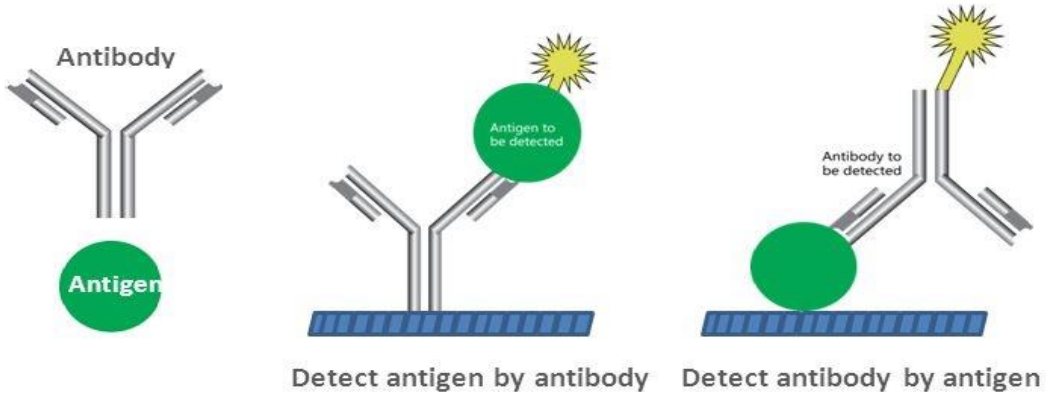
Immunoassay

- **An immunoassay is a biochemical test that measures the presence or concentration of an analyte (antibody or antigen) in a solution through the use of antibodies or antigens**



La reazione antigene-anticorpo produce un risultato analiticamente riscontrabile , eventualmente misurabile (es. variazione assorbanza) che permette di evidenziare, dosare un analita.

MEATHERAL R . J ANAL TOXICOL 1994 ; 18 (7) : 383-90

BENZODIAZEPINE SCREENING USING EMIT II AND TDX: URINE HYDROLYSIS PRETREATMENT REQUIRED.

Abstract

Urine specimens were collected from individuals prescribed oral doses of the following benzodiazepine tablets: diazepam, oxazepam, temazepam, lorazepam, alprazolam, flurazepam, and chlordiazepoxide. An aliquot was hydrolyzed using Helix pomatia beta-glucuronidase. Both the hydrolyzed and unhydrolyzed urine pairs were subjected to EMIT II and TDx immunoassay screening tests and to gas chromatographic-mass spectrometric quantitation. Hydrolysis is required to ensure adequate detection of oxazepam, temazepam, and lorazepam with either screening method.

A 100-ng/mL cutoff is required when screening for lorazepam, following therapeutic doses.

Test immunochimico per benzodazepine

Calibrazione del sistema utilizzando una determinata benzodiazepina

(es- lormetazepam)

Cut-off

espresso in ng/ml

come equivalenti della benzodiazepina utilizzata per la calibrazione
Es. test urina 200 ng/ml

Il "reale"cut off delle varie benzodiazepine , diverse da quella utilizzata per la calibrazione dipende da:
1) cross reattività
2) dal metabolismo delle varie benzodiazepine

Benzodiazepine : principi attivi

- alprazolam
- bromazepam
- brotizolam
- clobazam
- clonazepam
- clordiazepossido
- delorazepam
- diazepam
- estazolam
- etizolam
- flunitrazepam
- flurazepam
- lorazepam
- lormetazepam
- midazolam
- oxazepam
- triazolam

Benzodiazepine : livelli terapeutici

Benzodiazepina	Livelli terapeutici ng/ml	Livelli di allerta ng/ml
Lormetazepam	2-10	100
Triazolam	2-20	40
Diazepam + metaboliti	200-2500	3000

Test immunochimico (siero/urina) cut off 200 ng/ml :

negativo valore diagnostico ?

positivo, 900 ng/ml valore diagnostico ?

Metabolismo delle benzodiazepine

Via metabolica	Attività farmacologica dei metaboliti	Risposta dei metaboliti al test immunochimico	esempi
Fase I : ossidazione, idrossilazione, dealchilazione, riduzione	Formazione di metaboliti con ridotta, uguale o aumentata attività rispetto al parent drug	Cross reattività variabile rispetto al parent drug	diazepam flurazepam
Fase II: coniugazione con acido glucuronico, acetilazione	Formazione di metaboliti inattivi	Generalmente cross reattività ridotta/ quasi nulla	lorazepam

Valore diagnostico risultato test immunochimico !?

Aggravante : presenza di 2 o più diverse benzodiazepine

Improved Clinical Sensitivity of a Reflexive Algorithm to Minimize False-Negative Test Results by a Urine Benzodiazepine Immunoassay Screen

The Journal of Applied laboratory Medicine

Garrett R. Mullins et al. **DOI**: 10.1373/jalm.2017.024539 Published December 2017

Idrolisi : 30 μl β-glucuronidasi in 0.3 ml di urina, 2 ore a temperatura ambiente

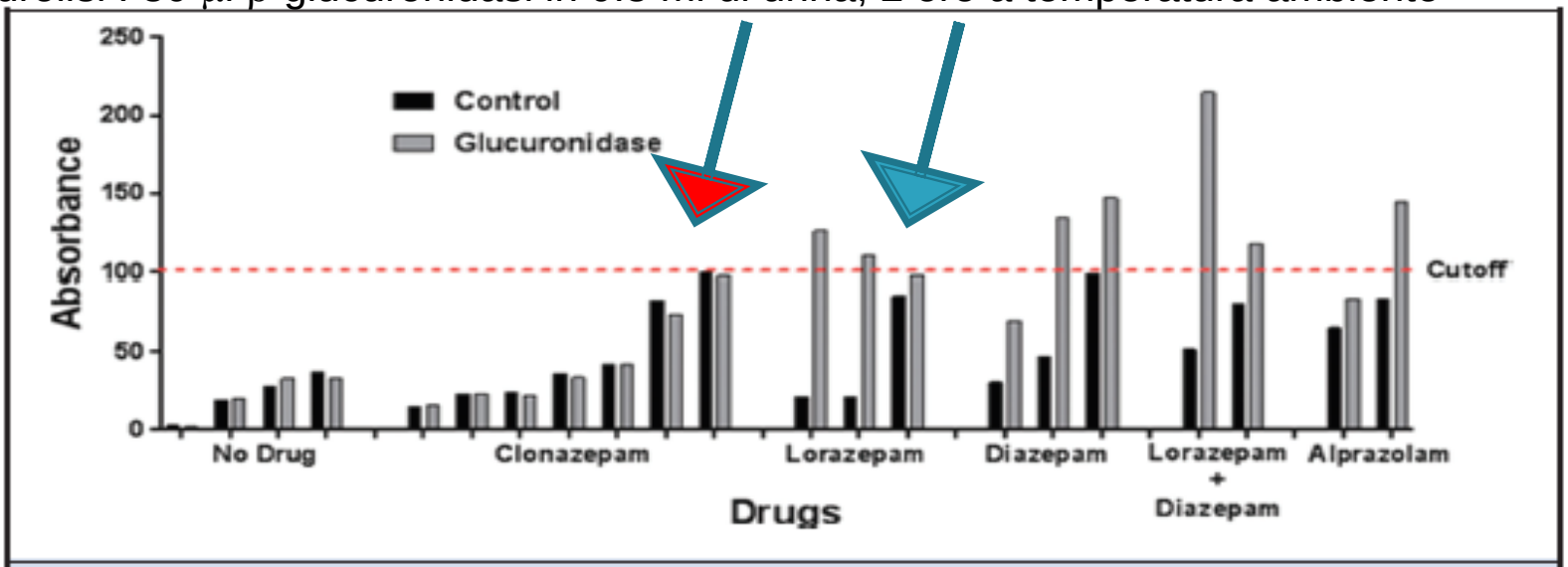


Fig. 1. The impact of a hydrolysis step before analysis by the benzodiazepine screen.
Urine samples were collected and analyzed by the benzodiazepine screen both with (gray bars) and without (black bars) prior hydrolysis by glucuronidase. Samples are grouped by drug taken. The red dotted line shows the manufacturer-recommended absorbance cutoff for a positive result.

Improved Clinical Sensitivity of a Reflexive Algorithm to Minimize False-Negative Test Results by a Urine Benzodiazepine Immunoassay Screen

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Table 2 . Comparison of the clinical performance of the benzodiazepines screen using the manufacturer recommended (100) or optimized (20) absorbance cut offs		
Absorbance cut off	100	20
Sensitivity	53 %	98 %
Specificity	87 %	64 %
False negative rate	47 %	2 %

Analisi benzodiazepine
Casistica Laboratorio Tossicologia Analitica
Fondazione IRCCS Policlinico San Matteo-Pavia
Caso 1

Femmina, 19 anni ricoverata per tentato sucidio con benzodiazepine

Test immunochimico urinario per benzodiazepine : negativo

Ricerca/dosaggio benzodiazepine nel siero con metodo cromatografico :

Bromazepam 342 ng/ml

(liv. Terapeutici < 170 ng/ml)

Diagnosi :

Intossicazione da benzodiazepine

Analisi benzodiazepine
Casistica Laboratorio Tossicologia Analitica
Fondazione IRCCS Policlinico San Matteo-Pavia
Caso 2

Bambino 2 anni, sospetta assunzione pastiglia del nonno (lorazepam) : astenia, confusione sonnolenza, difficoltà deambulazione

Test immunochimico urinario per benzodiazepine : negativo

Avviato iter diagnostico per patologia diversa da intossicazione:

Deficit neurologico ?

Esito negativo → riconsiderata intossicazione

Ricerca/dosaggio benzodiazepine nel siero con metodo cromatografico :

Lorazepam 42 ng/ml

.

Diagnosi :

Intossicazione da benzodiazepine

Analisi benzodiazepine
Casistica Laboratorio Tossicologia Analitica
Fondazione IRCCS Policlinico San Matteo-Pavia
Caso 3

Maschio, 78 anni , in trattamento con benzodiazepine: confusione, afasia, astenia

Diagnosi differenziale :

Intossicazione/ ischemia

Test immunochimico urina e siero per benzodiazepine : negativo

Ricerca/dosaggio benzodiazepine nel siero con metodo cromatografico :

Delorazepam 158 ng/ml

(liv. Terapeutici < 70 ng/ml)

Diagnosi :

Intossicazione da benzodiazepine

Analisi benzodiazepine
Casistica Laboratorio Tossicologia Analitica
Fondazione IRCCS Policlinico San Matteo-Pavia
Caso 4

femmina, 50 anni , in trattamento con benzodiazepine: confusione, afasia, astenia

Diagnosi differenziale :

Intossicazione/ ischemia

Test immunochimico urina per benzodiazepine :

POSITIVO, 849 ng/ml

VS cut off 200 ng/l

Ricerca/dosaggio benzodiazepine nel siero con metodo cromatografico :

diazepam e metaboliti in concentrazioni terapeutiche

Diagnosi :

ischemia

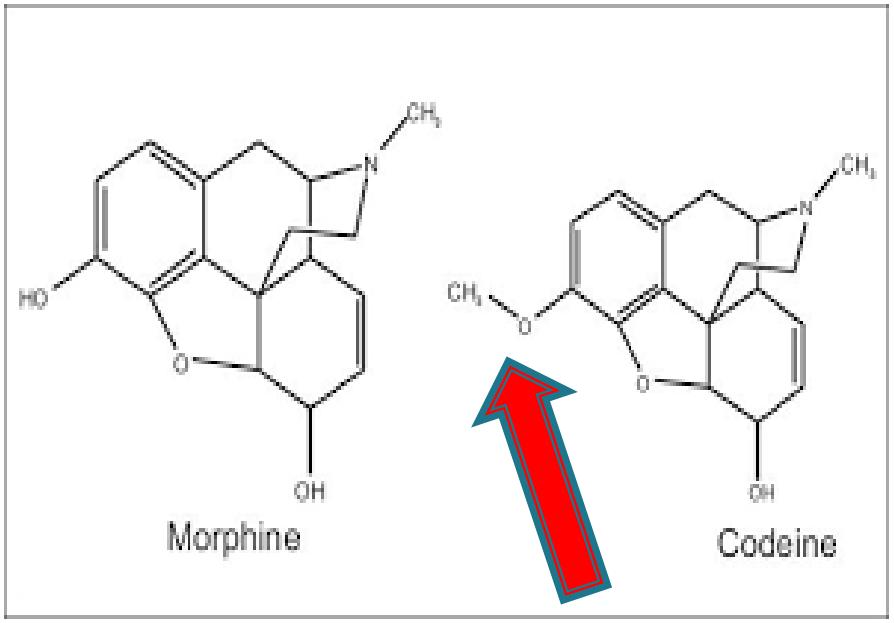
Immunoassay : false negative and false positive results

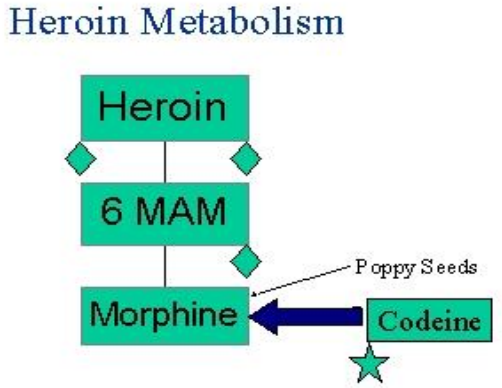
A false negative result occurs when immunoassay fails to detect a drug or metabolite within the targeted class.

A false positive result occurs when a positive results is caused by a compound outside of the targeted drug or drug class

Opiate Immunoassays

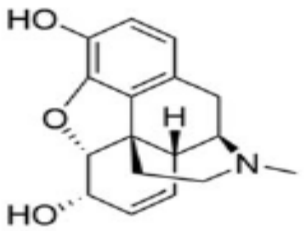
- Opiates immunoassays tipically target morphine and codeine



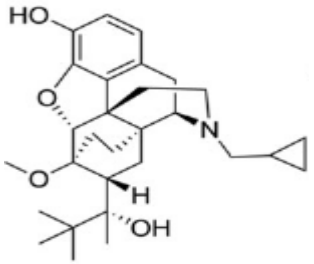


Opiate Immunoassays

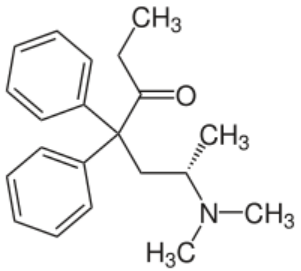
- Semisynthetic opiates similar in structure to morphine but requiring separates immunoassays for screening purposes



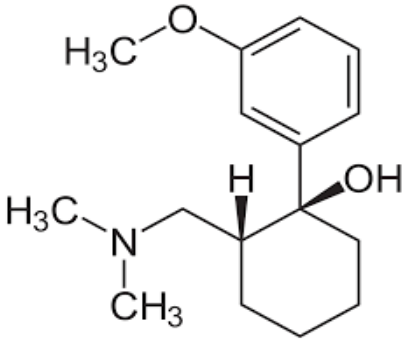
8: Morphine



11: Buprenorphine



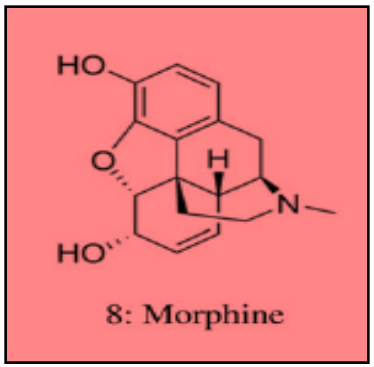
Metadone



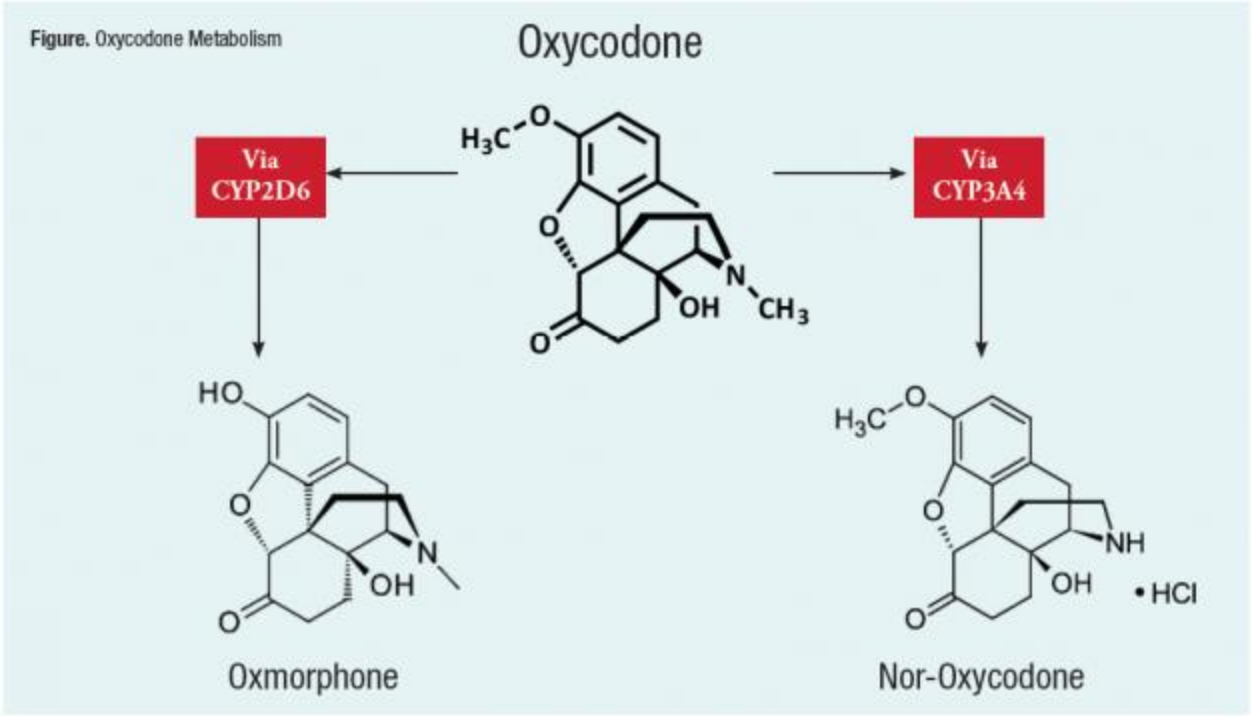
Tramadol

Opiate Immunoassays : oxicodone

- Semisynthetic opiates similar in structure to morphine but requiring separates immunoassays for screening purposes



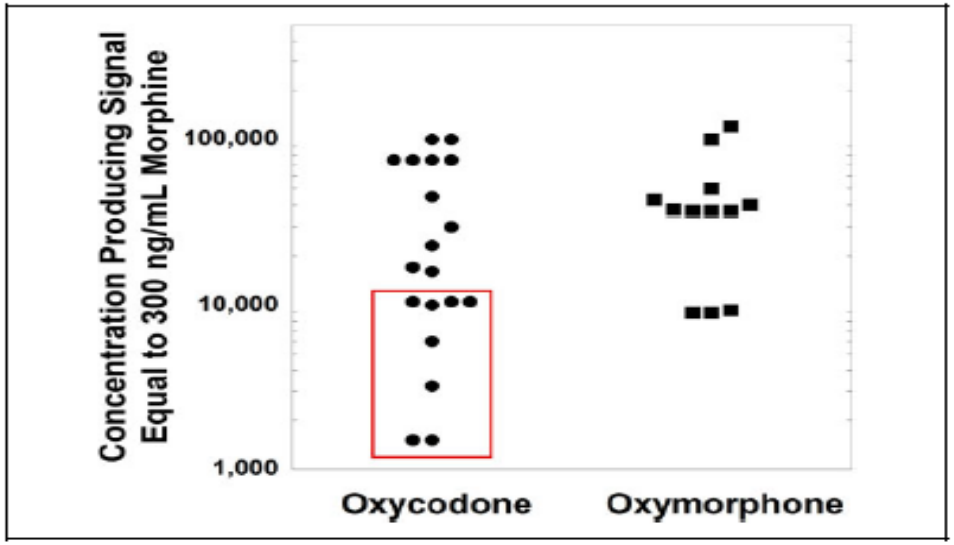
8: Morphine



Opiate Immunoassays : oxycodone

Cut off morphine 300 ng/ml :

equivalent cross reactivity concentration of oxycodone varies 2500 > 7500 ng/ml



Concentrations of oxycodone and oxymorophone producing equivalent signal to 300 ng/ml for morphine urine immunoassays, as reported in package inserts.

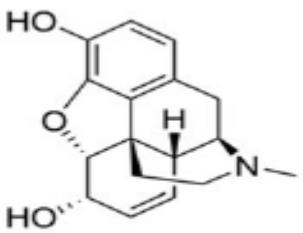
J.M.R. Krasowski et al ., Academic Pathology, vol 5: 1-10, 2018

Opiate Immunoassays

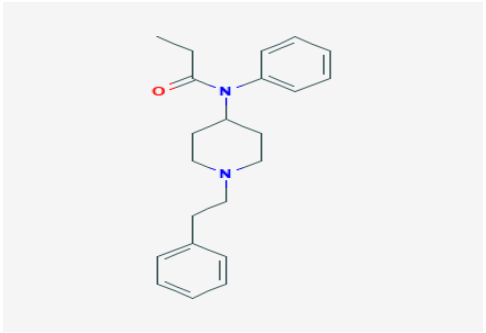
Fentanyl and analogs

therapeutic use

new psychoactive substances



8: Morphine



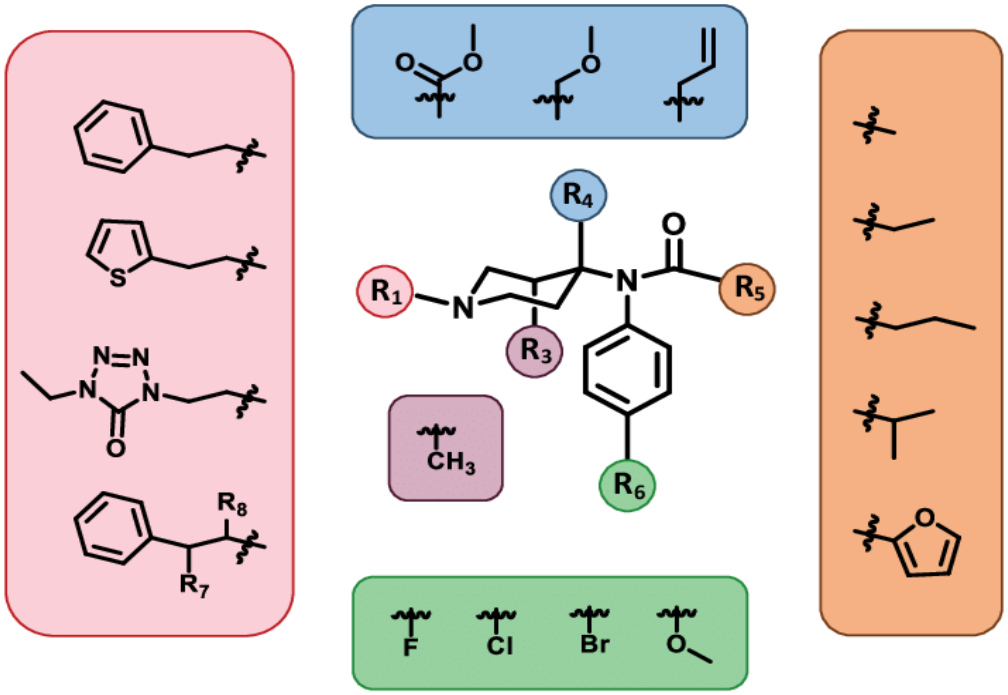
Fentanyl

Opiate Immunoassays

Fentanyl and analogs

therapeutic use

new psychoactive substances



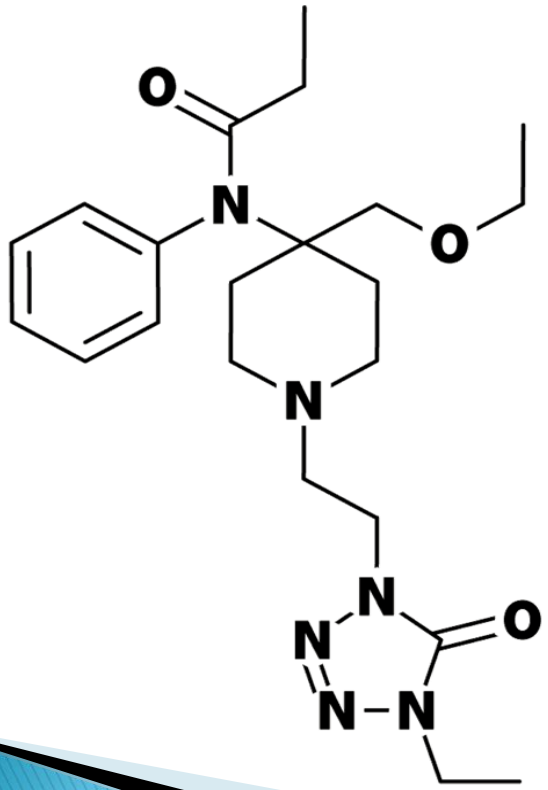
No cross reactivity
with opiates immunoassays

Opiate Immunoassays

Fentanyl and analogs

therapeutic use

new psychoactive substances



ALFENTANIL

Immunoassays

False positive results			
Cross-reacting drug	Immunoassay	Immunoassay manufacturer	Level wich false (+) occuredd
Bupropion	Siemens Dimension	Syva.	Occurred
Clorpromazine	CEDIA morphine, cutoff 300 ng/mL	Microgenics	60–1,700 μg/mL
Levofloxacine, ofloxacine, pefloxacine, enoxacine, gatifloxacine	EMIT II morphine, cutoff 300 ng/mL	Syva	40–600 μg/mL
Levofloxacine, ofloxacine, pefloxacine, lomefloxacine, moxifloxacine, ciprofloxacine, norfloxacine	Abuscreen OnLine morphine, cutoff 300 ng/mL	Roche	200–1,700 μg/mL

A. Saitman et al., Journal of Analytical Toxicology 2014;38:387 –396

Opiate Immunoassays : antibiotics

False positive results for opiates immunoassays			
Cross-reacting drug	Immunoassay platform (pos cut-off)	Immunoassay manufacturer	Level wich false (+) occuredd
Levofloxacine, oxfloxacine	AxSYM fluorescence polarization immunoassay morphine, cutoff 300 ng/ml	Abbott	1,700 μg/mL
Levofloxacine, ofloxacine, pefloxacine, enoxacine	CEDIA morphine, cutoff 300 ng/mL	Microgenics	60-1,700 μg/mL
Levofloxacine, ofloxacine, pefloxacine, enoxacine, gatifloxacine	EMIT II morphine, cutoff 300 ng/mL	Syva	40-600 μg/mL
Levofloxacine, ofloxacine, pefloxacine, lomefloxacine, moxifloxacine, ciprofloxacine, norfloxacine	Abuscreen OnLine morphine, cutoff 300 ng/mL	Roche	200-1,700 μg/mL

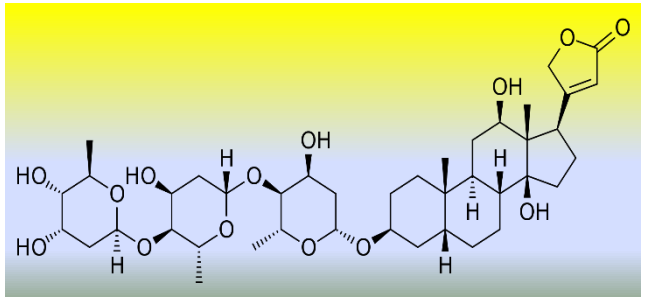
A. Saitman et al., Journal of Analytical Toxicology 2014;38:387 –396

- Journal of Analytical Toxicology 2014;38:387

- *Alec Saitman1*, Hyung-Doo Park and Robert L. Fitzgerald*

- **False-Positive Interferences of Common Urine Drug Screen Immunoassays: A Review**

Intossicazione da digossina



Intossicazione acuta	Intossicazione cronica
Paziente sano	Cardiopatie preesistenti
Nausea e vomito	Anoressia, nausea, vomito ,astenia, vertigini, dolori, disorientamento, delirio, disturbi visivi
ECG: aritmie sopraventricolari bradicardie con BAV	ECG: aritmie sopra e sottovetricolari, tachiaritmie
Digossinemia : valori elevati	Digossinemia : valori anche di poco superiori al range terapeutico

Antidoto : frammenti anticorpali-digitalici (FAB)

Dose da calcolare in relazione a : digossinemia
dose glucoside presuntivamente assunta

Digossinemia

- Metodi standard : immunochimici

Digossinemia dopo somministrazione di FAB
(FAB-digossina T ½ = 16-30 ore)

Interferenze analitiche

(es. sottostima)

Sequestro-richiamo digossina tissutale e intracellulare

→ Elevate concentrazioni digossina totale

Dato concentrazione digossina non attendibile

Eventualmente :
dosaggio quota libera digossina
(ultrafiltrazione campione ematico)

- Eur J Clin Chem Clin Biochem. 1997 May;35(5):369-70.

- Papa P, Rocchi L, Mainardi C, Donzelli G.

- **Buflomedil interference with the monoclonal EMIT d.a.u. amphetamine/methamphetamine immunoassay.**

Fondazione I.R.C.C.S. Policlinico San Matteo, Pavia

Grazie per l'attenzione