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# Immunoassay in Toxicology Diagnosis

Ewa Gomolka

*Jagiellonian University, Medical College, Department of Toxicology and Environmental Disease, Laboratory of Analytical Toxicology and Drug Monitoring, Krakow, Poland*

## 1. Introduction

Immunoassays are useful laboratory methods for clinical and forensic toxicology diagnostics. The methods are fast, sensitive, accurate and let determine poisons concentrations in different kinds of biological fluids. There are blood and urine or alternative: saliva, sweat and hair. The choice of the sample depends on the purpose of analysis. In acute poisoning the blood or serum poison concentration is important, but when the diagnosis is made more than 24 h post-ingestion, the urine sample should be collected and analyzed. The urine toxicology analysis let confirm the poison's ingestion. Alternative samples are more and more common, but they are used usually in forensic toxicology, in medical toxicology the results of hair or sweat determination are useful in the history of drugs overdosing or abusing research. Toxicological diagnostics is consequence, enables to make distinction if observed patient's symptoms are correlated to poisoning or other reasons. Fast quantitative measurement is important for poisoning confirmation, prognosis and decisions about specific treatment. All emergency rooms and poisoning treatment centers should have full access to such diagnostics. The most important immunoassay determinations are: the most often (e.g. alcohol, benzodiazepines, drugs of abuse) and the decision making ones (e.g. acetaminophen, phenobarbital, digoxin, amanitines).

The attributes of toxicological tests differ depending on the purpose of determination. When poisoning cause is to be identified – specificity is most important, to distinguish the poison from other exogenous or endogenous substances. When screening is made to check patient's abstinence – sensitivity of the test is most important. The sensitivity is usually set as a cut-off value – the concentration, above which the result is reported as positive. When test is used for therapeutic drug monitoring (TDM) purpose – test precision is most important, to follow changes of drug concentration in a narrow, therapeutic range. The most important points of toxicological tests are presented in Table 1.

The immunoassay method should be selected after consulting it's advantages and disadvantages. The choice of immunoassays is wide. There are simple qualitative cassettes or bar-tests and more accurate apparatus for semi-quantitative and quantitative measurements. They differ with sensitivity, cut-off values, kind of tested sample, result interpretation. All of them have some limitations and sometimes need confirmation by reference method.

Test	Indication	Preselection or Suspicion	Probability	Test Attribute	Example
Poisoning Diagnostics	Finding a Poisoning Cause	No	Moderate	Specificity	Looking for a poison
Screening	Checking	Yes	Low	Sensitivity	Abstinence Checking
Therapeutic Drug Monitoring	Monitoring Concentration Changes	Yes	High	Precision	Therapy

Table 1. Toxicological tests and their attributes

Aspects concerned with pre-analytical, analytical and post-analytical phases are important in a diagnosis process. The laboratory staff must know all immunoassay limitations and watch out for false results. The immunoassay methods became commonplace and toxicology diagnostics is performed in laboratories, where there are no reference and confirmation methods. Low cost and easy-automation encourage to start toxicology determinations in many clinical laboratories. Sometimes there are no toxicology specialists in the laboratories. Sometimes results interpretation cannot be performed properly without confirmation.

There are some analytical problems connected with toxicology diagnosis, especially when it is performed by immunoassay. One of the most important problem is result verification and creation result report. Immunoassay is not quite reliable. Analyst is responsible for false results, even when the misinterpretation is caused by limitation of immunoassay method. Some proceedings should be considered, for example, what to do when the result is doubtful and how to perform confirmation.

Most toxicology laboratories have access to reference methods and can confirm uncertain results. Analyst performing toxicology immunoassay in general laboratory should know all limitations of used method, know how to prepare reports, when to confirm results, how to store and transport sample for confirmation.

## 2. Immunoassays in toxicology

Acute intoxicated patient should be diagnosed as soon as possible. The biological samples are usually collected from poisoned patients in emergency rooms or in toxicology department. The collection time should be noted, because it influences the result interpretation. Medical laboratories usually use automatic immunoanalysers with methods: enzyme multiplied immunoassay technique (EMIT), fluorescent polarization immunoassay (FPIA), microparticle enzyme immunoassay (MEIA), cloned enzyme donor immunoassay (CEDIA), kinetic interaction of microparticles in solution (KIMS). They are useful for blood or serum therapeutic drug monitoring. They are also useful for serum and urine determinations of ethanol, medicines, drugs of abuse and other toxins. Other common immunoassays are cassette or strip rapid tests. Such tests are dedicated for urine or saliva determinations of drugs of abuse (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, ecstasy, methadone, opiates, phencyclidine, tricyclic antidepressants). Their sensitivity is usually set on a level appropriate for workers control or abstinence control of patients participating in drug substitution treatment.

Immunoassay methods do not enable to determine all poisons, drugs or biomarkers of exposition. In order to measure all of them, other methods are needed. There are spectrometry methods: ultraviolet/visible spectrometry, atomic absorption spectrometry (AAS); chromatography methods: thin layer chromatography (TLC), gas chromatography (GC), high performance liquid chromatography (HPLC) and chemical tests. They are complements to immunoassays, and they are used for confirmation positive immunoassay results. Examples of methods and determined substances in clinical and forensic toxicology are showed in Table 2.

Immunoassay	Chromatography	Spectrometry
RIA CEDIA EIA / EMIT ELISA FIA / FPIA, DELFIA KIMS MEIA POC (casette/strip rapid test)	TLC, HPTLC GC HPLC GC/MS LC/MS	Colorimetry Spectrophotometry UV Spectrophotometry VIS Spectrophotometry IR AAS AES
Amanitine Ethanol Drugs of abuse Therapeutic Drug Monitoring	Ethanol and other alcohols (Methanol, Ethylene Glycol, Izopropanol) Solvents Drugs of abuse Designer Drugs Medicines and metabolites Natural Toxins (e.g. Atropine, Scopolamine) Pesticides	Solvents Alcohols Medicines Pesticides Metals and Metalloids Biomarkers of exposition to chemical compounds (Carboxyhemoglobine COHb, Methemoglobine MetHb, Blood Acetylcholinoesteraze activity AChE)

RIA - Radioimmunoassay, CEDIA - Cloned Enzyme Donor Immunoassay, EIA / EMIT - Enzyme-Immunoassay / Enzyme Multiplied Immunoassay Technique, ELISA - Enzyme-Linked Immunosorbent Assay, FIA / FPIA - Fluorescence Immunoassay / Fluorescence Polarization Immunoassay, KIMS - Kinetic Interaction of Microparticles in Solution, MEIA - Microparticle Enzyme Immunoassay, POC - Point of Care  
TLC, HPTLC - Thin Layer Chromatography, GC - Gas Chromatography, HPLC - High Performance Liquid Chromatography, GC/MS - Gas Chromatography with Mass Spectrometry, LC/MS - Liquid Chromatography with Mass Spectrometry

Table 2. Examples of laboratory methods and determined substances in toxicology

### 3. Pre-analytical aspects of immunoassay in toxicology

#### 3.1 Collecting samples for immunoassay in toxicology diagnostics

Homogenous methods, in which a sample is added to mixture of reagents, immunological reaction goes between analyte and antibody in homogenous solution, (e.g. EMIT, FPIA, CEDIA, KIMS). They are useful for diagnostics of acute poisoned patients performed in blood, serum and urine. Heterogeneous methods, in which a sample is added to reaction

vessels with antibodies immobilized on the bottom (e.g. ELISA) can be used for forensic determinations of drugs in previous mentioned samples and in alternative samples (hair, sweat, saliva) after sample preparation. Rapid tests are usually dedicated for urine or saliva determinations. Drugs of abuse and some medicines are measured qualitative or semi-quantitative; TDM needs quantitative determinations (Table 3).

Qualitative tests (cassettes, strips)	Semi-quantitative tests	Quantitative tests
Urine or Saliva	Urine or Serum	Serum or Whole Blood
Acetaminophen Amphetamine Barbiturates Benzodiazepines Buprenorphine Cannabinoids (THC) Cocaine EDDP (methadone metabolite) Ecstasy (MDMA) Ethanol LSD Methadone Metamphetamine Morphine Opiates Phencyclidine (PCP) Tramal Tricyclic antidepressants	Amanitine Amphetamine/Metamphetamine Barbiturates Benzodiazepines Cannabinoids (THC) Cocaine Ecstasy (MDMA) LSD Tricyclic antidepressants	Acetaminophen Amikacine Carbamazepine Cyclosporine Digoxin Ethanol Gentamycine Lidocaine Phenobarbital Phenytoine Salicylates Theophylline Tobramycine Tricyclic antidepressants Valproic Acid Vancomycine

Table 3. Some substances determined by qualitative, semi-quantitative and quantitative immunoassays in different biological samples

Each kind of sample has a different detection time. The pharmacokinetics parameters of analyzed substances also influence the results interpretation. The detection window is the period of time when the substance can be detected in the sample. Blood, serum and saliva have a narrow detection window (1-24 h). Urine has a wider one (2-7 days) and hair has the widest one (several months). The detection window depends on the kind of collected sample, the kind of determined drug and the frequency of the drug ingestion (Table 4). Sometimes the detection time is prolonged, for example marijuana metabolites can be detected in urine for one to three weeks, depending on the size and frequency of marijuana ingestion. When the sample is collected in time out of the detection window (too early or too late) the result is negative in spite of visible patients symptoms connected with drug ingestion.

Collecting samples for therapeutic drug monitoring is different. Before starting TDM, the steady state of drug should be achieved. Depending on drug biological half time, the steady state is achieved in time from several hours to several days (Table 11).

Substance	Detection window	
	Occasionally	Chronic
Amphetamine derivatives	48 h	Up to 7 days
Barbiturates	Short acting: 24 h Long acting: 2-3 weeks	
Benzodiazepines	Short acting: 24 h Long acting : 3 days	
Cocaine	2-3 days	Up to 7 days
Methadone	2-3 days	1-2 weeks
Opiates	2-3 days	Up to 7 days
THC (Marijuana)	once: to 4 days occasionally: to 10 days passive exposition: not detected	4-6 weeks

Table 4. Urine detection window for drugs of abuse

The assay tests and reagents are dedicated for different kinds of samples. Every kind of sample contains specific background and it is not allowed to analyze serum using urine or saliva tests and vice versa.

The choice of the kind of sample depends on the purpose of determination. Blood and serum samples contain ingested substances. Urine samples contain mainly their metabolites. When acute poisoning diagnostics is made, the blood or serum samples are chosen. The blood and serum concentrations correlate with ingested dose of drug, correlate with poisoning symptoms and sometimes, give information about predictable poisoning effects. The diagnosis is useful when the physician wants to make decision about specific treatment with antidote or extracorporeal elimination. But when the time from drug ingestion to diagnosis is too long (longer than five biological halftimes) collecting the blood for poisoning diagnosis can be useless. The blood or serum results will be negative and urine sample should be collected. The urine concentration does not correlate the poisoning symptoms and does not have prediction value. But the urine presence of drug and it's metabolites confirms the patient has ingested the drug and the observed symptoms are connected with the poisoning.

Urine samples are collected when abstinence of patients and workers is analyzed. When the abstinence is checked, the urine adulteration or cheating is possible. Table 5 presents some possible urine cheating and ways of their recognition. Probably there are not all possible ways of cheating samples, that's why the best way of avoiding cheating is control the patients during collecting urine samples. Despite some disadvantages, the urine is quite good kind of sample for abstinence control. Urine is collected non-invasive and has a wide detection window. The most important differences between urine and blood (serum) samples are showed in Table 6.

Tests for urine determination of drugs of abuse and medicines have sensitivity on different levels. Laboratories should have tests with different cut-off levels. Cut-off levels in acute poisoning diagnosis should be the lowest, in patients of substitution therapy can be higher, in workers control can be the highest. High cut-off level means lower sensitivity and reduction of questionable or "false positive" results (caused by substances present in food or



supplements). Cut-off concentrations dedicated for qualitative urine and saliva drugs of abuse determination are showed in Table 7 and Table 8.

<b>Adulteration</b>	<b>Recognition</b>
Dilution	Evaluation of urine colour, temperature, creatinine concentration, density
Glutaric aldehyde	Urine strip test
Nitrates	Urine adulteration strip test
Soup, Bleach etc.	Evaluation of pH, look (foam), colour, fragrance
Acid, Alkali	Evaluation of pH, Urine adulteration strip test
Peroxides	Urine adulteration strip test
Vitamines, medicines	Chromatography analysis

Table 5. Urine adulteration and it's recognition

	<b>Urine</b>	<b>Blood (serum)</b>
Detection window	Wide (days, weeks)	Narrow (hours)
Concentration levels	High	Low
Analytes	Metabolites and toxins	Toxins
Correlation to intoxication symptoms	No	Yes
Cut off values	High	Low
pH influence	Yes	Yes
Adulteration risk	Yes	No
Cost	Low	High

Table 6. Differences between urine and blood (serum) samples

<b>Analyte</b>	<b>cut-off concentration ng/ml</b>
Amphetamine	1000, 500
Barbiturates	300
Benzodiazepines	200, 300
Cannabinoids (THC)	50
Cocaine	300
Extasy (MDMA)	1000, 500
Methadone	300
Methamphetamine	1000, 500
Opiates	300, 2000
Phencyclidine (PCP)	25
Tricyclic antidepressants	1000

Table 7. Cut-off concentrations dedicated for urine drugs of abuse determination by immunoassay

Analyte	cut-off concentration ng/ml	detection window
Amphetamine	50	10 min - 72 h
Cannabinoids (THC)	12	10 min - 72 h
Cocaine	20	10 min - 24 h
Methamphetamine	50	10 min - 72 h
Opiates	40	10 min - 72 h
Phencyclidine (PCP)	10	10 min - 72 h

Table 8. Cut-off concentrations and detection windows for saliva drugs of abuse determination by immunoassay

#### 4. Analytical aspects of immunoassay in toxicology

Immunoassay reagents are usually dedicated to closed auto-sampler systems, in which analyst's errors are minimized. Anyway, some aspects are important, and no automatic system can solve them. For example in situation of acute poisoned patient, when the measured poison concentration is out of calibration range. The necessity of sample dilution cause some manual manipulation. The diluent is usually distilled water or saline. Sometimes reagent kit contains a special diluent dedicated for a sample and analyte.

Cassette or strip immunotests are performed manually. Cassette is a device in which a sample is dropped into a special reservoir, strip is usually drown into liquid sample. Usually they are simple to do, but some errors are possible when the laboratory staff is unqualified. Too much or too little sample amount used for the test, inadequate reading time and invalid reading are possible. The factors can cause false test result. When the laboratory staff is experienced the error factors are reduced. Both cassette and strip contain a control bar placed in a distance from reading area. The control bar is getting colour when sample reaches the control area. It shows if the test is performed correctly. Laboratory staff must realize, the control bar is not a quality control. Quality assurance is realized only when controls samples (with known concentrations of measured substances) are analyzed.

##### 4.1 Quality controls in immunoassay in toxicology

All laboratories are obliged to keep internal and external quality control (EQC) systems. They are dedicated for the analyzed kind of sample (biological matrix), and the proper levels of analytes concentrations. Usually three levels of internal quality controls (low, medium and high) are performed. They let asses precision of the method on tree controlled levels. Precision is graphical illustrated as control charts (for example Levey-Jennings charts).

External quality controls let verify accuracy of the method. Laboratories participating in EQC programme get certificate of quality assurance which is necessary in laboratory accreditation procedure.

##### 4.2 Immunoassay sensitivity and specificity

Sensitivity reflects the lowest concentration giving positive result. Some immunoassays are useful for determination of a group of substances, for example benzodiazepines, barbiturates, amphetamines or opiates. The substances derivatives influence the results depending on their



sensitivity. Some of the derivatives have higher, and some have lower sensitivity, so they produce different results. For example 3,4-methylenedioxyamphetamine (MDMA) usually is not detected in immunoassays dedicated to amphetamines measurements because MDMA cross react when it's concentration in sample is nine times higher than amphetamine. We name the result "false negative".

Specificity is a property enabling to distinguish measured drug from other compounds. The drug metabolites, it's derivatives and other unknown compounds can interact the immunoassay reagents and produce elevated or "false positive" results. For example in therapeutic drug monitoring the physician is interested in the blood or serum concentration of the drug ingested by patient. But the result usually is elevated by the drug metabolites.

Another example of interfering substance is codeine, which cross react in morphine and opiates tests and produce positive results. We name them "false positive". The interfering substances can be medicines, supplements, drug components or endogenous compounds (for example DLIS - digoxin like immunoreactive substances). Other examples of interferences in immunoassays are showed in Table 9.

Analyzed compound or group	Interfering substance
Amphetamines	Fenfluramine, Ephedrine
Opiates (Morphine)	Codeine
Benzodiazepines	Oxaprozin
Cannabinoids	Niflumic Acid
Tricyclic Antidepressants	Carbamazepine, Phenothiazines
Digoxin	DLIS

Table 9. Examples of interferences in immunoassay

The positive results should be confirmed by specific chromatography methods. The confirmation is obligatory in forensic toxicology. Forensic toxicology use immunoassays as initial screening analysis; all the positive results must be confirmed by chromatography methods coupled with mass detection (GC/MS, LC/MS). The confirmation is optional in clinical toxicology. Most clinical laboratories do not have an access to time consuming and expensive chromatography methods.

## 5. Comparison of immunoassay and chromatography methods in some toxicology diagnostics

### 5.1 Alcohol determination

Alcohol is the most often abused drug and one of the most often cause of hospitalization in toxicology departments. Immunological methods became common in measurement of ethanol about 20 years ago. The methods are automated and reliable, they replaced manual Widmark's method and are comparable to gas chromatography and breathanalysis. Their sensitivity is usually less than 0,05 g/l. Interferences of non consumable alcohols (e.g. methanol, ethylene glycol, isopropanol) is insignificant (less than 1 %). The only interfering

alcohol can be n-butanol (18,5 %), but n-butanol is rather not present in any home products, and intoxication with the compound is hardly likely.

Correlation of alcohol results obtained by immunoassay and gas chromatography is acceptable. The only disadvantage of alcohol immunoassay test is a relatively narrow linearity range. In heavy drinkers blood the alcohol concentration can be higher than 3 g/l. When the linearity range is not wide enough, the sample dilution is needed.

The attention should be drawn to collection of blood sample for alcohol measurement. Blood can be contaminated when alcohol solution is used during draw blood.

Interpretation of blood and serum alcohol concentration should mind that serum alcohol concentration is higher than whole blood alcohol concentration. The difference is correlated to the concentration and can be calculated: blood alcohol concentration = serum alcohol concentration / 1,2. When serum ethanol concentration is not higher than 1 g/l, the difference is up to 0,16 g/l. Higher ethanol concentration implicate higher difference between serum and blood concentration.

## 5.2 Benzodiazepines determination

Benzodiazepines are often abused drugs. They are also used as date-rape drugs. The immunological methods measure concentration of benzodiazepines as a group of substances with basic shell of benzodiazepines rings. There are several known benzodiazepines and their metabolites that influence the immunoassay dedicated to this group of drugs. The interpretation of serum or urine benzodiazepines concentration result is not easy. They have different doses, applications, acting times and therapeutic ranges. Their affinities to immunoassay reagent are also different. In addition the immunoassay result is correlated to the sum of ingested benzodiazepines and their metabolites in biological sample. Some drugs (e.g. lorazepam, chlordiazepoxide) cross react with low efficiency. Other cross react with high efficiency (e.g. diazepam, oxazepam, alprazolam). The comparison of EMIT and HPLC showed, that benzodiazepines can produce results higher (alprazolam, diazepam), equal (nordiazepam) and lower (estazolam) than real drug concentration.

## 5.3 Carbamazepine determination

Carbamazepine is a common anticonvulsive drug. There are indications to measure carbamazepine concentration during therapy. Therapeutic drug monitoring make therapy safe, reduce risk of too low or too high dosage, and side effects. Immunoassay enables to control the blood carbamazepine concentration.

Comparison of EMIT and HPLC method showed differences in measured carbamazepine concentrations. EMIT results reflects sum of carbamazepine and it's metabolites concentrations. HPLC is a method enabling to separate carbamazepine and it's metabolites and quantify them separately.

The carbamazepine results interpretation should provide for the method. Anyway immunoassay is a good method for therapeutic drug monitoring, when the blood samples

are collected in a drug stationary phase and minimum drug concentration is measured (blood sample is collected before ingestion a dose).

Carbamazepine determination by immunoassay in acute poisoning does not let distinguish intoxication phase. Carbamazepine metabolites concentrations are low in absorption phase, later the equilibrium between drug and their metabolites concentration is established. Finally, in the elimination phase, carbamazepine metabolites concentrations are the highest. Immunoassays do not let demonstrate the changes, the result just reflects the sum of drug and it's metabolites.

#### 5.4 Tricyclic antidepressants determination

Tricyclic antidepressants (TCA) are the group of drugs used in psychiatric treatment. They are often abused by patients addicted to drugs of abuse. The drugs are toxic in elevated doses, but the correlation between serum TCA concentration and poisoning symptoms is rather weak. The serum TCA concentrations can be referred to ingested dose when analyst knows the name of drug. Table 11 shows variations among TCA therapeutic ranges.

The TCA concentration measured by immunoassay can be influenced by other substances. For example carbamazepine and some phenothiazines are common medicines elevating TCA immunoassay results (Table 9).

Substance	Volume of distribution	Elimination half-life
Amphetamines	3-33 L/kg	10-30 h Excretion pH dependent
Barbiturates (Phenobarbital)	0,7-1,5 L/kg	48-288 h
Benzodiazepines (Diazepam)	0,5-5 L/kg	20-40 h
Cannabis	10 L/kg	20-30 h
Cocaine	1,2-1,9 L/kg	0,5-1,5 h
LSD		3-4 h
Methadone	1-8 L/kg	15-55 h
Opiates (Morphine)	3-4 L/kg	1-7 h

Table 10. Pharmacokinetics parameters of drugs of abuse

## 6. Post-analytical aspects of immunoassay in toxicology

When the results are obtained, the staff must decide how to prepare the report. Quantitative blood and serum results seem to be easy interpreted. But for full interpretation the physician needs to know some more information, for example elimination half-life, volume of distribution. The pharmacokinetic parameters of some drugs of abuse are showed in Table 10. Other data also influence the results interpretation: poisoning circumstances, information about the dose of ingested compound, time from exposition or ingestion to collecting blood or urine sample, treatment started in ambulance.

Drug	Time to achieve steady state	Elimination half life	Protein binding	Usual sampling time	Usual therapeutic range
Acetaminophen	5-20 h	1-4 h	20-30 %	1 h	10-20 mg/l
Carbamazepine	2-6 days	6-25 h	65-80 %	before next dose (Cmin)	4-11 mg/l
Ethosuximide	5-15 days	30-60 h (adults) 20-56 h (children)	0 %	before next dose (Cmin)	40-100 mg/l
Phenobarbital	10-25 days (adults and adolescents) 8-20 days (infants and children)	50-150 h (adults) 40-130 h (infants and children) 60-200 h (newborns)	50 %	before next dose (Cmin)	10-40 mg/l
Phenytoin	2-6 h	20-30 h	92 %	before next dose (Cmin)	10-20 mg/l (adults and children) 6-14 mg/l (neonates)
Primidone	2-4 days	4-22 h	≤ 35 %	before next dose (Cmin)	5-15 mg/l
Valproic Acid	2-4 days	6-17 h (adults) 5-15 h (infants and children) 15-60 h (newborns)	90 %	before next dose (Cmin)	50-100 mg/l
Theophylline	2-3 days (adults) 1-2 days (children) 1-5 days (infants) 120 h (newborns)	3-12 h (adults non smokers) 4 h (adults smokers) 2-10 h (children) 3-14 h (infants) 24-30 h (newborns)	55-65 %	4-6 h after infusion beginning (max) 4-8 h after oral administration (Cmax) before infusion or next dose (Cmin)	8-20 mg/l (asthma) 6-11 mg/l (neonatal apnea)
Digoxin	5-7 days	20-50 h (adults) 12-24 h (children) 18-33 h (infants) 35-42 h (neonates)	20 %	8-24 h after administration	0,8-2,0 ng/ml

Drug	Time to achieve steady state	Elimination half life	Protein binding	Usual sampling time	Usual therapeutic range
<b>Tricyclic Antidepressants</b>					
Amitriptyline	3-8 days	17-40 h	90 %	before next dose (Cmin)	120-250 ng/ml
Clomipramine	7-14 days	19-37 h	90-98 %		Up to 700 ng/ml
Desipramine	2,5-11 days	12-54 h	75-90 %		125-300 ng/ml
Doxepin	9 days	8-25 h	68-85 %		150-250 ng/ml
Imipramine	2-5 days	6-28 h	63-96 %		150-250 ng/ml
Nortriptyline	4-20 days	18-56 h	87-93 %		50-150 ng/ml
Trimipramine	3-8 days	16-40 h	93-97 %		70-250 ng/ml
<b>Antibiotics</b>					
Amikacin	2,5-15 h (adults < 30 years) 7,5-75 h (adults > 30 years) 2,5-12,5 h (children) 10-45 h (neonates)	0,5-3 h (adults < 30 years) 1,5-15 h (adults > 30 years) 0,5-2,5 h (children) 2-9 h (neonates)	≤ 10 %	0,5-1 h after infusion (Cmax) before next dose (Cmin)	20-30 mg/l (Cmax) < 5 mg/l (Cmin)
Gentamycin					5-10 mg/l (Cmax) < 2 mg/l (Cmin)
Netilmicin					5-12 mg/l (Cmax) < 3 mg/l (Cmin)
Tobramycin					5-10 mg/l (Cmax) < 2 mg/l (Cmin)
Streptomycin	10-15 h	2-3 h	30 %	1-2 h after IM dose (Cmax) before next dose (Cmin)	15-40 mg/l (Cmax) < 5 mg/l (Cmin)
Vancomycin	20-30 h	4-10 h (adults) 2-3 h (children) 6-10 h (neonates)	30-55 %	1 h after infusion (Cmax) before next dose (Cmin)	20-40 mg/l (Cmax) 5-10 mg/l (Cmin)

Table 11. Pharmacokinetics parameters and chosen information about drugs determined for TDM purposes

Positive result usually means, that the patient ingested the determined substance. But positive result can be also generated by interfering compounds. The interferences are described in chapter about immunoassay tests specificity. When the time from substance ingestion and collecting blood sample is too short (shorter than time, when maximum blood concentration is observed), the determination should be performed once again. The second sampling time should be set correct, in order to reflect the tissues concentration. For example after digoxin ingestion the drug is distributed to muscle tissues and the time of setting equilibrium between serum and tissues is 6-8 h. Blood digoxin concentration measured earlier does not correlate the medical symptoms and severity of poisoning.

Negative result usually means, that the patient didn't ingest the determined substance. But negative result are obtained when the sample is collected in wrong time, too early or too late (out of the detection window). Negative result is obtained, when the method is not sensitive enough or cut-off concentration is too high. The next reason of negative result is limited sensitivity of the test for the measured analyte, when the immunoassay is dedicated for the group of substances. For example diazepam and nordiazepam are detected in benzodiazepine immunotests in low concentration. But lorazepam and chlordiazepoxide are detected in benzodiazepine test in high concentration, sometimes three or four times higher than diazepam. The implication of that can be false negative result, despite drug ingestion and observed poisoning symptoms.

So the result report should contain not only the determination result but information about assay method, its sensitivity (limit of detection, cut-off value), specificity (some most important interfering compounds) and recommendation for confirmation of the result.

## 7. Summary

Immunoassay methods are commonly used in laboratories. Toxicologists use them as useful screening in medical diagnosis of acute poisoning, checking abstinence, forensic purposes and therapeutic drug monitoring. When short time of analysis is important, immunoassay is the best method.

Despite many positive points, the method has some disadvantages. Commonness and low costs encourage performing the assays by unqualified staff. Forensic toxicologists always perform results confirmation. In medical laboratories the confirmation is not obligatory, and usually is not necessary, when interpretation is made in correlation to patient's condition.

Reporting rapid test result is sometimes dicey, there is a risk of false result. That is why the result report must contain information about the immunological methods used to perform determination, it's sensitivity, specificity and possible interferences.

This would keep confidence in laboratory results.

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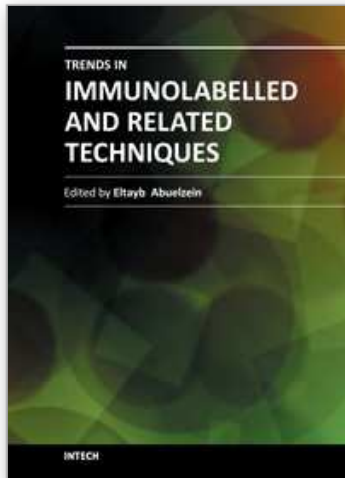
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## **Trends in Immunolabelled and Related Techniques**

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The book is coined to provide a professional insight into the different trends of immunoassay and related techniques. It encompasses 22 chapters which are grouped into two sections. The first section consists of articles dealing with emerging uni-and-multiplex immunolabelled methods employed in the various areas of research. The second section includes review articles which introduce the researchers to some immunolabelled techniques which are of vital significance such as the use of the conjugates of the Staphylococcus aureus protein "A" and the Streptococcus Spps. protein "G" in immunolabelled assay systems, the use of bead-based assays and an overview on the laboratory assay systems. The book provides technological innovations that are expected to provide an efficient channel for developments in immunolabelled and related techniques. It is also most useful for researchers and post-graduate students, in all fields, where immunolabelled techniques are applicable.

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Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
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### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

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