

The Effect of Urine Manipulation on Substance Abuse Testing*

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1. Introduction

Substance abuse analytics has become a commonly used tool in drug abuse therapies, for intoxication testing in the clinical field, for preliminary forensic examinations (road accidents, criminal offences) and in various companies, where newly recruited staff, but also long-serving employees whose work involves an above-average degree of risk are tested.

Various factors must be taken into account in the interpretation of the test results, including the objective of the test, e.g. medical diagnosis (substitution therapy, withdrawal therapy, differential diagnostics in emergency situations), legal aspects and social issues [1, 2]. Therefore, it does not come as a surprise that addicts who are in employment or are about to get a job, individuals in prison and persons, who have been banned from driving after having been found under the influence of a substance are determined to ensure that they pass their drugs test. For this purpose there are a number of products available, which claim to eliminate traces of drugs from the urine, or otherwise modify the urine so that certain substances are not detected. In many cases, such attempted manipulation is unsuccessful, as these products work only for certain analytical methods, while being ineffective with others. Many of the products have even no effect whatsoever on the test result, and their marketing is simply fraud.

On the other hand, the results of urine tests might be affected inadvertently and without any fraudulent intent. Table 1 shows a number of products that, when consumed, might affect the results of urine tests.

Table 1: Types and methods of manipulation

Interferences in Drug of Abuse Immunoassays
Unintentional
- <i>Interferences after Intake of therapeutic drugs (Neuroleptics, Antidepressants, Multivitaminpreparations) [3]</i>
- <i>Alimental Influences (Poppy seeds, Liquid intake shortly before void of Urine)</i>
Intentional Urine Manipulation
- <i>Urine Exchange (foreign Urine, artificial Urine etc.) [4]</i>
- <i>Deception: "Poppy seeds", Vitamines [5,41,43]</i>
- <i>Excessive Liquid intake, Stimulation of Diuresis [6,11]</i>
- <i>External Dilution of the Urine</i>
- <i>Chemical Manipulation (see manipulation methods)</i>
- <i>Adding drugs to the tube "falsely accusing"</i>

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2. One hour surfing the internet

By surfing the internet for only an hour, one comes across a number of sites giving detailed information on how to manipulate urine after the consumption of illicit drugs or alcohol, so that these substances are not detected with standard tests (based on immunoassays).

Such an internet search is even enlightening for persons who are specializing in urine testing for drugs.

The following examples show what inexperienced analysts are likely to overlook, unless they keep up to date with the latest manipulation methods.

A web site called "URINE LUCK" [7] promotes products and contains information, instructions and detailed descriptions on how urine test and manipulative products work. On linked pages, visitors find articles covering all principal issues to be considered in order to successfully achieve a negative test result.

For analysts, it is sobering to learn from such information that manipulation is possible, and that the producers of products for this purpose are very well informed about any aspect of the applied testing methods. The pages reveal details about which tests are most commonly used, how drug tests are carried out, and how labs attempt to detect manipulation. They also provide tips on how to cover up attempts of tampering with the urine.

It becomes obvious from viewing this information that the people behind it are trained in the field, and must at some stage have passed their masters or doctorate exams. Specialists working in the field of drug testing and analytics and who try to carry out these tests properly and diligently (including examination re. potential tampering) might even lag a few steps behind their adversaries. In the US, where testing for drugs is more common than everywhere else, some specialists fear that the field has become a stage for a showdown between chemists, namely those developing new manipulation products and methods, and those improving the test systems.

Chemists are caught up in a race: As soon as a new urine modifying product has been developed, labs are coming up with a respective detection kit, which in turn leads to the development of yet the next manipulation product.

The methods and products for the manipulation of urine tests are the product of highly creative thinking, and both sides (i.e. manipulator and analyst) might benefit by having a close look at what is going on from a medical-chemical point of view. Analysts might also consider socio-economic aspects of the issue (e.g. are tests and analyses still useful, even if the costs of secondary analyses are extremely high). The interests of both the producers of manipulation products and the manufacturers of analysis products for drug testing are primarily commercial. This is most obvious from the fact that commercially run laboratories carry out tens of thousands of drug test analyses every year, specializing in illicit drugs. The issue of drug abuse and testing is thus not only driven by social concerns. The products on offer on the internet, can be classified as shown in Table 2.

Not all of the information about manipulation methods found on the internet is correct. It is for example not possible to modify all drugs of abuse with pyridinium chromates in order to get a negative result by the immunoassays or the confirmation analyses. Some of the products offered on web pages claim to detoxify the body [6,8,17], but are more likely to cause other medical problems (dehydration, vitamin overdose, damage from artificial not approved drinks, etc.). Most manipulation substances are designed for addition to urine samples, as urine remains the most tested sample. On the other hand, it is less possible to interfere with other samples materials, such as blood, saliva or hair.

Table 2: Methods and substances of Manipulations, Interferences in Immunoassays [6,9-32,41,60]

Household products are most used as specific adulteration for one special method, (old fashioned manipulation), [Literature 6,9 – 18]]

Method or substance	Frequency of use	Can be tested by
– Urine exchange	frequently used today	Should be checked during void (related to chain of custody)
– External urine dilution	?	Should be checked during void (related to chain of custody)
– Sodium chloride		Sodium or chloride analyses
– Bleaching agent	seldom	pH, smell, color, ACR
– Drain cleaner	seldom	pH
– Detergents	seldom	foaming, ACR, pH
– Vinegar, acid	seldom	pH, ACR
– Baking soda	seldom	pH, ACR
– Ammonia	seldom	pH, ACR
– Visine, Coloring agents → liquid sol.	seldom	Chromatography

Oral intake, not checkable during void of urine, Literature [8,11,14,15,41]

Method or substance	Frequency of use	Can be tested by
– Golden Seal (Herbal tea)	?	Creatinine, spec. gravity, ACR
– Quick Caps (Herbal powder as capsules)	?	Creatinine, spec. gravity, ACR
– Test Clean	?	Creatinine, spec. gravity, ACR
– Zydor Ultimate Blend (liquid)	?	Creatinine, spec. gravity, ACR
– Vitamines (Ascorbate, B ₂ , B ₆ ,) Multivitaminpreparates)	frequent	color, ascorbic acid with pH, chromatography, ACR
– Dilution of urine by excessive fluid intake	most frequent	Creatinine, spec. gravity

Substitution and chemical adulteration of urine, in general checkable during void (C-O-C)**Literature [6,14,15,18-32]**

Method or substance	Frequency of use	Can be tested by
– Substitution of urine by solubilize a lyophilisated commercial urine and filling in a sample container e.g. void through artificial genitals)	seldom	checking during void
– Chromate, Pyridiniumchromates	? (US more often)	Colortest or AA for chromate, ACR
– Peroxide und Peroxidases	presumed: often	pH, ACR
– Glutaric aldehyde	seldom	ACR
– Nitrite	?	ACR

ACR = Adulteration screening reagents (Stix or wet chemistry). AA = Atomic Absorption, C-O-C = Chain of custody

3. Urine manipulation - a serious problem [33]?

In order to assess the frequency of urine sample manipulations, let us have a look at the current fields of application of drug tests:

In the US and the UK, most drug test samples are gathered in workplace testing campaigns. In continental Europe, this type of testing is currently still of minor importance, with varying

figures for the different countries. Only a few companies are using it, and, in general, only employees in high-risk workplaces are tested. Some European companies have introduced tests for new apprentices. Tests for illicit drugs and alcohol are also a standard method in addiction treatment and substitution programs. They are further used in forensics in relation to criminal offences and road accidents [35]. Drugs of abuse testing in prisons, on parties, in volunteers before participating in payed pharmacokinetic studies and in special cases at psychiatric patients and in cases of Munchhausen (by proxy).

In case of analyses carried [34] out in connection with criminal offences or road safety, it is nearly impossible to adulterate the urine sample. For drug addicts participating in withdrawal therapy, the high price of the manipulation products on offer is certainly an obstacle. But even here, cases of manipulated urine were identified (resulting from curiosity or "of impers to surprise"). In the US, the frequency of such samples is approximately 2 to 5 per cent of all urine samples tested for drugs [56].

The only figures available for Europe originate in Germany and are based on estimates [36]. Here, the frequency varies between 2 per cent of samples taken from persons in drug substitution programs, to 50 per cent of all samples taken in relation to possible driving bans. For the US, see Quest drug testing index (60).

More reliable figures for European countries will only be available, when testing for manipulation becomes a standard procedure in the sample analysis. It will however not be easy to establish such an approach, as there are many different ways of manipulating a sample, as described above. Comprehensive testing is also very costly, and requires each sample to be analyzed for drugs and adulterants (by immunoassays and chromatography).

4. Frequently used methods of analysis for the detection of illicit drugs [37]

As readers will be aware, most analytical screening methods in this field are based on the principle of antigen-antibody reactions known as immunoassays (immunochemical screening).

Adulterants which affect the proteins in general or the binding between antibody and antigens are adulterants to all immunoassays (strong acids, bases etc.). Other adulterants like glutaraldehyde are used only to spoil one specific immunoassay (EMIT). A third category of adulterants belong to the class of acting on specific measuring systems influencing directly the tracer or the tracer determination (masking, destroying etc.). This three adulteration methods are adulterants for all substance analyses of one specific method or the immunoassay systems in general. Most of the old fashioned adulterating agents (Household products) belong to this category (changing pH, changing protein structure etc.).

The immunochemical methods available in the form of quick tests as in strips, tabs, etc. or automated assays for analyzers are prone to interference, due to the underlying method. Chromatographic methods, which, from an analytical point of view, are the only methods capable of detecting specific substances with (generally) high sensitivity, are only used by a few, highly specialized laboratories. Accordingly, they are less likely to be tampered with. As chromatographic methods are very expensive, most labs specializing in screening use immunochemical procedures. For forensic purposes and other samples taken in relation to a legal procedure, only the results of chromatographic analyses are accepted (this also applies to the confirmation analysis).

From an analytical point of view the question rises, is the result positive or negative? Based only on the results of the analysis, is it possible to come to a final conclusion regarding the

existence of a substance in the sample (specificity, cross-reactivity in relation to immunochemical on-site tests and standard wet chemical quantitative methods, interpretation)?

The range of parameters is determined by the methods used in workplace testing in the US. These methods include applications used in clinical-chemical and forensic laboratories, such as wet chemical, quantitative and automated procedures, as well as test strips and quick tests in general. Tests can be classified into "broadband" tests for substance groups such as opiates or benzodiazepine on the one hand, and substance-specific tests for THC carboxylic acid, methadone, LSD, etc. on the other [1,2]

Often, the tests on offer are based on the mandatory SAMHSA (NIDA) test programs, which primarily target amphetamines (methamphetamine), THC carboxylic acid (cannabinoids), benzoylecgonine (cocaine), opiates and phencyclidine (PCP). The test for barbiturates, benzodiazepines, LSD, methadone and tricyclic antidepressants are mainly designed for special cases such as compliance and intoxication testing.

One disadvantage of quick tests is their fixed cutoff value, leaving no leeway for interpretation of the results. Also, there are no quantitative test results available for progress tests (i.e. assessment of renewed consumption, e.g. of cannabis [44,45]). For this type of examination, immunochemical methods that can be run automatically on analyzers are with some exceptions more suitable. Progress tests always include the analysis of creatinine, leading to higher expenses.

5. Manipulation methods [6, 9-32] (Figure 1)

It is the primary objective of any manipulation to generate test results consistent with drug abstinence. There were few cases where the manipulation was aimed at producing a positive test result. This occurred in the context of forensic examinations (diminished responsibility) or compliance screening in therapies (Methadone). The most common and extensively documented method of manipulation the dilution of the urine [6,11,39,40] is only successful in connection with THC carboxylic acid testing, producing an incorrect negative result. Other parameters are only affected, if the concentration of the queried substance is near the cutoff point, which generally means that the drug was consumed some time ago [8,16,17,41].

More sophisticated methods of dilution include the consumption of diuretics combined with vitamins and creatine (to simulate a normal creatinine concentration only successful if enzymatic creatinine determination is used), in the form of infusions. The effect is however often overstated. Vitamins can however mask certain tests, due to their colour, leading to non measurable analysis or inadvertent incorrect positive results [8,41].

„UrinAid“ (Glutaraldehyde) [30]

Glutaraldehyde is an agent that was originally used to adulterate the Syva EMIT II test (concentration dependent). The effect on other tests is shown in figure 1. (in Europe not often used). Glutaraldehyde can be detected with Dip Stick or wet chemistry methods.

Oxidizing agents

The intention of using oxidizing adulterants mostly is to pass the confirmation tests for THC-carboxylic acid (beating the drug and or the internal standard). Opiates and very seldom the cocaine metabolite benzoylecgonine are the subjects of these adulterations in a lesser extent. Most of these adulterants (Class of oxidants) are commercially available through the internet.

One of the recently detected adulterant is iodine which acts similar to chromate and peroxidase as oxidant [42].

	<u>Effects</u>							
	CEDIA		EMIT		KIMS		FPIA	
Acids	—		↓↓		↓↑		↓↑	
Bases	↓↓		↓↓		↓↓		—	
Bleaching agent	↓↓		↓↓		↓↑		↓↑	
Soap	NA		↓↓		↓↑		↑↑	
Dilution	↓↓		↓↓		↓↓		↓↓	
Salts	—		↓↓		↓↓		↓↑	
Glutaraldehyde	↓↓		↓↓		↓↑		↓↑	
Nitrite*	Cannabinoids	↓	Cannabinoids	↓	Cannabinoids	↓	Cannabinoids	↓
Chromate	Cannabinoids	↓	Cannabinoids	↓	Cannabinoids	↓	Cannabinoids	↓
	Opiate	↓	Opiate	↓	Opiate	↓	Opiate	↓
Peroxides/ Peroxidases	Cannabinoids	↓	Cannabinoids	↓	Cannabinoids	↓	Cannabinoids	↓
	Opiate	↓	Opiate	↓	Opiate	↓	Opiate	↓

Figure 1: Immunoassays: effect of different manipulation substances/methods. ↓↑ = Differ from one substance assay to another. * In confirmation analyses nitrite often influences the internal standard used for GC/MS analyses.

“Urine Clear”

Literature [23,24,25,26,27] describes the results of manipulation with nitrite in solution in an attempt to prevent the detection of THC-carboxylic acid. In one paper THC acid "cannabis-positive" urine samples were tested in replicates for several days on THC-carboxylic acid (after the addition of 2500 mg/l nitrite, with or without acidification. Under acidic conditions, THC-carboxylic acid cannot be detected by several immunoassays after a short period of time, and never by chromatographic methods [25]. After alkalization of the urine sample, the chromatographic methods will detect THC-COOH (affected is the internal standard). Other drugs are not affected by Nitrite.

„Stealth“ (Peroxide/Peroxidase)

Agents containing peroxides in connection with peroxidases are adulterants which have to be added to urine after void (e.g. this is also the case for glutaraldehyde, oxidizing agents, etc.) Therefore these products are not easy to use, if the period of void is watched. These agents change the structure of THC-COOH, LSD and morphine [8,20,21,22,51]. These drugs are masked and can not be detected nor by immunoassays neither by chromatography (dependent on the the peroxide and peroxidase concentration). This type of manipulation can be detected by several Dip Sticks and wet chemistry tests (detection is dependent on the time lag between addition of agent and testing for the adulteration).

“Urin Luck” (Chromate, pyridinium chromate)

This agent is based on pyridinium chromate and the action is comparable with that of peroxides/peroxidases. Chromate is an oxidant and acts as a adulterant on several ways. As an example the response rates of all EMIT drug assays are decreased [29]. This is an action on the

whole assay system (pyridinium or chromate or both?). Most of the THC-carboxylic acid and opiate assays are affected, dependent on the chromate concentration. The results will appear as negative. The detection of this adulterants is possible by Dip Sticks, wet chemistry tests, chromium determination by Atomic Absorption and pyridinium by chromatography. This manipulation is however easily exposed by means of specially designed test strips.

6. Definition of manipulation

In Table 3, the definitions of SAMHSA (NIDA) have been summarized: (These definitions are also recommended by the Swiss working group on drugs of abuse) [1,2,38].

Table 3: SAMHSA (NIDA) definitions. Definitions in Europe mmol/l, by the US-Government SAMHSA in (mg/dl)

-
- **Diluted**
 - Creatinine <2 mmol/l (10 mg/dl) but >0,45 mmol/l (5,0 mg/dl)
 - Specific gravity \leq 1.003 kg/l, but \geq 1.001 kg/l Changes in SAMHSA US Federal Register, 13 April 2004, 69 (71): 19644-19673.
 - **Substituted**
 - No regular components of human urine contained
 - Creatinine concentration \leq 0.16 mmol/l (2 mg/dl) and specific gravity \leq 1.001
 - Density \leq 1.001 kg/l or \geq 1.020 kg/l
 - **Adulterated**
 - pH-value \leq 3 or \geq 11
 - Nitrite concentration over 500 μ g/ml
 - Evidence of exogenous and endogenous substance out of the range
-

SAMHSA = U.S. Substance Abuse and Mental Health Services Administration (formerly NIDA), NIDA = National Institute for Drug Affairs.

The previously discussed attempts of manipulation are mainly targeted at immunochemical methods. Most common is probably excessive liquid consumption to try to dilute the urine. In this respect, it must be taken into account that urine samples taken in the evening may show creatinine concentrations close to the limit value of 1.8 mmol/l (20 mg/dl). In drug screening, urine with creatinine concentrations below this value are considered.

7. Accidental interference (Definition) [3,46,58]

Less well known is the issue of cross-reactivity with prescribed medicaments in immunochemical tests. Such interference often only comes to light in confirmation analyses. The results for opiates analyses from different manufacturers are known to be affected by neuroleptics dependent on the substance and their concentrations. The effect of specific drugs (generally non-steroid analgesics) on various test methods (i.e. various parameters and/or different test products) has to be considered because of the widespread use of these drugs.

Pathological biochemical pathways related to metabolic diseases can produce substances which are eliminated in urine and lead to false negative drug tests in urine [59]. Incorrect positive results (to a lesser extent: incorrect negative results) of unspecific tests often lead to considerable extra costs for retesting. We estimate that approximately 1 to 4 per cent of the routine screening analyses with immunoassays lead to such incorrect positive results [inhouse studies, 3]. (Wet chemical tests or spot tests).

8. Detection of manipulations [4,43,47-55]

As described above, common manipulations (like dilution) can only be reliably detected by carrying out additional analyses for creatinine in urine, specific gravity and pH, and assessment of the urine colour.

If specific substances, as available on the internet, are used, which destroy the actual drug in the urine, mask the test or directly influence a specific, generally known test method, it can be very difficult to prove that manipulation took place.

Even the test strips designed to detect manipulations are not beyond all doubt. If manipulation is suspected, supervision during void is important, as it generally makes the addition of any interfering substance impossible.

In the case of a positive test result for opiates, where the person in question claims that this is due to the consumption of poppy seed cake or poppy seed bread [5,43], it is very difficult to prove otherwise, as morphine is actually released when these foods are consumed. The opiate content of poppy seed varies greatly (depending on the actual harvest). The highest measured concentration in our experience is 4500 ng/ml (cutoff according to Swiss Working Group for Drugs of Abuse Testing Guidelines AGSA: 300 ng/ml). According to the literature, thebaine is the only potential poppy seed marker (accurate when positive, but not otherwise).

The identification of a urine, supposed to be substituted, is possible by DNA analyses comparison in blood versus the urine test. Confirmation of specific human proteins by immunoassay analyses helps to confirm the existence of a human urine. Other proposals to prevent substitution "are based on use of marker substances like polythylenglycols. These substances have to be given under control about half an hour before void of urine. The substances are analyzed in urine by chromatography (HPLC) [57].

Alle these tests are expensive and some are also time consuming.

The costs for the detection of urine that has been manipulated with any of the commercially available additives, after automated wet chemical analysis or quick test lead to considerably increased prices for analysis, depending on the assessed parameters (additional approx. 50 to 100 per cent of the total analysis costs). In certain countries, such as Switzerland, some of the additional analyses cannot be charged to the client.

Prior to introducing a program for the manipulation testing, the expected frequency of attempted manipulations and the related consequences must be examined in detail. Depending on the nature of the testing body (forensic laboratory, substitution therapy center, employer, training facility), the share of manipulated samples is often below one per cent.

Conclusion

In conclusion immunoassays can be easily automated and adapted to equipment but are very delicate to interferences and adulterants can produce a lot of extra work and expenses. Adulterants are used in most cases to produce false negative screening tests. They can be used as specific adulterants for a substance (THC-carboxylic acid), a testmethod or the immunoassays in general. Unexpected positive results are often produced through prescribed drugs, vitamins etc. This effects produce costs because of additional confirmation testing and the search for interfering substances.

In the future methods for testing drugs of abuse should be developed which are more resistant to interferences and adulterants. Perhaps toxicologists should reflect about the existing cutoff systems which often lead to critical situations.

Last but not least, manipulation is a problem, but one should not overestimate the frequency of these cases.

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Wahlen in der Schweizerischen Gesellschaft für Rechtsmedizin

An der Mitgliederversammlung der Schweizerischen Gesellschaft für Rechtsmedizin (SGRM) vom 20. November 2004 wurde der bisherige *Vorstand für die kommenden 2 Jahre* im Amt bestätigt. Es wurden wiedergewählt:

Präsident:	Dr. phil. Thomas Briellmann, IRM Basel
Vizepräsident:	Prof. Dr. med. Volker Dittmann, IRM Basel
Sekretär:	Dr. med. Thomas Plattner, IRM Bern
Vorsitzender der Sektion Forensische Medizin:	Prof. Dr. med. Volker Dittmann, IRM Basel
Vorsitzender der Sektion Forensische Genetik:	Dipl. biol. Christian Gehrig, IML Genève
Vorsitzender der Sektion Forensische Chemie und Toxikologie:	Dr. chem. Christian Staub, IML Genève

In der **Sektion Forensische Chemie und Toxikologie** wurden die *Gruppenleiter* ebenfalls für weitere 2 Jahre bestätigt:

Gruppe Toxikologie:	Dr. Marc Augsburger, IUML Lausanne
Gruppe Forensische Chemie:	Dr. Michael Bovens, Wissenschaftl. Dienst, Zürich
Gruppe Blutalkohol:	Karl Sutter, IRM St. Gallen
Gruppe Leiter der toxikologischen Labors der IRM:	Dr. chem. Christian Staub, IML Genève