

False-positive cannabis results in Italian workplace drug testing

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Introduction

Since 1990, Italian legislation planned a regulation to control drugs of abuse and psychotropic substances issuing a law (D.P.R. n.309/1990^[1]) that includes the protection of workers employed in risky jobs.

After many years of occasional application of this law, an agreement between the government and the regions (Conferenza Unificata - Provvedimento 30 October 2007^[2]) together with the publication of the sanitary procedures (Procedura per gli accertamenti sanitari - Provvedimento 18 September 2008^[3]) allowed the realization of the dispositions of Article n.125 of D.P.R. 309/90.^[1] The order, issued on 18 September 2008, refers to guidelines for clinical tests for drugs of abuse, including modality of collection, storage, the whole chain of custody of biological samples, and the analytical techniques to be used.

The Ministry of Labour and Social Security, together with the Ministry of Health, established the need to assess the absence of drug addiction by performing workplace drug testing.

The law identifies the professional categories to be inspected for the use/abuse of drugs, prescribing the control of workers through sanitary inspection by the employer. The professional categories to be verified include mainly public or private transporters (e.g. bus drivers, train and airplane, flight controllers, pilots, and railway and maritime staff), workers handling explosives such as the manufacture of fireworks, technical nuclear system direction and workers employed in production, packaging, detention, transport, and sale of explosive material and toxic gases.

The verification of the absence of addiction must be performed before the beginning of the specific function and periodically during the employer-employee relationship. For this purpose, drug detection in biological samples (urine and/or hair) and clinical tests including medical examination are usually carried out. The health facilities qualified for these purposes were identified in the Health Authority of the Region of the employer. This institution locates a specialist, namely the *Medico Competente* (expert physician) asked to verify worker health following dispositions of the Legislative Decree n. 81 of the year 2008^[4] about job security. If the task of the worker is included in the professional categories reported on the *Conferenza Unificata*,^[2] the expert physician must verify eventual drug addiction. For this purpose, he or she has to perform clinical tests, including anamnestic, medical, and psychiatric examinations, and biological specimen collection (urine and/or hair) to be analyzed in authorized toxicological laboratories. The physician could carry out immunochemical screening for drugs of abuse, but each positive result must be confirmed by chromatographic techniques in reliable and authorized laboratories.

At this point, if the worker fails to appear without good cause, the employer is obliged to make him stop his work duties addressing to

other jobs. A new request for sanitary verifications has to be arranged within 10 days; another refusal obliges the employer to definitively stop the task.^[3] Positive immunochemical tests compel the temporary stoppage of the risky job; results confirmed true positive bind the employer to change the employee's function and a rehabilitation route is undertaken.^[3]

In this context, the Forensic Toxicological Laboratory of Catholic University of Rome usually performs confirmation tests on biological samples (urine and hair) coming from workers already submitted to first-level tests.

The aim of this paper is to report the false-positive cannabis results observed in Italian workplace drug testing.

Material and methods

Immunochemical screening for the most common drugs of abuse – opiates, methadone, cannabinoids, benzodiazepines, amphetamine, and cocaine – is carried out using Kinetic Interaction of Microparticles in Solution (KIMS) technology.^[5,6] Urine specimens are collected under the supervision of skilled health personnel and checked for adulteration while respecting the dignity of the donors. The samples are split in three aliquots: the first one is employed for immunochemical screening; the second for the possible confirmatory test; while the third is frozen (–20 °C) for any further analysis of review according to European guidelines.^[7] Each aliquot is introduced in a sealed container to which a numeric code is assigned to ensure the privacy of the employee.^[7]

The KIMS procedure uses a drug-bound microparticle conjugate that forms a lattice when an antibody to the drug is added. The absorbance increases as the conjugate binds to the antibody and when a urine sample containing the drug of interest is added, free drug in the sample competes with the conjugate for antibody-binding sites. This reaction inhibits lattice formation and decreases the absorbance in proportion to the amount of free drug.

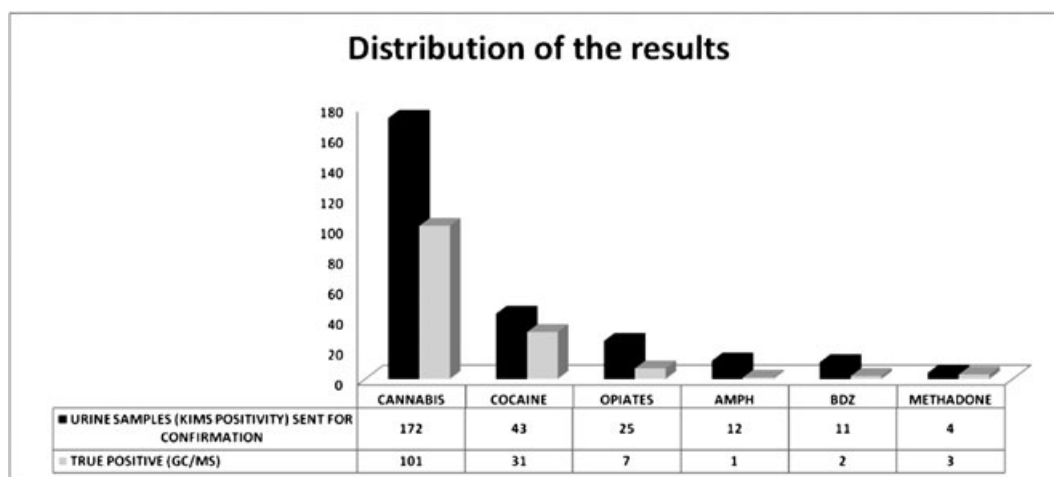
Second-level tests are performed by chromatographic techniques (gas chromatography–mass spectrometry; GC-MS) using pre-validated in-house methods^[8,9] while respecting the chain of custody. Limit of detection (LOD) and limit of quantitation (LOQ) of the methods employed, are respectively 5 and 20 ng/ml for opiates (morphine, codeine, and monoacetylmorphine), cocaine and benzoylecgonine, methadone and its metabolite, and amphetamines

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Table 1. Cut-off values of initial and confirmatory tests given by legislative decree

SUBSTANCES	INITIAL TEST (ng/ml)	CONFIRMATORY TEST (ng/ml)
Opiate metabolites	300	<i>Morphine, Codeine, 6-Acetylmorphine</i> 100
Cocaine and metabolites	300	<i>Cocaine, Benzoylcegonine</i> 100
Cannabinoid metabolites	50	15
Amphetamine and analogous	500	<i>Amphetamine, Methamphetamine, MDMA, MDA, MDEA</i> 250
Methadone	300	100

**Figure 1.** Analysis performed on 267 urine samples by KIMS and GC/MS technology.

(Amphetamine, Methamphetamine, Methylenedioxyamphetamine, Methylenedioxymethylamphetamine, Methylenedioxyethylamphetamine), 2 and 5 ng/ml for 11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid (THC-COOH). Cut-off concentrations used for screening and confirmatory tests are given by the legislative decree^[3] (Table 1).

Results and discussion

In the period 2006–2010, about 100 000 urine samples were collected by the Air Force Laboratory in the workplace area and submitted for immunological screening. Specimens found positive and questioned by the workers were sent to our laboratory for the confirmation analysis. We examined 267 urine samples for the different classes of drugs by GC-MS.^[9] In Figure 1, a graphical representation of the results obtained both with KIMS and GC-MS is explained. False negative results can be related to dilution of the sample, and the presence of adulterants and/or endogenous compounds. Cannabis is the most common drug encountered on the Italian illicit market, and the compound most revealed in biological samples. Only 59% of positively screened cannabis samples were confirmed by GC-MS. False-positive results can be due to chemically related substances (e.g. codeine in the opiate test), interferences by some medicine (e.g. non-steroidal anti-inflammatory drugs) as referred by the literature.^[10–12] For this purpose, it is of utmost importance, as recommended by the forensic societies and the European WDT guidelines to confirm all positive screening

results by most specific and sensitive chromatographic techniques such as GC-MS^[7,13]

However, further investigation is needed to identify the cause of such high number of false-positive samples.

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