

FAST SCREENING OF 1° LEVEL FOR LABELLED ABUSE DRUGS

in urine/hair/whole blood by LC/MS

Code SC1000





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This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD).

The declaration of conformity (CE) is available upon request.

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INTRODUCTION

The screening of Drugs of Abuse in biological liquids (above all in urine) is generally carried out using immunometric techniques with the utilization of monoclonal antibodies and following marking with various reagents. These methodologies are considered to be screening.

The present method allows the semi-quantitative determination of Morphine, Codeine, 6-Monoacetylmorphine, 3,4-MDA, 3,4-MDE, 3,4-MDMA, MBDB, Amphetamine, Methamphetamine, Benzoylecgonine, Cocaine, Methadone, EDDP, Norbuprenorphine, Buprenorphine, delta-9-THC, delta-9-THC-COOH, Cocaethylene, Ecgonine Methyl Ester, 11-OH-THC, Dihydrocodeine, Ketamine, Oxycodone, Morphine-6-beta-glucuronide, Morphine-3-beta-glucuronide, Norbuprenorphine glucuronide, Codeine-6-beta-glucuronide, delta-9-THC-COOH glucuronide, Buprenorphine glucuronide by LC-MS/MS.

This method aims to present itself as an alternative to the classic methods of immunoenzymatic screening, allowing to carry out a semi-quantitative analysis on the panel of labelled substances of abuse on urinary matrix, keratin and whole blood.

COMPONENTS OF THE KIT (200 TESTS)

Reagent A – Diluting Solution	1 x 50 ml
Reagent B – Stabilizing Solution for urine	1 x 100 ml
Reagent C – Deuterated Internal Standards for urine	1 x 4 ml
Reagent D – Stabilizing Solution for hair	1 x 100 ml
Reagent E – Deuterated Internal Standards for hair	1 x 10 ml
Reagent F – Wash Solution for hair	1 x 400 ml
Reagent G – Stabilizing Solution for whole blood	1 x 20 ml
Reagent H – Deuterated Internal Standards for whole blood	1 x 60 ml
Reagent M1 – Mobile Phase M1	2 x 500 ml
Reagent M2 – Mobile Phase M2	4 x 500 ml

All the reagents are ready to use and stable 3 years at 2-8 °C,
except the Reagent C, Reagent E and Reagent H that must be stored at -20 °C.
As regards the preservation method of the Lyophilized Calibration Standard, it is described in the dedicated
technical sheet.

Screening and confirmation cut-off values in Urine

Substance Class or substance (screening target substance)	Screening cut-off (ng/ml)	Confirmation cut-off (ng/ml)
Opiates (free morphine) Total Morphine ^a Total Codeine ^a 6-Monoacetylmorphine	300	100 100 10
Cocaine metabolite (benzoylecgonine) Cocaine Benzoylecgonine	300	100 100
Amphetamines and congeneris Amphetamine Methamphetamine	500	200 200
3,4-Methylenedioxymethamphetamine and congeneris MDMA MDA MDEa MBDB	500	200 200 200 200
Methadone (Methadone) Methadone EDDP	300	100 100
Cannabinoids (THC-COOH) THC-COOH	50	15
Buprenorphine Total Buprenorphine ^a Total norbuprenorphine ^a	5	5 5

a- the value is reported to the sample subjected to hydrolysis (to check the efficiency of the hydrolysis reaction of morphine-3-glucuronide in the process of method validation is mandatory)

Ref. GUIDELINES FOR LABORATORIES SEARCHING FOR SUBSTANCES OF ABUSE ON BIOLOGICAL SAMPLES TAKEN FROM THE LIVING SUBJECTS WITH "TOXICOLOGICAL AND FORENSIC-MEDICAL-LEGAL PURPOSES" [Rev. n. 4, 6 December 2012 from the Scientific Association Quality Commission "Group of Italian Forensic Toxicologists" (GTFI)].

Cut-off values according to Society of Hair Testing (SOHT)

Screening cut-offs according to SOHT recommendations			Confirmation cut-off according to SOHT recommendations	Confirmation cut-off in accordance with measure 9/18/2008 on workers with jobs at risk
Class of substance	Cut-off (ng/mg)	Analyte	Cut-off (ng/mg)	Cut-off (ng/mg)
Amphetamines	0,2	Amphetamine Methamphetamine MDA MDMA MDE	0,2 0,2 0,2 0,2 -	0,2 0,2 0,2 0,2 0,2
Cannabinoids	0,1	THC THC-COOH	0,05 0,0002	0,1 -
Cocaine	0,5	Cocaine BEG, EME, CE, NC*	0,5 0,05	0,2 0,05
Opiates	0,2	Morphine Codeine 6-MAM	0,2 0,2 0,2	0,2 0,2 0,2
Methadone	0,2	Methadone EDDP	0,2 0,05	0,2 -
Buprenorphine	0,01	Buprenorphine Norbuprenorphine	0,01 0,01	0,05 -
BEG: Benzoyllecgonine, EME: Ecgonine Methyl Estere, CE: Cocaethylene, NC: Norcocaine				

GUIDELINES FOR THE DETERMINATION OF SUBSTANCES OF ABUSE IN THE HAIR MATRIX
 Edited by Simona Pichini and Roberta Pacifici

The guidelines of the cut-off values for first level screening in blood are not available.

However, each laboratory should define its own screening cut-off values according to the required diagnostic sensitivity and specificity.

The present method has been developed according to screening cut-off values of 10 ng/mL for cannabinoids, 20 ng/mL for opiates, methadone and for the molecules belonging to the class of cocaine and 40 ng/mL for those belonging to the class of amphetamines.

MINIMUM INSTRUMENTAL EQUIPMENT REQUIRED

LC-MS/MS Triple Quadrupole

ESI mode

OPTIONAL EQUIPMENT

Autosampler

Operational Computer

URINE COLLECTION PROCEDURE

Take 10 ml of urine

If stored at -20 °C, the sample is stable about 3 months.

Centrifuge the urines before processing.

HAIR COLLECTION PROCEDURE

Recommended length: 5 cm, starting from the scalp. Is cut a strand, not torn (the bulb has no purpose at the end of these investigations) in the region of the vertex back of the head, of at least 200 mg which is divided into 2 aliquots of similar weight (A and B) of each of which is fixed to the proximal end. These aliquots are placed in separate containers non-transparent bearing caps airtight and sealed with tape irremovable and stored at room temperature. The cut of the hair must be performed with clean scissors with a small amount of alcohol and dry well.

HAIR COLLECTION PROCEDURE

It is necessary to cut 200 mg of hair from the pubic region; the hairs thus collected is divided into two aliquots (A and B).

WHOLE BLOOD COLLECTION PROCEDURE

Tube with anticoagulant EDTA

Stable for about 4 weeks at 2 – 8 °C or 4 months at –20°C.

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
SC1006U	Calibrator in urine for Tabeled Abuse Drugs Screening	4 x 1 ml
SC1009U	Control in urine for Tabeled Abuse Drugs Screening – Levels 1 and 2	2 x 5 x 1 ml
SC1006H	Calibrator in hair for Tabeled Abuse Drugs Screening	1 x 1000 mg/ 1 x 3 x 0,5 ml
SC1009H	Control in hair for Tabeled Abuse Drugs Screening - Levels 1 and 2	1 x 1500 mg/ 1 x 3 x 0,4 ml/ 1 x 25 ml
SC1006B	Calibrator in whole blood for Tabeled Abuse Drugs Screening	4 x 1 ml
SC1009B	Control in whole blood for Tabeled Abuse Drugs Screening - Levels 1 and 2	2 x 5 x 1 ml
SK1000	Starter kit for Tabeled Abuse Drugs	35 x 1 ml
TF25402052130	Hypersil GOLD PFP (50 x 2,1mm x 1,9um) Analytical Column	1 Pc
Z1636/26	10 ml Pirex Tubes with cap SWL (for hydrolysis, till 300 °C-washable and reusable)	1 x 40 Pcs
L-30-210	Eppendorf Tubes by 1,5 ml with caps	1 x 1000 Pcs
S51843550	Clear Glass Vials with reduced volume (goblet form)	1 x 100 Pcs
S51820717	Caps for glass Vials with reduced volume (goblet form)	1 x 100 Pcs

ANALYTICAL PROCEDURE FOR URINE

Add in sequence:

- 450 μ l of **Reagent B – Stabilizing Solution for urine**
- 50 μ l of Urine Sample/Calibrator/Controls
- 20 μ l of **Reagent C – Deuterated Internal Standards for urine**

INJECTION

- Inject 5 μ l of solution into LC/MS system

PREANALYTICAL PROCEDURE FOR HAIR

PREPARATION of unknown samples

STEP 1

Wash hair intact inside a glass tube of 10 ml with 2 ml of **Reagent F – Wash Solution for hair**

Seal the tube with the cap

Vortex for 1 minute

Centrifuge at 3.500 rpm for 5 minutes

Open glass tube

STEP 2

Evaporate to dryness the solvent with the aid of a light stream of helium or nitrogen paying attention to remain in the hair / fur on the bottom of the tube. Alternatively remove with a clean forceps with Acetone (not supplied in the kit), the hair / fur from the tube and place on paper towels until completely dry. In this last case the blotter paper should be changed for each sample.

Repeat steps 1 and 2 for two consecutive

STEP 3

Cut your hair, washed and dried, in segments of 1-2 cm with scissors cleaned with Acetone (not supplied in the kit)

STEP 4

Weigh an aliquot of sample, between 20 and 50 mg depending on the availability of the sample, in a Pyrex glass tube with screw cap clean and previously named.

ANALYTICAL PROCEDURE FOR HAIR

STEP 1

ANALYTICAL PROCEDURE FOR WHOLE BLOOD

STEP 1

Add in sequence:

- 100 µl of Whole Blood Sample/Calibrator/Controls
- 300 µl of **Reagent H – Deuterated Internal Standard for whole blood**

STEP 2

Centrifuge at 14.000 rpm for 10 minutes

STEP 3

Take 100 µl of supernatant and add 100 µl of **Reagent G – Stabilizing Solution for whole blood**

INJECTION

- Inject 5 µl of solution in HPLC

SCREENING OF 1°LEVEL BY LC/MS - Warnings

COLUMN CONDITIONING

Install a new analytical column Hypersil Gold PFP (50 x 2,1 mm, 1,9 µm), thermostated at 40°C. Disconnect the detector and flux 30 ml of a solution of H₂O : Methanol (20 : 80 v/v) set flow at 300 µl / minute. Condition further flux a solution of H₂O : Mobile Phase M2 (95 : 5 v/v) set flow at 400 µl for 15 minutes. **Don't recycle the washing solutions. Two injection of 50% H₂O HPLC grade / 50% Acetonitrile before proceeding to the analytical series.**

It ISN'T possible to make analysis at recycling phase

If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

COLUMN CLEANING

Flux a solution of H₂O : Methanol (20 : 80 v/v) for 30 minutes discharge it. Store the column in this solution.

INJECTION NEEDLE WASHING

Wash with Methanol.

GRADIENT SETTING

Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	FLOW (µl/min)
0.2	95	5	400
8.0	25	75	400
8.1	0	100	400
10.0	0	100	400
10.1	95	5	400
15.0	95	5	400



eureka kit



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