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**THE DETECTION OF ETG IN URINE:
COULD THIS MARKER BE USEFUL IN
WORKPLACE CONTROL?**

Alcohol and drugs represent a serious problem for a significant percentage of the working population (5%–20% of workers) causing negative consequences

- ⦿ People involved
- ⦿ Factory
- ⦿ Economy

The majority of European Countries have
general legislation or agreement
to forbid or regulate the consumption of alcohol
at the workplace

The issue is dealt with

- ⦿ labour code
- ⦿ occupational safety and health legislation
- ⦿ social dialogue

and

Has the intent to
ban the alcohol use at work or during working
hours
as well as the presence of drunk people at the
workplace

Alcohol tests, in a first instance, typically involve
breathalyzers; in case of positive result, blood
analysis for the confirmation may be required

In Italy

the law 125/2001 and the legislative decree 81/08
regulate
alcohol consumption and alcohol abuse
at the workplace

State–region agreements (2006) specify categories of workers considered at risk and set out a series of preventive measures.

The occupational physician can carry out breath analysis at discretion

In this study

A new enzyme immunoassay for the determination of the Ethylglucuronide (EtG) in urine samples was **VALIDATED**.

EtG in urine is a reliable indicator of recent drinking

- in follow up of people with alcohol related problems
- when “zero” tolerance is requested

This method was APPLIED on different types of
real samples

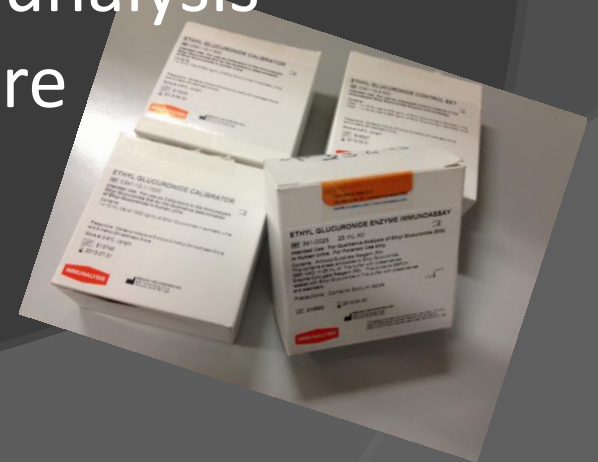
in order to
critically check for the possible use of
EtG in urine as the marker
in workplace Alcohol Test.

Immunochemical analysis

- Siemens Healthcare Diagnostic VIVA-E Analyzer



- Ethyl Glucuronide Enzyme Immunoassay, Calibrators and Controls set Immunoanalysis sold in Italy by Siemens Healthcare Diagnostic



Evaluation was done taking into account
Matrix effect, Precision and Accuracy

Analyses were carried out on

Real samples (n=100) coming from patients
monitored in a Public Treatment Unit

Workers (n=25) submitted to drugs of abuse
urinalysis with selfdeclaration of recent
alcohol consumption

Normal population with known alcohol
consumption

Confirmation method LC-MS-MS

Sample preparation :

- Dilution 1:50 in mobile phase containing D₅-EtG (IS);
- Centrifugation at 13000 RPM and direct injection in the LC-MS/MS system;

Liquid Chromatography:

- Chrompack Inertsil ODS-3 (100 × 3 mm, 3 μm i.d.);
- 0.1% formic acid/ACN (99/1);
- 200 μl/min;
- 100 μl/min ACN post-column.

Tandem mass spectrometry

- Turbo V™ ion source in negative ionization;
- 4000 QTrap: MRM reactions:

EtG	m/z	221.1 → 75.1	D_5 -EtG	226.1 → 75.1
		221.1 → 85.1		226.1 → 85.1
		221.1 → 221.1		226.1 → 226.1

LOD 25 ng/ml LLOQ 50 ng/ml

Validation parameters

Calibration curve

0, 375, 500, 625, 1000 ng/ml n=2 /5 days

R² > 99%

Limit of quantitation

negative sample n=5 /5 days

70 ng/ml

Intraday imprecision

375, 500, 625 ng/ml n=5 twice a day /5 days

CV% < 5%

100 urine samples from patients coming from a Detoxification Treatment Unit

cutoff 500 ng/ml

TP 49	TN 39
FP 12	FN 0

cutoff 1000ng/ml

TP 49	TN 51
FP 0	FN 0

Real samples – 25 Workers

N = 19 sampling in the afternoon (13:00-15:00)

4 have not drunk

NEGATIVE

4 had alcoholic beverages at lunch

POSITIVE > 1000 ng/ml

2 have winy breath

>>>>> 1000 ng/ml

9 drank during the evening before (beer or wine)

6 NEGATIVE (beer)

1 POSITIVE (EtG 660 ng/ml)

2 FP between 500-600 ng/ml < 500 LC-MS-MS (wine)

Real samples – 25 workers

N = 6 sampling in the morning (8:00 – 9:00)

3 did not drink the evening before

NEGATIVE

2 drank a glass of wine and 1 beer respectively

POSITIVE < 1000 ng/ml

1 declared 2 beer

POSITIVE >>> 1000 ng/ml

Real samples – known alcohol consumption

1 beer at dinner

N=4 3 POSITIVE >> 1000 ng/ml

first urine in the morning

1 POSITIVE < 1000 ng/ml (690 ng/ml)

sampling at 10:00

2 beer at dinner

N = 2 POSITIVE >>>>> 1000 ng/ml

first urine in the morning

Cocktail at night

N=2 POSITIVE >>>>> 1000 ng/ml

sampling at 10:00

2 glass of wine at lunch (30 g of alcohol)

Sampling at

15:30	POSITIVE >1000 ng/ml
21:00	POSITIVE > 1000 ng/ml
7:00	POSITIVE > 500 ng/ml
11:00	NEGATIVE < 500 ng/ml
15:30	NEGATIVE < 500 ng/ml

In conclusion

Immunochemical analysis for the detection of EtG in urine is the optimum when zero tolerance was requested

As regard to the use of this marker for the first screening at the workplace

We have to focus on two main critical aspects

Suitable Cutoff

1000 ng/ml

Time for sampling

in the afternoon ???

Certainly, this study is a starting point

- to set the standard for the use of EtG in urine as the marker in workplace test
- to be ready when laws and protocols will enter into force
- to actively cooperate as “expert” when laws and protocol will be enacted

Thank you very much for your attention

