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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 breathalysers; 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 carried 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 Immunalysis sold in Italy by Siemens Healthcare Diagnostic
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.

Evaluation was done taking into account Matrix effect, Precision and Accuracy.
Confirmation method LC-MS-MS

Sample preparation:
- Dilution 1:50 in mobile phase containing D<sub>5</sub>-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  
  221.1 → 85.1 
  221.1 → 221.1

  D<sub>5</sub>-EtG  226.1 → 75.1  
  226.1 → 85.1 
  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

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cutoff 1000 ng/ml

<table>
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Real samples – 25 Workers

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

4 have not drunk \hspace{2cm} \text{NEGATIVE}

4 had alcoholic beverages at lunch \hspace{2cm} \text{POSITIVE > 1000 ng/ml}

2 have winy breath \hspace{2cm} \text{>>>>>> 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 drunk the evening before

2 drank a glass of wine and 1 beer respectively

1 declared 2 beer

NEGATIVE

POSITIVE < 1000 ng/ml

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
<table>
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<td>15:30</td>
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<td>21:00</td>
<td>POSITIVE &gt; 1000 ng/ml</td>
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<td>7:00</td>
<td>POSITIVE &gt; 500 ng/ml</td>
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<tr>
<td>11:00</td>
<td>NEGATIVE &lt; 500 ng/ml</td>
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<tr>
<td>15:30</td>
<td>NEGATIVE &lt; 500 ng/ml</td>
</tr>
</tbody>
</table>

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

Sampling at
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