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**Abuse of alcohol and volatile compounds:  
modern diagnostic tools**

**April 20-21, 2018 Verona, Italy**





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## Scientific committee:

**Franco Tagliaro**, University of Verona  
**Sergey Savchuk, Svetlana Appolonova**,  
Sechenov University, Moscow

## Organizing committee:

**Federica Bortolotti**, University of Verona  
**Anna Bertaso**, University of Verona  
**Daniela Sorio**, University of Verona

In the frame of the collaboration between the **University of Verona** and the **I.M. Sechenov First Moscow State Medical University** we are pleased to announce the first pharmacotoxicological joint meeting entitled

## Abuse of alcohol and volatile compounds: modern diagnostic tools



## Dates

**April 20<sup>th</sup> 2018, 16:00– 18:30**

**April 21<sup>st</sup> 2018, 09:00– 18:00**

## Venue

Ex Gavazzi, via Bengasi 7  
37134-Verona, Italy

## Topic

Diagnostic methods for objective determination of alcohols, volatile and alcohol abuse biomarkers and their application in the clinical and forensic context and in epidemiological studies

## List of participants:

**Anna Bertaso** *anna.bertaso@univr.it*  
Univ of Verona  
**Federica Bortolotti** *federica.bortolotti@univr.it*  
University of Verona  
**Artem Gil** *artyoum5@mail.ru*  
I.M.Sechenov First Moscow State Medical University  
**Maria Gofenberg** *hoffenberg@yandex.ru*  
Regional Psychiatric Hospital, Yekaterinburg, Russia.  
**Oksana Gorina** *pu1ssme@gmail.com*  
Bureau of Forensic Medical Examination of the Saratov Region, Russia  
**Dmitry Kudriashov** *79213214877@yandex.ru*  
Chem.-toxicological Lab. of the "City narcological hospital", St. Petersburg, Russia.  
**Daniil Kuznetsov** *danmbf@mail.ru*  
Chem.-toxicological Lab. Volgograd Narcological Dispensary, Volgograd, Russia  
**Andrey P. Novikov** *zvlN00005@gmail.com*  
Surgut Clinical Psychoneurological Hospital, Surgut, Russia  
**Natalya Nikitina** *wfi-spb@yandex.ru*  
Chem.-toxicological lab Narcological of Pskov Region, Russia.  
**Luca Morini**, *luca.morini@unipv.it*  
University of Pavia  
**Giacomo Musile** *giacomo.musile@univr.it*  
University of Verona  
**Elena Pipina** *redgold@yandex.ru*  
Forensic Dept. of the Tomsk bureau of forensic-medical examination, Tomsk, Russia  
**Nadia Porpiglia** *nadia.porpiglia@univr.it*  
University of Verona  
**Lilla Rizvanova** *Germiona-kdl@yandex.ru*  
Chem.-toxicological lab Nijnevartovsk Psychoneurological Hospital, Khanty-Mansiysk, Russia  
**Sergey Savchuk** *serg-savchuk@yandex.ru*  
Sechenov First Moscow State Medical University  
**Daniela Sorio** *daniela.sorio@univr.it*  
University of Verona  
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University of Verona



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## Abuse of alcohol and volatile compounds: modern diagnostic tools

April 20-21, 2018 Verona, Italy

Friday, April 20<sup>th</sup> 2018

**OPENING**  
16:00-16:30

- Prof. Mario Pezzotti  
*Vice Rector*
- Prof. Franco Tagliaro  
*Director of the PhD Program in Nanosciences and Advanced Technologies*

**FIRST SCIENTIFIC SESSION**  
16:30- 18:30

**Alcohol and volatile abuses and related deaths**  
Chairperson: Svetlana Appolonova

- Affordability of alcohol and its consumption in European countries in transition. Alcohol consumption and premature mortality in Russia  
**A. Gil** (*Russian Federation*)
- Alcohol and technical fluids in the structure of mortality in the Saratov region in-2017  
**O. Gorina** (*Russian Federation*)
- Alcohol associated traffic injuries in the province of Verona, a 15 year survey.  
**F. Tagliaro** (*Italy*)
- Cases of abuse with mixtures of propane-butane by juvenile  
**N. Nikitina** (*Russian Federation*)

19:30 WELCOME COCKTAIL  
CHIOSTRO S. MARIA DELLE VITTORIE  
Lungadige Porta vittoria, 37129 Verona

Saturday, April 21<sup>st</sup> 2018

**SECOND SCIENTIFIC SESSION**  
09:00- 15:30

**Biomarkers of alcohol abuse**  
Chairperson: Sergey Savchuk

- Biomarkers of chronic alcohol abuse  
**F. Bortolotti** (*Italy*)
- Diagnostic and clinical significance of the CDT results for the testing of large population groups in the KhMAO region  
**A. P. Novikov** (*Russian Federation*)
- Clinical, methodological and organizational problems of CDT method application in the Volgograd region  
**D. Kuznetsov** (*Russian Federation*)

10:30- 11:00 Coffee Break

- Association of CDT and other biomarkers of alcohol abuse with alcohol related traffic accidents  
**F. Tagliaro** (*Italy*)
- Novel sample preparation for CDT analysis by capillary zone electrophoresis  
**N. Porpiglia** (*Italy*)
- Dried Blood Spot in combination with capillary electrophoresis a new way for CDT determination  
**A. Bertaso** (*Italy*)
- Determination of CDT by new method by HPLC coupled with fluorescence detector  
**D. Sorio** (*Italy*)
- CDT and fluorescence developments.  
**G. Musile** (*Italy*)

12:30- 14:00 Lunch

Saturday, April 21<sup>st</sup> 2018

- Diagnosis of alcohol consumption through the evaluation of ethyl glucuronide, a direct ethanol biomarker, in different biological matrices  
**L. Morini** (*Italy*)
- Comparison of sample preparation methods for the determination of ethyl glucuronide in blood by high-performance liquid chromatography with mass-selective detection  
**L. Rizvanova** (*Russian Federation*)

15:00- 15:30 Coffee Break

**THIRD SCIENTIFIC SESSION**  
15:30- 17:30

**Diagnosis of acute intoxication with alcohol and volatiles**  
Chairperson: Federica Bortolotti

- Laboratory diagnostics of acute poisoning with ethanol and volatile organic compounds in Sverdlovsk Region  
**M. Gofenberg** (*Russian Federation*)
- The diagnosis of intoxication with derivatives of GHB and 1,4 - butandiol in clinical practice of acute poisoning with ethanol.  
**D. Kudriashov** (*Russian Federation*)
- Differentiation of alive consumption and postmortem formation of ethanol in the body at the forensic investigation  
**S. Savchuk** (*Russian Federation*)
- Forensic chemical identification of toxicants in poisonings with alcohol, volatile poisons, and alcohol substitutes in the Regional Bureau of Forensic Medicine of the Tomsk region  
**Elena Pipina** (*Russian Federation*)



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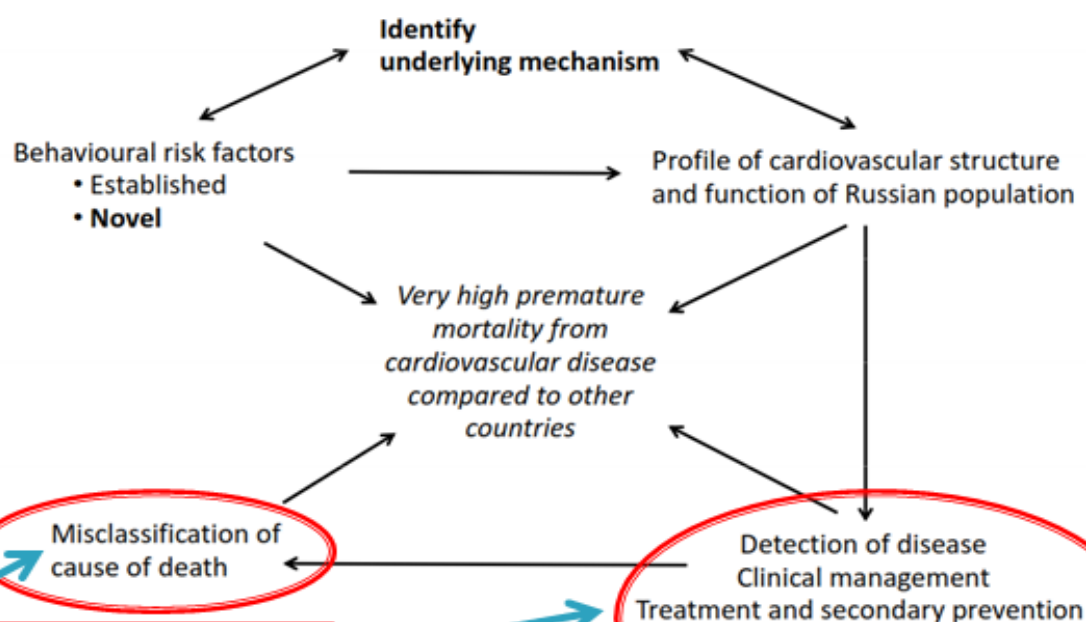


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# Affordability of alcohol and its consumption in European countries in transition. Alcohol consumption and premature mortality in Russia.

**Currently going and planned research in the field of alcohol and health is focused on the following comprehensive approach**



*The role of biomarkers of alcohol consumption*





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The population of the Region, according to the Russian Statistics Service data for 2 500 000 people (2018).

The density population – 24 km<sup>2</sup> (2018)





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*ABUSE OF ALCOHOL AND VOLATILE COMPOUNDS:  
MODERN DIAGNOSTIC TOOLS, Verona April 20<sup>th</sup>-21<sup>st</sup>, 2018*

# ASSOCIATION OF CDT AND OTHER BIOMARKERS OF ALCOHOL ABUSE WITH ALCOHOL RELATED TRAFFIC ACCIDENTS

**F. Tagliaro**

*Dept. of Diagnostics and Public Health, University of Verona,  
Verona, Italy*

## ALCOHOL AND TRAFFIC ACCIDENTS

In many western countries the fitness-to-drive is assessed on the basis of the following

### Axiom

chronic alcohol abuser



high number of occasions  
of excessive alcohol intake



▲ risk of driving under the influence



▲ risk of car crash



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# Cases of abuse of butane-propane compounds by undergrowth



Chemical-toxicology laboratory  
Narcological Clinic of Pskov region  
Natalia Nikitina

## VOLATILE COMPONENT ANALYSIS EQUIPMENT



### vapor-phase gas chromatographic analysis

- gas chromatograph Agilent 6890N with a flame ionization detector
- column capillary HP-Blood ALC  
7m, 0.32mm, 20  $\mu\text{m}$
- hydrogen generator, compressor
- Carrier gas-nitrogen of high purity
- Gas chromatographic microsyringes with a capacity of 100  $\mu\text{l}$ .




- gas chromatograph Agilent 6890N and Maestro 7820A with a mass-selective detector
- column capillary HP-FFAP  
50m, 0.32mm, 0.50  $\mu\text{m}$
- Carrier gas- helium of high purity
- Gas chromatographic microsyringes with a capacity of 10  $\mu\text{l}$ .





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The Pskov region is a small territory bordering Estonia, Latvia and Belarus. The population is small. The region is fully subsidized by the state. The region has its own productions. Therefore, the region is extremely small, and there are no favorable conditions for the development of the region.



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**ABUSE OF ALCOHOL AND VOLATILE COMPOUNDS:  
MODERN DIAGNOSTIC TOOLS**, Verona April 20<sup>th</sup>-21<sup>st</sup>, 2018

## BIOMARKERS OF CHRONIC ALCOHOL ABUSE

**F. Bortolotti**

*Dept. of Diagnostics and Public Health, University of Verona,  
Verona, Italy*

### BIOCHEMICAL MARKERS OF CHRONIC ALCOHOL ABUSE

<b>Non-oxidative metabolites of ethanol</b>	<b>Ethyl glucuronide (EtG)</b>
	<b>Ethyl sulfate (EtS)</b>
	<b>Fatty Acid ethyl esters (FAEE)</b>
	<b>Phosphatidylethanol (Peth)</b>
	<b>Cocaethylene</b>
<b>Acetaldehyde Products</b>	<b>Acetaldehyde Protein Adducts</b>
	<b>Salsolinol</b>
<b>Markers of alcohol related metabolic changes</b>	<b>Carbohydrate Deficient Transferrin (CDT)</b>
	<b>5- hydroxytryptophol (5-HTOL)</b>
<b>Markers of alcohol related organ damages</b>	<b>Aspartate Amino transferase (AST)</b>
	<b>Alanine amino-transferase (ALT)</b>
	<b>Gamma Glutamyl transferase (GGT)</b>
	<b>Mean Corpuscular Volume</b>



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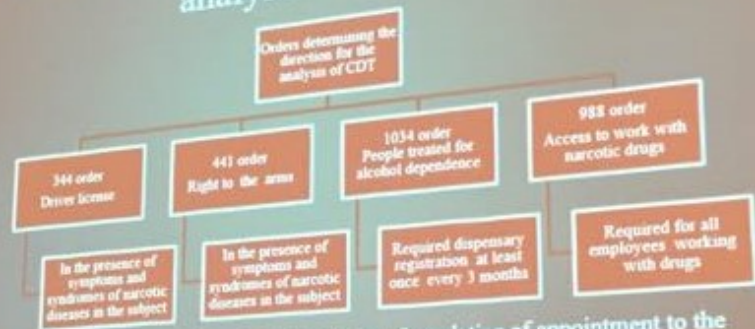




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## Orders determining the direction for the analysis of CDT



344 and 441 orders don't work properly as the formulation of appointment to the analysis is blurred (A person can look quite healthy though he takes 40 mg of ethanol alcohol per day). Also the wording of these orders can make people come to the conclusion that the appointment for the analysis is given unreasonably.





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MODERN DIAGNOSTIC TOOLS**, Verona April 20<sup>th</sup>-21<sup>st</sup>, 2018

## NOVEL SAMPLE PREPARATION FOR CDT ANALYSIS BY CAPILLARY ZONE ELECTROPHORESIS (CZE)

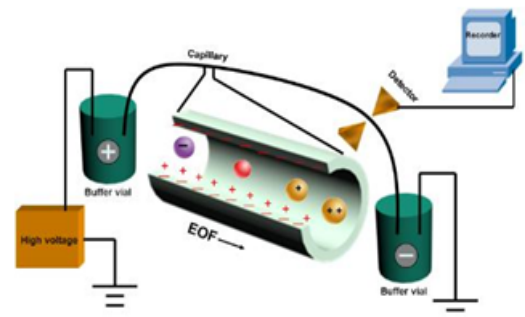
**N. Porpiglia**

*Dept. of Diagnostics and Public Health, University of Verona,  
Verona, Italy*

### CZE method routinely used in our lab

#### **Analytical conditions:**

- ⚡ Capillary features: 30  $\mu\text{m}$  i.d. x 60 cm T.L.
- ⚡ Separation voltage: 30 kV
- ⚡ Running buffer: 120 mM  $\text{H}_3\text{BO}_3$ , pH 8.2 + 6 mM DAB (1,4-diaminobutane)
- ⚡ Hydrodynamic injection: 0.5 psi x 25 seconds
- ⚡ Detection: UV absorbance at 200 nm
- ⚡ Cut-off: 1.8%
- ⚡ Serum dilution 1:8 with ferric solution



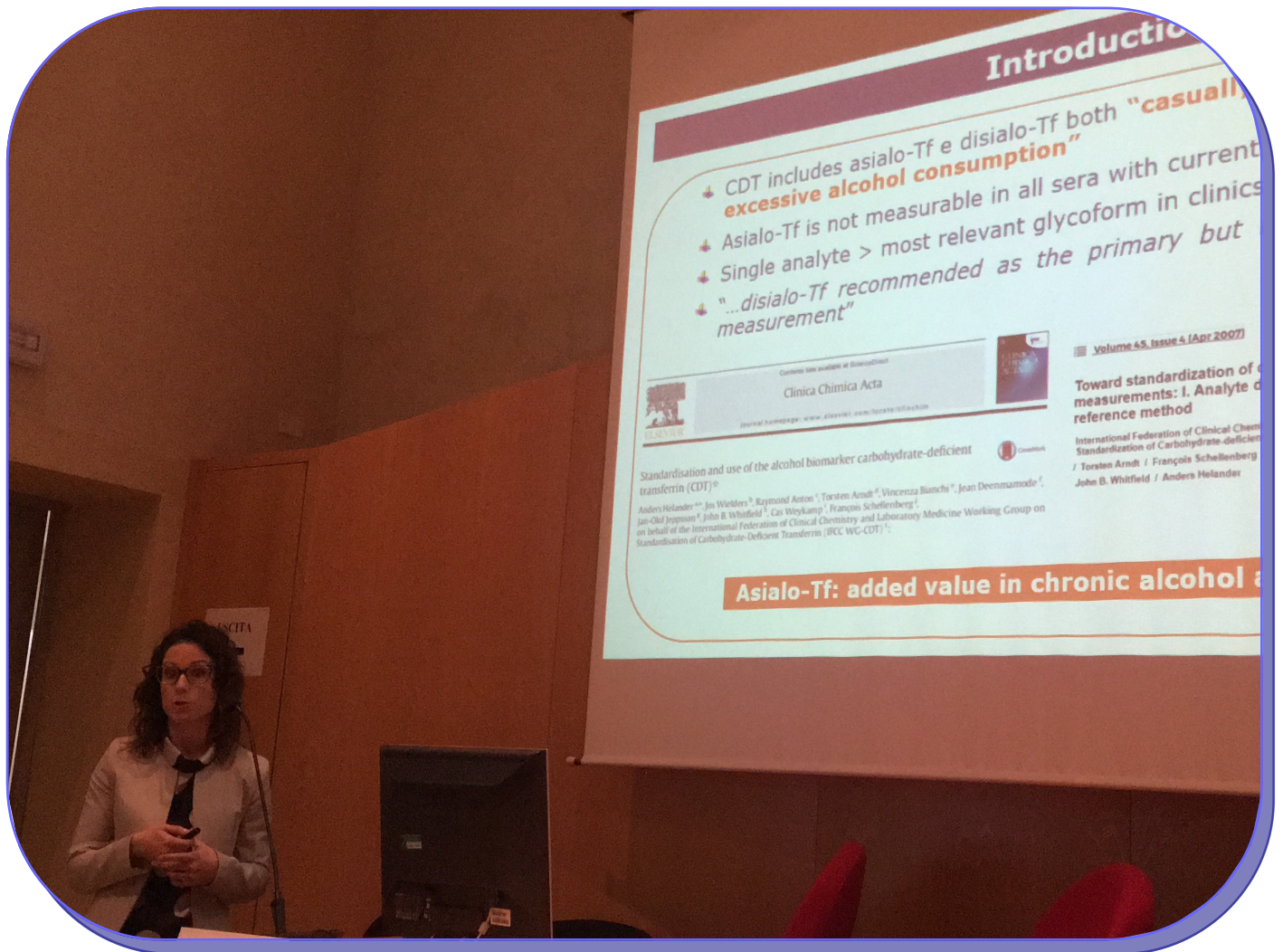
**Complete resolution of  
Tf glycoforms peaks**

Improved capillary electrophoresis determination of carbohydrate-deficient transferrin including on-line immunosubtraction

Jennifer P Pascali PhD\*, Federica Bortolotti MD PhD\*, Daniela Sorio PhD\*, Mariela Ivanova MD\*, Timothy M Palmbach JD-MS<sup>2</sup> and Franco Tagliaro MD PhD\*



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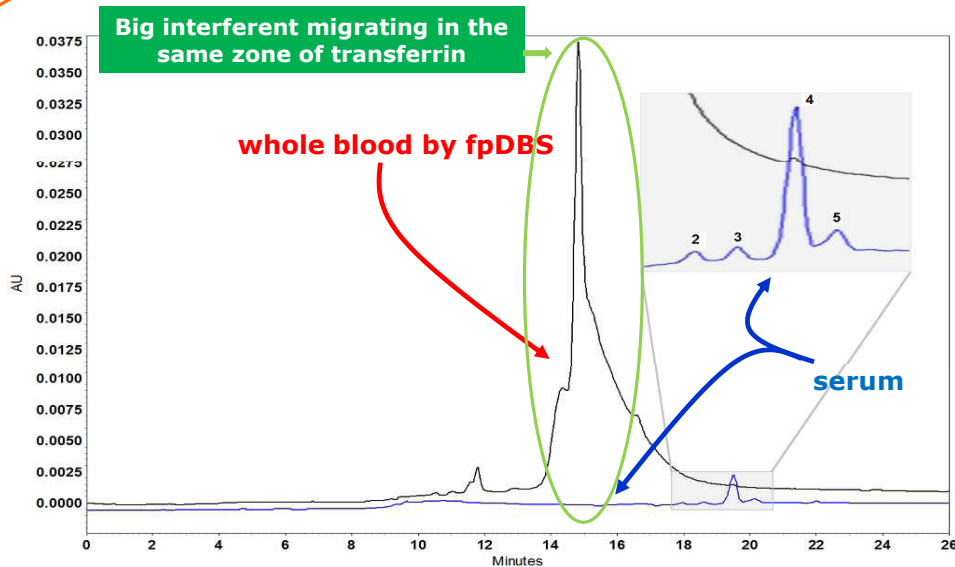
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MODERN DIAGNOSTIC TOOLS**, Verona April 20<sup>th</sup>-21<sup>st</sup>, 2018

## DRIED BLOOD SPOT IN COMBINATION WITH CAPILLARY ELECTROPHORESIS A NEW WAY FOR CDT DETERMINATION

**A. Bertaso**

*Dept. of Diagnostics and Public Health, University of Verona,  
Verona, Italy*

### fpDBS CZE vs serum CZE



In the insert a zoom of the normal pattern of CDT .  
2: disialo-Tf; 3: trisialo-Tf; 4: tetrasialo-Tf; 5: pentasialo-Tf.

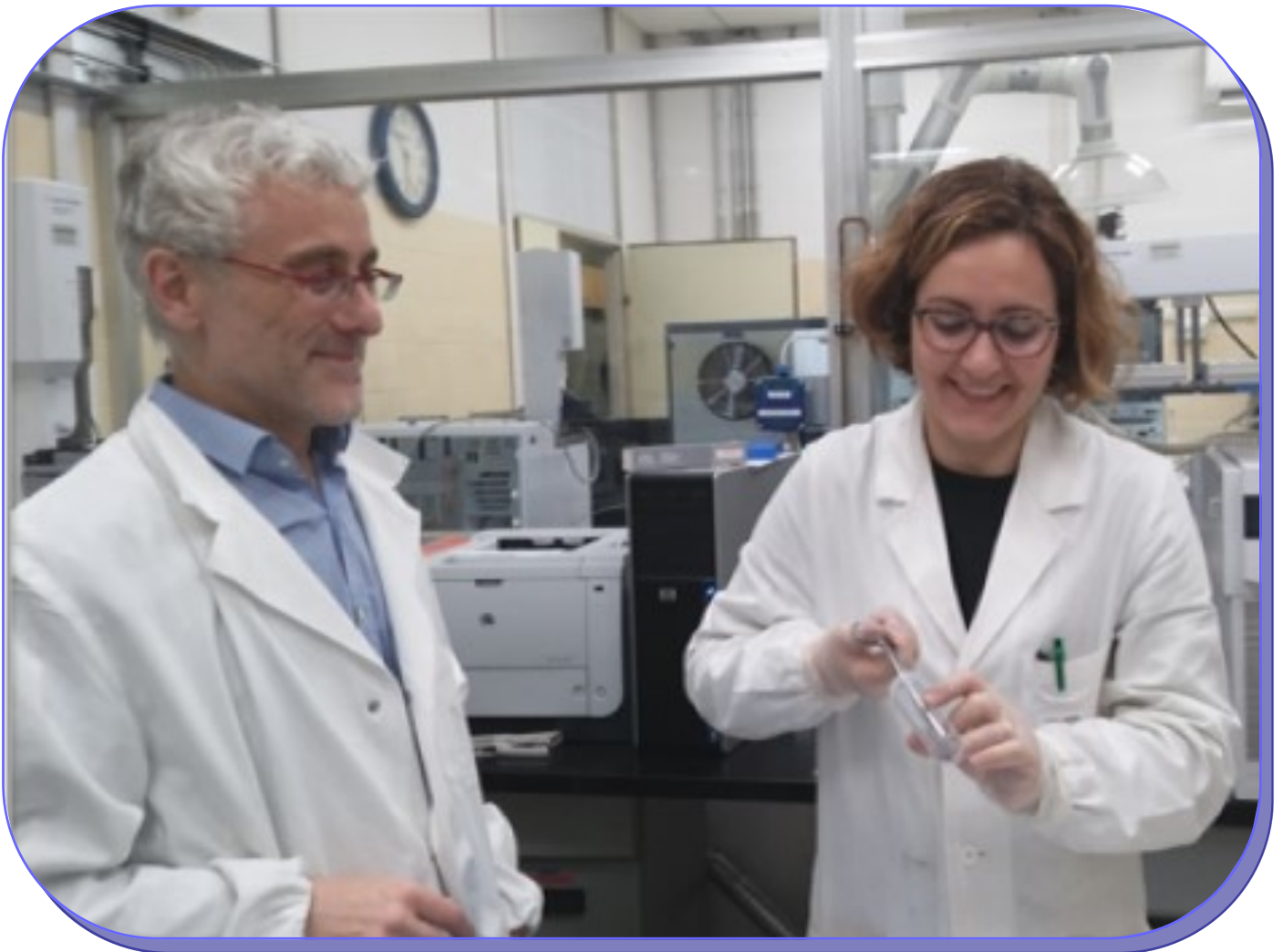


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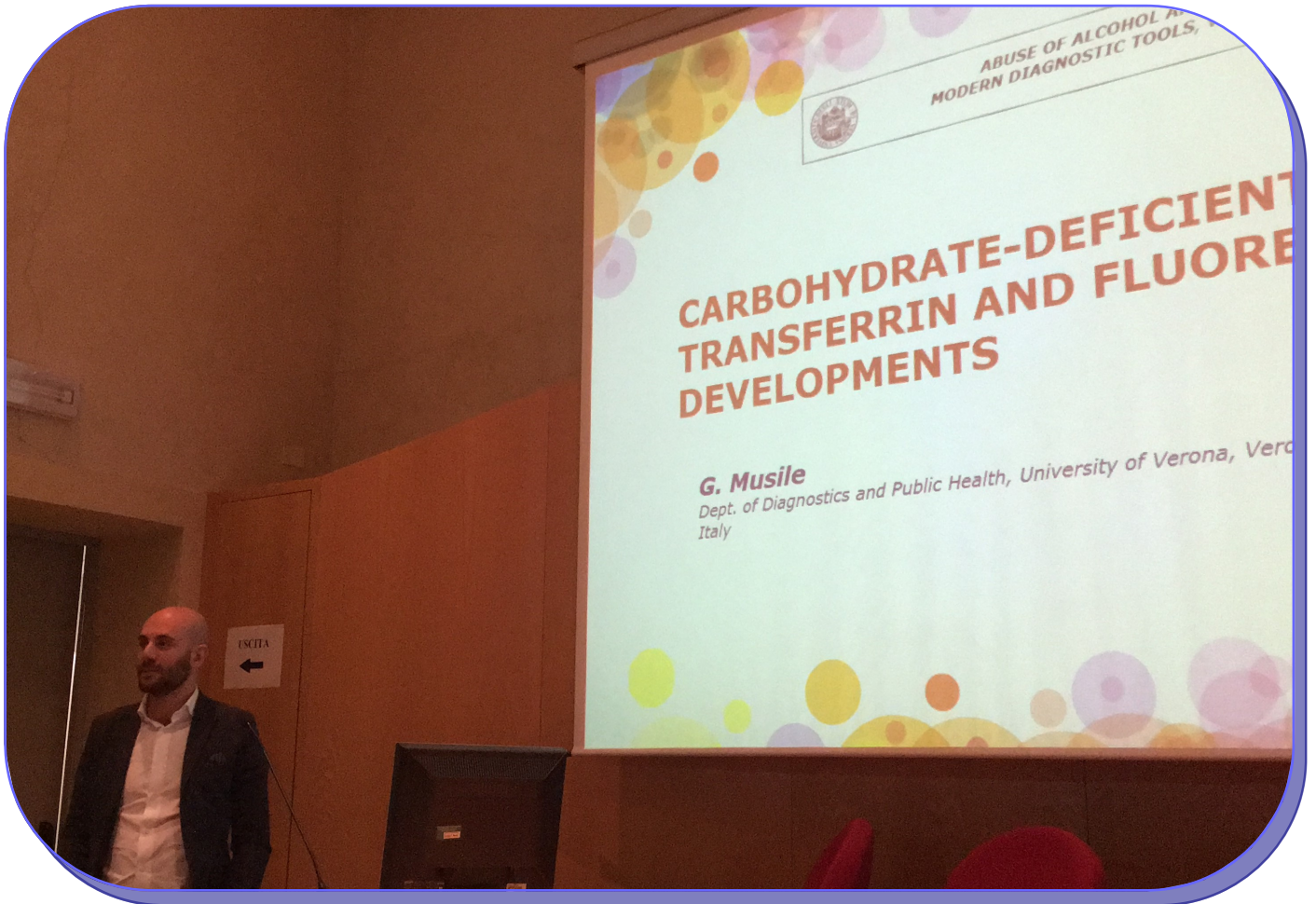


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Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Italy



## Diagnosis of alcohol consumption through the evaluation of ethyl glucuronide, a direct ethanol biomarker, in different biological matrices

Luca Morini,  
Francesca Freni, Matteo Moretti

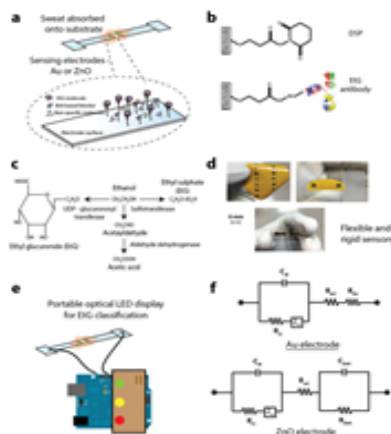
Department of Public Health, Experimental and Forensic Medicine, University of Pavia, via Forlanini 12, 27100, Pavia, Italy

Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Italy



## When and Why in sweat

- Longer kinetics compared to blood
- Concentrations 100 times lower than those measured in blood



**A wearable biochemical sensor for monitoring alcohol consumption lifestyle through Ethyl glucuronide (EtG) detection in human sweat.** *Sci Rep.* 2016 Mar 21;6:23111. doi: 10.1038/srep23111.  
Selvam AP1,2, Muthukumar S2, Kamakoti V1, Prasad S1.

**Quantitative determination of ethyl glucuronide in sweat.** *Ther Drug Monit.* 2008 Aug;30(4):536-9. doi: 10.1097/FTD.0b013e318180c83d.

Schummer C1, Appenzeller BM, Wennie R.



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### Comparison of sample preparation procedures

for the determination of ethyl glucuronide and ethyl sulfate in blood serum  
by high-performance liquid chromatography with mass-selective detection

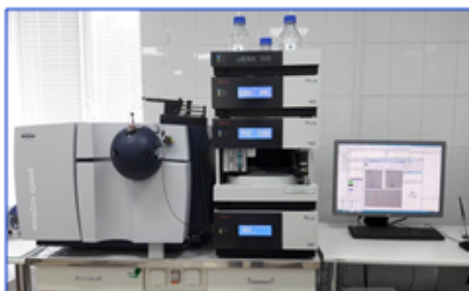
Lilyia Rizvanova

Nizhnevartovsk Psychoneurological Hospital,  
Khanty-Mansiysk Autonomous District -Yugra, Russian Federation

Verona, April 2018

## Analytical equipment and LC-MS/MS conditions

The same equipment  
for drugs/NPS analysis and EtG/EtS analysis



HPLC-MS/MS (ion trap) Bruker Toxtyper



Dionex UltiMate 3000 HPLC system coupled to an Amazon speed Bruker mass spectrometer

Capillary voltage, 4500V. Drying gas, 159°C.  
Nebulizing gas pressure, 29.3 psi

Column: Acclaim® RSLC 120 C18 2.1 x 100 mm (Dionex)  
Particle size 2.2 µm. Pore diameter 120Å. Surface area 340 m<sup>2</sup>/g.

Mobile phase A: 2mM ammonium formate, 0.1% formic acid,  
1% acetonitrile in deionized water

Mobile phase B: 2mM ammonium formate, 0.1% formic acid,  
1% deionized water in acetonitrile

Flow rate, 0.5 ml/min.

Column oven: 40 °C. Autosampler: 12 °C.

**Method for drugs and NPS  
markers**

Gradient mode:  
0-1 min 1% eluent B,  
1-8 min gradient up to 95% eluent B,  
8-9 min 95% eluent B,  
Final for 2 min 1% eluent B

MS1, MS2, MS3 (full scan)  
detection mode; mass range, 70–800  
m/z. Simultaneous registration of  
positive and negative ions.

**Method for EtG and EtS**

Isocratic mode:  
5% eluent B

The detection using MRM in  
negative registration mode:  
EtG m/z 221->203  
EtS m/z 125->97



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Sverdlovsk Regional Poison Treatment Centre, Regional Psychiatric Hospital,  
Yekaterinburg, Russian Federation

Regional Narcological Clinic, Yekaterinburg, Russian Federation



The Urals State Medical University of the Ministry of Healthcare  
of the Russian Federation



# Laboratory diagnostics of acute poison with ethanol and volatile organic compounds in Sverdlovsk region

**Mariia Gofenberg**

Head of Sverdlovsk Regional Poison Treatment Centre: A. Chekmarev

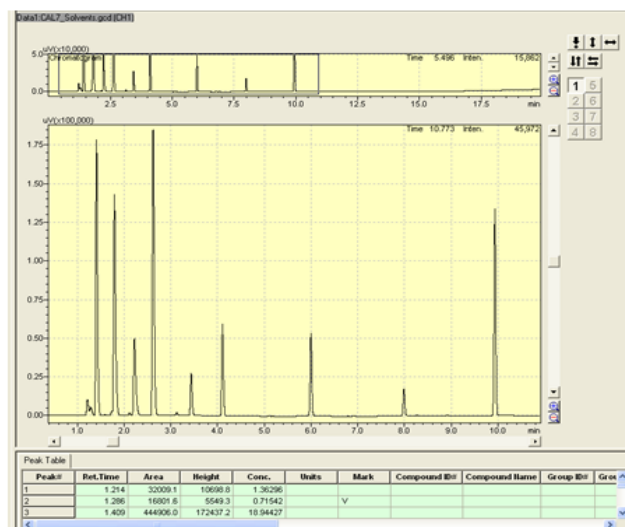
Head of Chemical-Toxicological Laboratory: I. Varlamov

## GC-FID conditions for screening for volatile organic compounds

- HP-FFAP column (Agilent, 25 m, 0.32mm i.d., and 0.5 $\mu$ m).
- Column temperature program:

Rate	Temperature	Hold Time
–	50,0	1,00
7,0	175,0	1,00

- Temperature of injector – 200 °C
- Injection mode – split 3:1
- Carrier gas – Helium
- Flow control mode – linear velocity



- Flow rate – 2 ml/min
- Temperature of detector – 250 °C
- The injection volume – 3 $\mu$ l



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THE ESTIMATION OF INTOXICATION  
DIAGNOSTICS BY SODIUM  
HYDROXYBUTYRATE, BY 1,4 BUTANEDIOL IN  
CLINICAL PRACTICE OF ACUTE POISONING  
WITH ETHANOL.

OPTIMIZATION OF THIS RESEARCH FOR  
SCREENING PURPOSES.

STUDY OF INTOXICATION BY GAMMA SALT  
OXYDUTYRATE, 1,4 BUTANEDIOL AS THE  
COMPONENT PART OF THE COMBINED  
PROGRAM CHEMICAL TOXIC EXAMINATION  
DURING THE SCREENING IN THE TREATMENT  
PRACTICE OF PATIENTS WITH ACUTE  
POISONING.

THE CITY NARCOLOGICAL CLINIC





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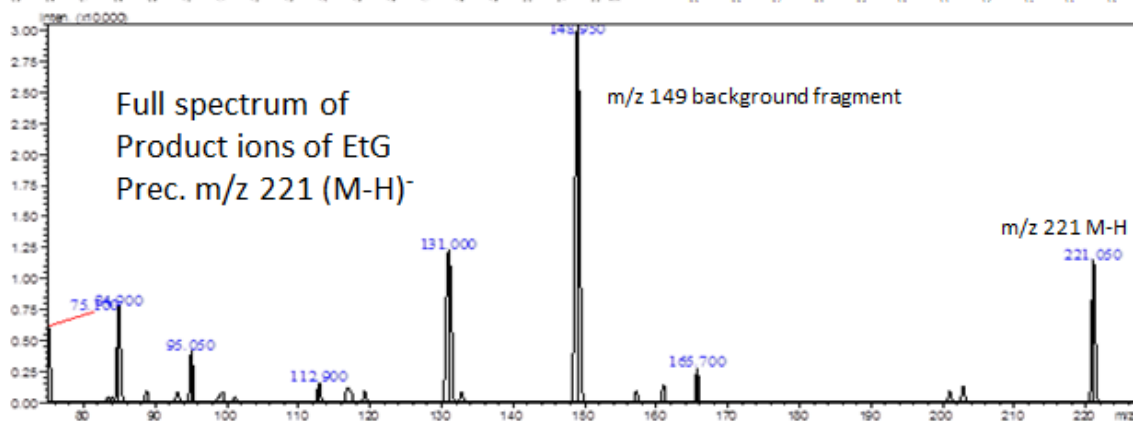
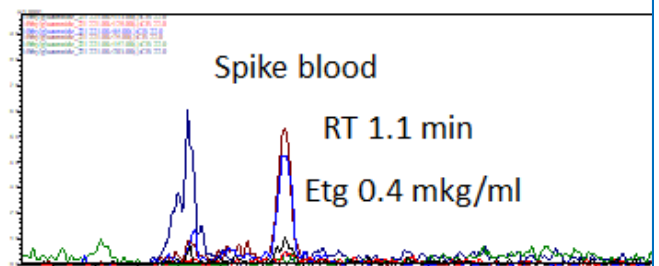
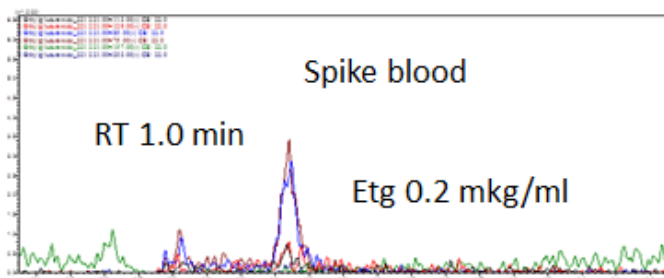
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## Differentiation of alive consumption and post-mortem formation of ethanol in the body at the forensic investigation

Sergey Savchuk, PhD, dr.sci.

- Russian center of forensic-medical expertise
- laboratory and metabolic analysis Institute of Pharmacy and Translational Medicine of Sechenov University



Background m/z 149 presented in all mass spectra of blood samples



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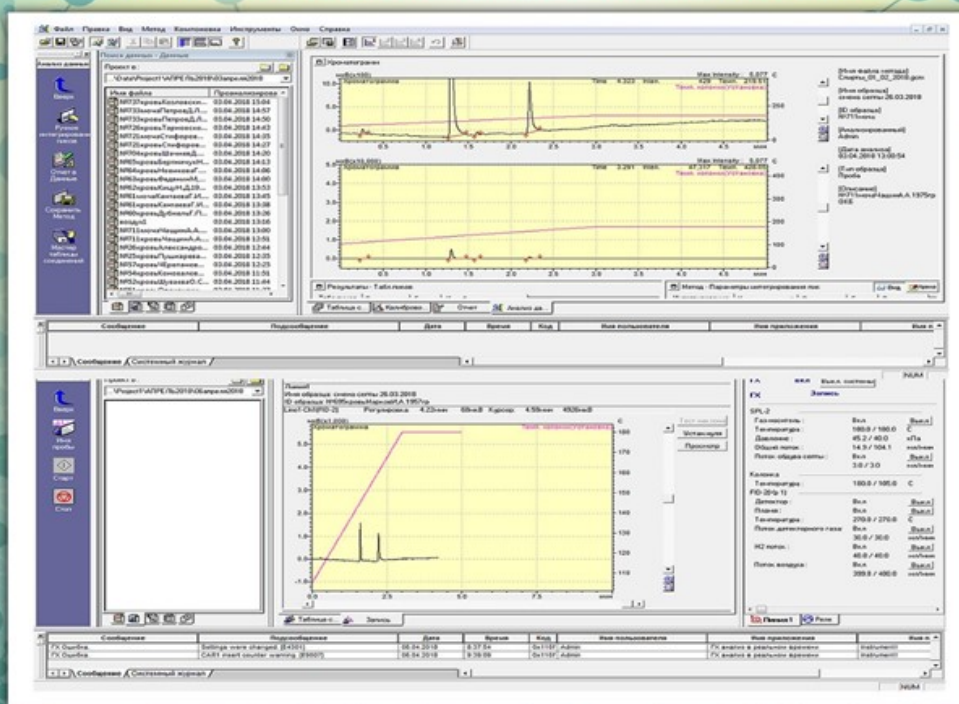
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**Forensic chemical identification of toxic agents in alcohol intoxication by volatile poisons and alcohol substitutes in Regional State Healthcare Institution.**

Pipina E.B. / Labutin A.V. / Pushkina V.V.

**Chromatograms of volatile compounds acetonitrile, butane**







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**Forensic chemical identification  
of toxic agents in alcohol intoxication  
by volatile poisons and alcohol  
in Regional State Healthcare**

Pipina E.B. / Labutin A.V. / Pus



E. PIPINA



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