ANALYTICAL DIAGNOSIS IN THE ACUTE POISONING.

EFFICIENCY EVALUATION

Ana Ferrer Dufol
DIAGNOSIS STEPS

• Clinical history
• Circumstantial evidence
• Signs y symptoms
• Antidotal essays
• Analytical tools:
  – Biochemical ==> Clinical diagnosis
  – Toxicological ==> Etiological diagnosis
• Other complementary tools: Rx, SCAN, Endoscopy
BIOMARKERS

• Direct BMs ➔ TOXICOLOGICAL ANALYSIS
  – Detection/quantification of the toxic and/or metabolites in biological fluids ➔ Biomarkers of toxic exposure

• Indirect BMs ➔ BIOCHEMICAL ANALYSIS
  – Detection/quantification of biological disfunctions produced by the toxic and/or metabolites ➔ Biomarkers of effect
BIOMARKERS

• Direct BMs ➔
  – Detection ➔ exposure
  – Quantification ➔ toxic exposure

• Indirect BMs ➔
  – Detection/quantification of biological disfunctions produced by the toxic and/or metabolites ➔ Biomarkers of effect
¿What do we need an analytical toxicology diagnosis for?

To confirm the etiologic diagnosis

To establish a prognosis as accurate as possible

To implement some therapeutic measures in some cases

IT'S FUNDAMENTAL DUE TO LEGAL AND SCIENTIFIC REASONS
¿Where and when do we need an analytical toxicology result?

To confirm the etiologic diagnosis
To establish a prognosis as accurate as possible
To implement some therapeutic measures in some cases

EFFICIENCY CRITERIA
EFFICIENCY

• Measured positive balance between efforts, in terms of energy, time or money, and benefits obtained in the performance of a task.
EFFICIENCY

- Measured positive balance between efforts, in terms of energy, time or money, and benefits obtained in the performance of a task

- Reasons
  - Limited resources
  - Risks of overuse of diagnostic measures
UNIT OF CLINICAL TOXICOLOGY

Hospital Clínico Universitario “Lozano Blesa” de Zaragoza
ARAGON

1,350,000 inhabitants
50,000 km$^2$

10 Public Hospitals
5 in Zaragoza

3 hours

1 hour
Clinical relevancy

Frequency of poisoning

Analytical availability
EPIDEMIOLOGICAL PROFILE

ED Clinic University Hospital: 1995-2010
TOTAL 17151

- suicides
- overdoses
- domestic
- occupational
- iatrogenic
- other
- uk

Abuse drugs

- ethanol
- opiates
- cocaine
- cannabis
- anfetamines

Medicines

- Bzd
- Bb
- Atd
- Nl
- AAS
- PCT
- AINES

other

- CO
- IG
- Other G
- Solvents
- Pestic
- Caust
- Mushr

other

- Bzd
- Bb
- Atd
- Nl
- AAS
- PCT
- AINES
ANALYTICAL TOOLS

• **COLORIMETRIC** (visual or spectrophotometer measurement)
  – Identification and quantification (serum or urine)
    • Trinder reaction $\Rightarrow$ ASA
    • Plasmatic cholinesterases activity $\Rightarrow$ O-P Insec.

• **ENZIMATIC** (spectrophotometer measurement)
  – Identification and quantification (serum or urine)
    • NAD-ADH $\Rightarrow$ Ethanol

• **IMMUNOENZIMATIC** (visual or spectrophotometer measurement)
  – Identification and semi-quantification (serum or urine)
    • Screening of drugs (opiates, cocaine…), therapeutic families (Bzd, Tatd)
AUTOMATIC ANALIZERS

Can be present at a moderate cost at the Emergency Labs
INSTRUMENTAL TECHNIQUES

• CHROMATOGRAPHY
  – Thin layer
  – Column
    • HPLC/MS ===> therapeutic drugs, abuse drugs
    • GC/FID ===> alcohols, solvents
    • GC/MS ===> therapeutic drugs, abuse drugs, chemicals

• SPECTROPHOTOMETRY
  – Molecular Absorption ===> lecture of colorimetric, enzymatic and immunoenzymatic techniques
  – Atomic Absorption ===> metals
<table>
<thead>
<tr>
<th><strong>Strengths</strong></th>
<th><strong>Weaknesses</strong></th>
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</thead>
<tbody>
<tr>
<td>• high clinical relevance</td>
<td>• low clinical relevance</td>
</tr>
<tr>
<td>• analytical accuracy (sensitivity,</td>
<td>• low predictive value</td>
</tr>
<tr>
<td>specificity and predictive value)</td>
<td>• methodological complexity</td>
</tr>
<tr>
<td>• methodological simplicity</td>
<td>• high economic cost</td>
</tr>
<tr>
<td>• short turnaround time</td>
<td></td>
</tr>
<tr>
<td>• low economic cost</td>
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</table>
CLINICAL CRITERIA

• ESPECIFICITY AND URGENCY OF TREATMENT
  – The analytical result modifies the therapeutic decision

• POISONING SEVERITY

• POISONING FREQUENCY
¿When and where?
LESS COMMON POISONS

Methanol   27/ 11
O-P Insecticides  35/ 7
Amanitines  11/ 6
Paraquat  6/ 3
HCN 10 / ?
ANTIDOTES

- Ethanol: 8525
- Bzd: 3040
- Cocaine: 1033
- Antidepressant: 838
- Opiates: 662
- Caustics: 448
- Amphetamines: 446
- CO: 317
- PCT: 296
ANTIDOTES

Methanol:  27/ 11
O-P Insecticides  35/ 7
Amanitines  11/ 6
Paraquat  6/ 3
HCN  10/ ??
Molecular Absorption Espectrofotometry: enzymatic technique NAD-ADH

Cromatografía de Gases / FID
SERUM ALCOHOL CONCENTRATION

\[ C_2H_5OH + NAD^+ \xrightarrow{\text{ADH}} CH_3CHO + NADH + H^+ \]

ethanol + nicotinamide adenine dinucleotide \rightarrow acetaldehyde

- ADH: Alcohol Dehydrogenase
SERUM ALCOHOL CONCENTRATION

Analytical accuracy
Simple method
Low cost
Short turnaround

[EtOH]_s > EtOH]_b
Low clinical relevance
High risk of overuse

Very high frequency ➔ Differential diagnosis

Could be installed in the Emergency Lab
BLOOD ALCOHOL CONCENTRATION

10’

ethanol

4’

isopropanol

GC/ FID
BLOOD ALCOHOL CONCENTRATION

GC/ FID

Analytical accuracy
Blood sample
Short turnaround
Gold st. for legal purpose

Complex method
Instrument high cost

Must be installed in the Toxicology Lab
Screening

Immunoenzymatic techniques (POCT)

DRUGS

Immunoenzymatic techniques (espectrofotometric lecture): EMIT, CEDIA
DRUGS

Simple method
Short turnaround
Wide range of analytes

- Very low specificity
- Very low + predictive value
- Low clinical relevance
- High risk of overuse

Not recommended to be used in any ED
DRUGS

Simple method
Low cost
Short turnaround

Low specificity
Low predictive + value
Low clinical relevance
High risk of overuse

Could be installed in the Emergency Lab
But…
DRUGS

confirmation/quantification

GC/MS

HPLC/MS
Development, validation, and application of a fast and simple GC-MS method for determination of some therapeutic drugs relevant in emergency toxicology.

Meyer MR, Welter J, Weber AA, Maurer HH.

Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, University of Saarland, Homburg (Saar), Germany. markus.meyer@uksh.de

Abstract

BACKGROUND: To date, immunoassays are commercially available for quantification of valproic acid, salicylic acid, paracetamol, phenobarbital, phenytoin, and primidone. As they are no longer available, a fast, simple, and cost-effective quantitative gas chromatography-mass spectrometry (GC-MS) method was developed and fully validated for these drugs.

METHODS: After simple and fast liquid-liquid extraction, the samples were analyzed by GC-MS using the selected ion monitoring mode. The method was validated including the parameters selectivity, calibration model, precision, accuracy, and extraction efficiency.

RESULTS: The above-mentioned analytes were separated within 8.5 minutes and sensitively detected. No interfering peaks were observed in blank samples from 8 different sources. The linearity ranges were 20-200 mg/L for valproic acid, 100-1200 mg/L for salicylic acid, 10-200 mg/L for paracetamol, 10-200 mg/L for phenobarbital, 4-20 mg/L for primidone, and 2.5-30 mg/L for phenytoin. Generally accepted criteria for accuracy and precision were fulfilled for all analytes using 6-point calibration. Even 1-point calibration was applicable for all analytes. The assay was successfully applied to analysis of real plasma samples and proficiency testing material.

CONCLUSIONS: The assay described allowed fast and reliable determination of analytes relevant in the diagnosis of poisonings. Furthermore, time- and cost-saving 1-point calibration was shown to be suitable for daily routine work, especially in emergency cases.

Remane D, Meyer MR, Wissenbach DK, Maurer HH.

Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Saarland University, Homburg, Saar, Germany.

Abstract

For fast and reliable screening, identification, and quantification of as many analytes as possible, multi-analyte approaches are very useful in clinical and forensic toxicology. Using ultra high performance liquid chromatography-tandem mass spectrometry, such an approach has been developed for blood plasma analysis after simple liquid-liquid extraction. In the present paper, validation and application is described for 31 neuroleptics, 28 benzodiazepines, and Z-drugs (zaleplone, zolpidem, and zopiclone). The validation parameters included recovery, matrix effects, process efficiency, ion suppression/enhancement of co-eluting analytes, selectivity, crosstalk, accuracy and precision, stabilities, and limits of quantification and detection. The results showed that the approach was selective, sensitive, accurate, and precise for 24 neuroleptics and 21 benzodiazepines and Z-drugs. The remaining analytes were unstable and/or too low dosed. Cost- and time-saving one-point calibration was applicable only for half of the analytes. The applicability was successfully shown for most of the drugs by analyzing authentic plasma samples and external quality control samples.

PMID: 21773738 [PubMed - indexed for MEDLINE]

Publication Types, MeSH Terms, Substances

LinkOut - more resources
DRUGS

GC/MS

HPLC/MS

Must be installed in the Toxicology Lab

+ Analytical accuracy
  Broad spectrum
  Gold st. for legal purpose

- Complex method
  Instrument high cost
Bzd and Opiates

- Diagnosis though clinical criteria
- Therapeutic decisions fully independent of analytical results
- Typical cases of antidote’s assay

\[ [\text{Bzd}]_s > 5000 \text{ ng/mL doesn't imply the use of Flumazenil} \]

A respiratory arrest caused by opiates doesn't allow waiting for any analytical result
CO

• ANTIDOTE ➔ Oxygen 100% or hiperbaric

• CLINIC AND ANALYTIC CRITERIA ➔

COHb % is a good biomarker for CO poisoning
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<th>Uds.</th>
<th>Measure Range</th>
<th>Test Range</th>
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<td>pH</td>
<td>escala pH</td>
<td>6.300-8.000</td>
<td>6.85-7.55</td>
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<tr>
<td></td>
<td>nmol/L</td>
<td>10.0-501</td>
<td>28-141</td>
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<tr>
<td></td>
<td>mmol/L</td>
<td>5.0-250</td>
<td>17-160</td>
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<tr>
<td></td>
<td>kPa</td>
<td>0.67-33.3</td>
<td>2.27-21.3</td>
</tr>
<tr>
<td></td>
<td>torr</td>
<td>5.0-250</td>
<td>17-160</td>
</tr>
<tr>
<td></td>
<td>mmHg</td>
<td>0.00-107</td>
<td>2.67-77.3</td>
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<tr>
<td></td>
<td>kPa</td>
<td>0.00-800</td>
<td>20-580</td>
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<tr>
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<td>0.0-800</td>
<td>20-580</td>
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<td>pCO₂</td>
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<td>0.67-33.3</td>
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<td>torr</td>
<td>0.0-800</td>
<td>20-580</td>
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<tr>
<td>Electrolites</td>
<td>cCl⁻</td>
<td>mmol/L</td>
<td>7-350</td>
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<tr>
<td></td>
<td>meq/L</td>
<td>7-350</td>
<td>95-150</td>
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<tr>
<td></td>
<td>mmol/L</td>
<td>0.20-9.99</td>
<td>0.51-2.2</td>
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<td></td>
<td>meq/L</td>
<td>0.40-19.98</td>
<td>1.0-4.4</td>
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<tr>
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<td>2.0-8.8</td>
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<tr>
<td></td>
<td>mmol/L</td>
<td>0.5-25.0</td>
<td>2-8</td>
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<tr>
<td></td>
<td>meq/L</td>
<td>0.5-25.0</td>
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<td>mg/dL</td>
<td>7-350</td>
<td>120-180</td>
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<td>120-180</td>
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<tr>
<td></td>
<td>mmol/L</td>
<td>0.0-60</td>
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<td></td>
<td>mg/dL</td>
<td>0-1081</td>
<td>9.0-270</td>
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<tr>
<td></td>
<td>mmol/L</td>
<td>0.0-30</td>
<td>0.5-15</td>
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<td>0.5-15</td>
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<td>0-1081</td>
<td>9.0-270</td>
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<td>mmol/L</td>
<td>0.0-30</td>
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<td>0.0-30</td>
<td>0.5-15</td>
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<tr>
<td></td>
<td>µmol/L</td>
<td>10-1800</td>
<td>50-1500</td>
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<tr>
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<td>mg/dL</td>
<td>0.1-20.3</td>
<td>0.57-17.0</td>
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<tr>
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<td>µmol/L</td>
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<td>0-400</td>
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<td>mg/dL</td>
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<td>0.0-23.4</td>
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<td>mg/L</td>
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<td>Oximetry</td>
<td>ctHb</td>
<td>g/dL</td>
<td>0.00-27.7</td>
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<tr>
<td></td>
<td></td>
<td>mmol/L</td>
<td>0.00-17.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>g/L</td>
<td>0.0-277</td>
</tr>
<tr>
<td>-</td>
<td>sO₂</td>
<td>%</td>
<td>0.0-100.0</td>
</tr>
<tr>
<td></td>
<td>Fraction</td>
<td></td>
<td>0.000-1.000</td>
</tr>
<tr>
<td>-</td>
<td>FO₂Hb</td>
<td>%</td>
<td>0.0-100.0</td>
</tr>
<tr>
<td></td>
<td>Fraction</td>
<td></td>
<td>0.000-1.000</td>
</tr>
<tr>
<td>-</td>
<td>F COHb</td>
<td>%</td>
<td>0.0-100.0</td>
</tr>
<tr>
<td></td>
<td>Fraction</td>
<td></td>
<td>0.000-1.000</td>
</tr>
<tr>
<td>-</td>
<td>F MetHb</td>
<td>%</td>
<td>0.0-100.0</td>
</tr>
<tr>
<td></td>
<td>Fraction</td>
<td></td>
<td>0.000-1.000</td>
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<tr>
<td>-</td>
<td>FHHb</td>
<td>%</td>
<td>0.0-100.0</td>
</tr>
<tr>
<td></td>
<td>Fraction</td>
<td></td>
<td>0.000-1.000</td>
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<tr>
<td>-</td>
<td>FHbF</td>
<td>%</td>
<td>0.0-100</td>
</tr>
<tr>
<td></td>
<td>Fraction</td>
<td></td>
<td>0.00-1.00</td>
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</table>
Must be installed in the Emergency Lab

Must be installed in the triage point and in emergency ambulances
PARACETAMOL

PCT

GLYCO-CONJUGATES

SULPHO-CONJUGATES

NAPBQ + GLUTATHIONE

+ MACROMOLECULES

HEPATOCYTES

NAC

CYTOLISIS
GUIDELINES TREATMENT FOR PCT POISONING

- Serum PCT concentration (4 h. post ingestion)
  - \([\text{PCT}] < \text{line100} \Rightarrow \text{stop antidote}\)
  - \([\text{PCT}] \text{ between line 100 and 150} \Rightarrow \text{Patients with presumed enzymatic induction or glutathione depletion} \Rightarrow \text{Full antidote course}\)
    - \(\Rightarrow \text{Non risk patient} \Rightarrow \text{stop antidote}\)
  - \([\text{PCT}] > \text{line 150} \text{ or repeated supra-therapeutic doses} \Rightarrow \text{Full antidote course}\)

Biological signs of hepatic toxicity \(\Rightarrow\) antidote maintenance course
PCT

- Simple method
- Low cost
- Short turnaround
- High clinical relevance

+ Analytical interferences
- Low frequency

Must be installed in the Emergency Lab
LES S COMMON POISONS

Methanol: 27/11
O-P Insecticides 35/7
Amanitines 11/6
Paraquat 6/3
HCN 10/??
**METHANOL**

- **ANTIDOTES** (ethanol and fomepizole)

\[
\text{CH}_3\text{CH}_2\text{OH} \quad \rightarrow \quad \text{CH}_3\text{CHOH}
\]

\[
\text{CH}_3\text{OH} \quad \leftrightarrow \quad \text{CH}_2\text{O} \quad \rightarrow \quad \text{HCOOH}
\]
METHANOL

- Direct Biomarker ➔ Methanol quantification
- Effect Biomarkers ➔
  - pH
  - Anion gap
  - Osmolal gap
- Treatment monitoring ➔ ethanol quantification
Methanol/Ethanol evolution

- Blood methanol
- Blood ethanol

Time (h)
Methanol and metabolism

Ethanol

Methanol

Ethylene glycol

Alcohol dehydrogenase

Formaldehyde

Acetaldehyde

Glycol aldehyde

CO₂ + H₂O

Folnic acid

10-formyl tetrahydrofolate synthetase

Calcium oxalate

Knut Erik Hovda & Ken McMartin
NAACT 2007

Aldehyde dehydrogenase

Glycolic acid

Glycater oxidase

SERUM FORMATE CONCENTRATION

\[ \text{FDH} \]

\[
\text{HCOOH} + \text{NAD}^+ \rightleftharpoons \text{CO}_2 + \text{NADH} + \text{H}^+
\]

formate
LESS COMMON POISONS

Methanol: 27/11
O-P Insecticides 35/7
Amanitines 11/6
Paraquat 6/3
HCN 10/??
O-P INSECTICIDES

- Direct Biomarker ➔ chromatographic identification/quantification
- Effect Biomarkers ➔
  - Cholinesterases activity
LESS COMMON POISONS

Methanol: 27/11
O-P Insecticides 35/7
Amanitines 11/6
Paraquat 6/3
HC 10
Mushrooms poisoning
1982 - 2011

419 episodes
(1028 patients)

Dr. J. Piqueras
Hospital Valle de Hebrón
Barcelona
AMANITA PHALLOIDES

• BIOMARKER: Amanitine quantification

• Wieland colorimetric reaction
LESS COMMON POISONS

Methanol: 27/11
O-P Insecticides 35/7
Amanitines 11/6
Paraquat 6/3
HCN 10
• BIOMARKER: Paraquat quantification

• Na ditionite colorimetric reaction
LESS COMMON POISONS

Methanol: 27/11
O-P Insecticides 35/7
Amanitines 11/6
Paraquat 6/3
HCN 10/??
HCN

• BIOMARKER ➔ CN⁻ quantification
• Thiosulphate quantification

BUT

! ABSOLUTE URGENCY !

Antidote treatment by clinical criteria
• **IN WHICH POISONINGS YOU SHOULD MAKE AN ANALYTICAL DIAGNOSIS?**
  – We should confirm all systemic poisonings

• **IN WHICH CASES IT SHOULD BE MADE URGENTLY?**
  – In those in which it changes significantly relevant therapeutic decisions

<table>
<thead>
<tr>
<th>Emergency Lab</th>
<th>Toxicology Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>Methanol</td>
</tr>
<tr>
<td>COHb / MetHb</td>
<td></td>
</tr>
<tr>
<td>Cholinesterases</td>
<td>24/24 h</td>
</tr>
<tr>
<td>Litium</td>
<td></td>
</tr>
<tr>
<td>Digoxine</td>
<td></td>
</tr>
</tbody>
</table>
• Toxicology Laboratory (normal working hours)

  \[
  \begin{align*}
  & \text{GC/FID} \quad \text{Ethanol} \\
  & \text{EIA/ Colorimetric} \quad \text{Drugs} \\
  & \text{GC/MS} \quad \text{Medicines}
  \end{align*}
  \]

• External reference Laboratory (normal working hours)

  \[
  \begin{align*}
  & \text{Amanitines} \\
  & \text{Metals}
  \end{align*}
  \]
UK NPIS/ACB

24h in all acute hospital
- Carbohy/MethHb
- Digoxine
- Ethanol
- Iron
- Li
- PCT
- Paraquat
- Salylate
- Theophyline
- Valproate

specialis, infrequent assays
- Arsenic
- Carbamazepine
- Cholinesterase
- Ethylene Glycol
- Lead, Mercury
- Methanol
- Methotrexate
- Paraquat (quantif)
- Phenobarbital, Phenytoin
- Thallium
- Tyroxine
WHAT WE CURRENTLY DO

24h in all acute hospital
- Carbohy/MethHb
- Digoxine
- Ethanol
- Iron
- Li
- PCT
- Paraquat
- Salylate
- Theophyline
- Valproate

specialis, infrequent assays
- Arsenic
- Carbamazepine
- Cholinesterase
- Ethylene Glycol
- Lead, Mercury
- Methanol
- Methotrexate
- Paraquat (quantif)
- Phenobarbital, Phenytoin
- Thallium
- Tyroxine
This study could be carried out in other countries to establish what analyses are available for the treatment of poisoned patients.
CONCLUSION

• All acute systemic poisoning counting with specific biomarkers should be confirmed by means of toxicological analysis.

• Before getting the analytical confirmation diagnosis and treatment of most current acute poisoning should be performed including “strong” antidotes as ethanol or atropine.

• Every health care level has to decide the panel of required analytical devices to meet its analytical needs following the mentioned criteria of usefulness and cost-effectiveness.
THANKS FOR YOUR ATTENTION

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