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Résumés des communications
Abstracts

1. Evidence based point of care testing for drugs and ethanol

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Point of Care Testing [POCT] has been adopted in many clinical situations because its immediacy. POCT for drugs has almost exclusively been focussed on substance abuse either in clinical workplace or legal situations. The devices typically are for use in urine and are presented as cartridges or strips; the former may be for single or multiple substances in a fixed combination.

The use of these devices is covered by the IVD [in Vitro Diagnostic] Devices regulations [ISO 15189 Appendix D; ISO 22870], which address training, quality, service organisation, etc. However the obvious characteristic of POCT devices is the unit cost i.e. the widespread use of POCT devices is costly. For drugs the devices are immunoassay based and therefore any positives are presumptive and must be confirmed by mass-spectrometry. POCT can be viewed as a method of immediate screening, which may provide sufficient 'evidence' for an action to be taken or withheld.

Amazingly there has to date been no attempt to systematically investigate the evidence-base for POCT in general and drugs in particular. The National Academy of Clinical Biochemistry [USA] is developing such evidence. I present to you today the information my expert panel has adduced following a systematic search of the literature. This may be summarised as: there is little evidence that POCT for drugs affects outcomes. However we are of the view that 'absence of evidence is not evidence of absence'. We seek informed, substantiated, peer-reviewed evidence to aid our deliberations. Our draft is available for comment on http://www.nacb.org/lmpg/poct/chp7_abuse.doc and we would be delighted if you would contribute to our

information. The slides from this presentation are available on:
http://www.nacb.org/lmpg/am04/Drug_Testing_Group_Watson.pdf

2. A rapid and sensitive liquid chromatography-apci-tandem mass spectrometry method for the determination of amphetamine and related designer drugs in urine

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A method for the direct analysis of six amphetamine compounds in urine was developed using liquid chromatography tandem mass spectrometry (LC-MS/MS). Amphetamine and related designer drugs included were: amphetamine (AMP), metamphetamine (MET), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymetamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB).
Deuterated internal standards used were d_5-AMP, d_5-MET, d_5-MDA, d_5-MDMA and d_5-MBDB. Samples were prepared by addition of 90 µl of the internal standard solution (1 µg/ml of each d_5-deuterated analogue) to 10 µL urine, followed by centrifugation and direct injection into LC-MS/MS. Chromatographic separation was performed on a reversed phase C18-column using gradient elution. The LC-MS/MS system was equipped with an APCI interface. Detection of the amphetamine compounds was carried out in the multiple reaction monitoring mode, using two MRM transitions for each analyte. Separation and detection of all compounds was acclompished within eight minutes. Linearity was established for all compounds, from 78 to 100000 ng/mL. Correlation coefficients (r) for all analytes exceeded 0.998. The lower limit of quantification was 10 ng/ml for all compounds, except for AMP and MDA (78 ng/mL). Within-day imprecision (CV %) and between-day CVs ranged from 2.62 to 16.26 % and from 0.86 to 11.98 %, respectively. Accuracy (bias %) lay between 0.16 and 7.17 %. The peak areas of the amphetamines added to urine fell in the range 85-115 % compared to the standard solutions in methanol/water, except for AMP and MDA. Carry-over was negligible and stability of the vials in the autosampler at room temperature for up to 24h was acceptable. In conclusion, the presented method allows the accurate, precise and rapid determination of six amphetamine compounds in urine samples over a wide analytical range after a minimal sample preparation.

3. Clinical comparison of IMx® Sirolimus (MEIA) assay with LC-MS/MS reference method

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Introduction: Sirolimus (Rapamune®, rapamycin) is an immunosuppressive drug indicated for renal transplant immunosuppressive therapy, usually in synergy with cyclosporin. Monitoring of sirolimus in post-transplant patients is important for assessing the risk of drug toxicity.

The semiautomated IMx method incorporates microparticle enzyme immunoassay (MEIA) for sirolimus measurement in whole blood, making monitoring possible without the need for specialised and expensive laboratory equipment (e.g. LC-MS/MS).

Our goal was to evaluate the performance of the MEIA assay in a routine laboratory setting in comparison with a liquid chromatography-tandem mass spectrometry (LC-MS/MS) reference method.

Materials and methods: The IMx MEIA analysis was performed according to the manufacturer's instructions. The LC-MS/MS measurement was on an API 2000 system (Applied Biosystems) with an Atlantis C18 column (2,1 x 20mm). Analytical performance (within- and between-run CV) of the IMx assay was assessed and the results of 126 consecutively collected routine whole blood specimens from renal transplant recipients (n=67; age 54.1 ± 24.4 years, mean ± 2sd) were correlated.

Results: Measurement of imprecision of the MEIA method (%CV) gave the following results:

	Within-run (n=10)		Between-run (n=10)		
Mean (ng/mL)	5,2	9,8	4,9	10,9	28,0
SD	0,05	0,44	0,43	0,29	1,80
CV (%)	0,9	4,4	8,7	2,6	6,4

The range of concentrations in whole blood samples was 1,1-30,0 and 0,5-35,4 for MEIA and LC-MS/MS, respectively. Passing-Bablok linear regression analysis yielded the equation: MEIA = 0,54 + 1,04 x LC-MS/MS (r = 0.80). Overall MEIA results were on average 11 % higher.

Conclusion: Our results for imprecision and bias are comparable to, even better than, those found in literature. So, in a routine drug monitoring laboratory setting, the IMx® Sirolimus (MEIA) assay yields comparative results to the reference LC-MS/MS method offering an easier and faster sample preparation resulting in a shorter turn-around-time.

4. Analysis of γ-hydroxy butyric acid, dl-lactic acid, glycolic acid, ethylene glycol and other glycols by a direct injection gas chromatography mass spectrometry assay for wide use

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Analysis of blood of severely intoxicated patients always requires a prompt investigation. Diagnosis of intoxications with ethylene glycol, γ-hydroxybutyric acid or d-lactic acid takes hours since several different procedures have to be started. A fast derivatisation of the common hydroxyl function may resolve this analytical problem.

Here we describe a fast method for simultaneous measurement of ethylene glycol, glycolic acid, γ-hydroxybutyric acid and racemic lactic acid. Only 20 µL of serum, plasma or urine is immediately derivatised at 70°C with 750 µL of bis-N,O trimethylsilyl trifluoroacetamide after adding 20 µL of the internal standard solution (1,3-propylene glycol) and 20 µL of the catalyst dimethylformamide. After centrifugation an aliquot is transferred to the gas chromatographic system and analysed with electron impact mass spectrometry in selective ion monitoring mode.

The derivatised acids and ethylene glycol are well separated and detected with a good sensitivity. The method is linear from 0.5 to 1800 mg/L blood for ethylene glycol, from 0.7 to 1200 mg/L for lactic acid, from 1.2 to 1800 mg/L for glycolic acid, and from 3.2 to 200 mg/L for γ-hydroxybutyric acid with analytical recoveries, accuracy, day to day and within day precision well within required limits. Total analysis time with one calibrator was 30 min, derivatisation time included.

This method is very suitable for emergency toxicology since several toxic substances can be quantified simultaneously in a fast and sensitive manner.

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5. The myth of the Fates challenged: five times poisoned and still the thread of life couldn't be cut. A case report and comment on a present drug of abuse problem.

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Introduction: In Greek mythology our destiny lies in the hands of the Fates. There are three Fates. Clotho, who spins the thread of life. Lachesis, who chooses the lot in life and measures off how long it is to be. Atropos, she who cannot be turn, who at death with her shears cuts the thread of life. Atropine (Atropos) is only used as an oral drug for special psychiatric indications. Atropine as a cause of severe anticholinergic poisoning therefore is rare. We describe a case of an 82-year-old woman with repetitive severe anticholinergic poisoning where the toxidrome was only recognized at the third presentation and the cause of poisoning after the fifth presentation.

Case report: a 82-year-old woman was admitted in October 2001 for reasons of dizziness and transient dysarthria. During hospitalisation an episode of "convulsions" of arms and legs, dysarthria, stupor, itching and plethora developed and diagnosed as a transient ischemic attack. A myxoma in the left atrium was found and designated as embolic focus. Cardiac surgery for myxoma removal was performed. One day after discharge the patient was readmitted with fever, stupor, dysarthria, and restless legs. Vital signs were: pulse 110/min, blood pressure 200/80 mmHg, respiration 30/min, and temperature 38°C. Dilated pupils, extreme restlessness, itching and plethora of the face were noticed. Differential diagnosis included: postoperative sepsis, encephalitis and epilepsy. She needed to be sedated with a propofol infusion. All investigations (labs, brain imaging, cerebrospinal fluid analysis, tox screen) were normal and the patient recovered completely the next morning. After two weeks on neurology without new signs she was discharged with a diagnosis of "transient ischemic attack". The next day she was admitted again with the same clinical signs. Now the toxidrome pentad: "hot as a hare", "blind as a bat", "dry as a bone", "red as a beet" and "mad as a wet hen" was recognized and a diagnosis of anticholinergic poisoning was stated although neostigmine 2 mg iv changed nothing. She recovered after 12 hours. Despite repetitive questioning and toxicological screening for medication with anticholinergic properties no cause could be found. After another two admissions the family finally remembered an old prescription for headache containing caffeine 100 mg and atropine 10 µg pro tablet. Toxicological analysis of these recently renewed tablets showed 10 mg atropine pro tablet. There was a 1000 fold concentration mistake made by a pharmacist.

Conclusion: This case report shows the high cost and morbidity due to not recognizing a toxidrome and withholding drug intake information by patient and family. Note that presently in Belgium, Holland, France and Italy there have been numerous case reports of severe anticholinergic toxidroma due to cocaine intake "contaminated" with atropine.