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The promise of microsampling, delivered

the microsampling workshop

Application of Mitra Microsampling for the quantification of antibiotics: possible use for therapeutic drug monitoring in pediatrics

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Certificazione ISO 9000 n IT231277
Accreditamento Joint Commission International dal 01/12/2007

Istituto Giannina Gaslini

Istituto Giannina Gaslini

We are here!

Why do we need to measure antibiotics levels in children?

For the individualization of antimicrobial therapy, especially in critically ill patients

C. Adembri, A. Novelli. Clin Pharmacokinet. 48 (2009) 517–28.

R. Admiraal, C. van Kesteren, J.J. Boelens. Arch Dis Child. 99 (2014) 267-272.

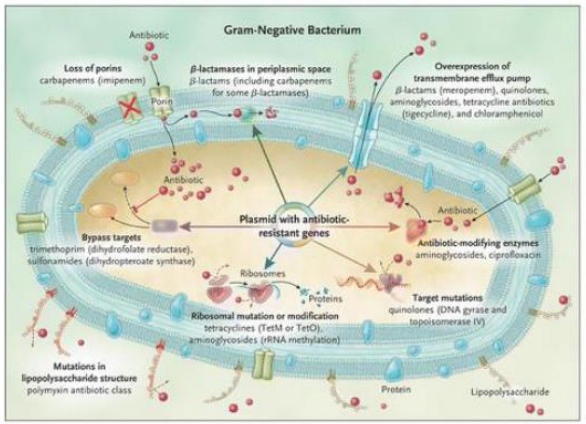
Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America

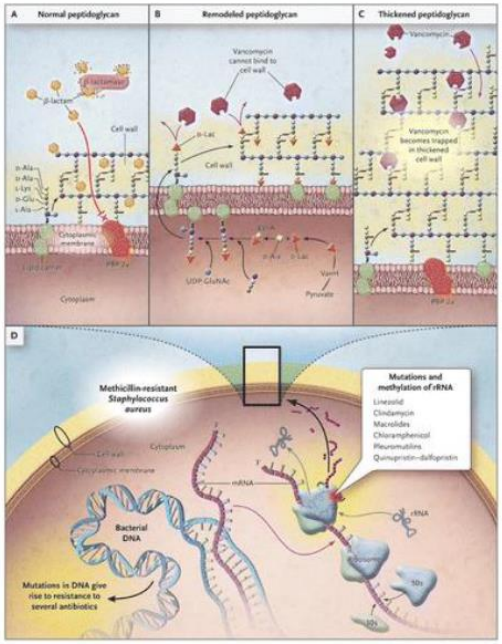
Clinical Infectious Diseases 2009; 48:1–12

1058-4838/2009/4801-0001$15.00
DOI: 10.1086/595011

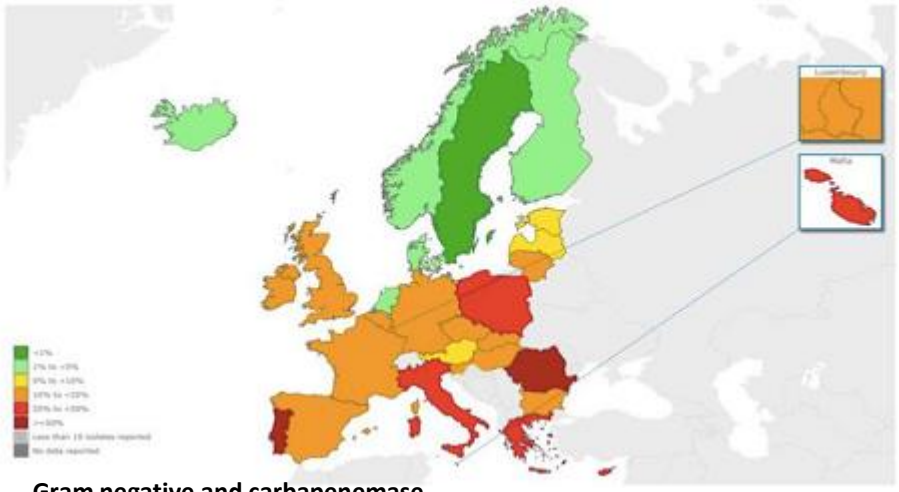
Clinical Infectious Diseases 2009; 49:992–3

1058-4838/2009/4906-0031$15.00
DOI: 10.1086/605539

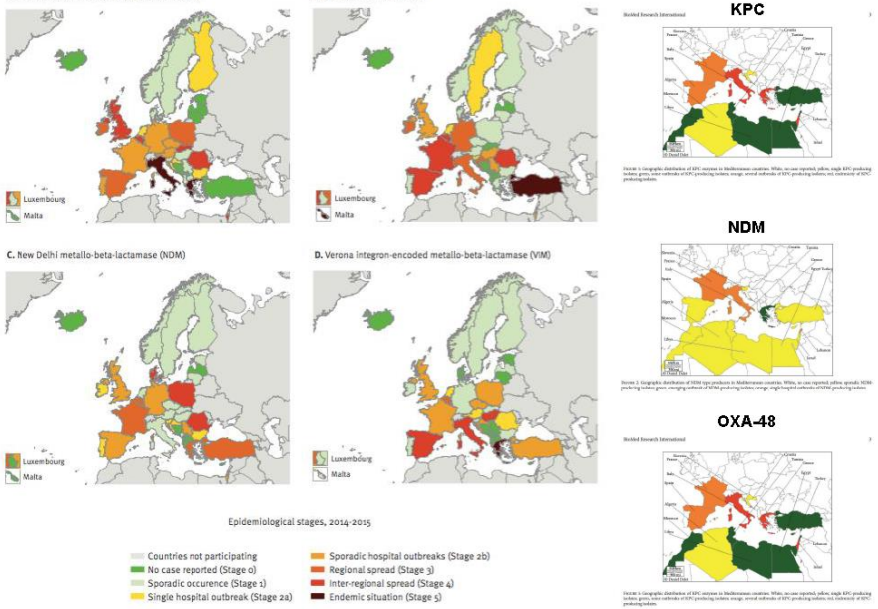




***Staphylococcus aureus*: percentage (%) of invasive (blood and cerebrospinal fluid) isolate resistant to meticillin (MRSA), EU/EEA, 2012**



Gram negative and carbapenemase



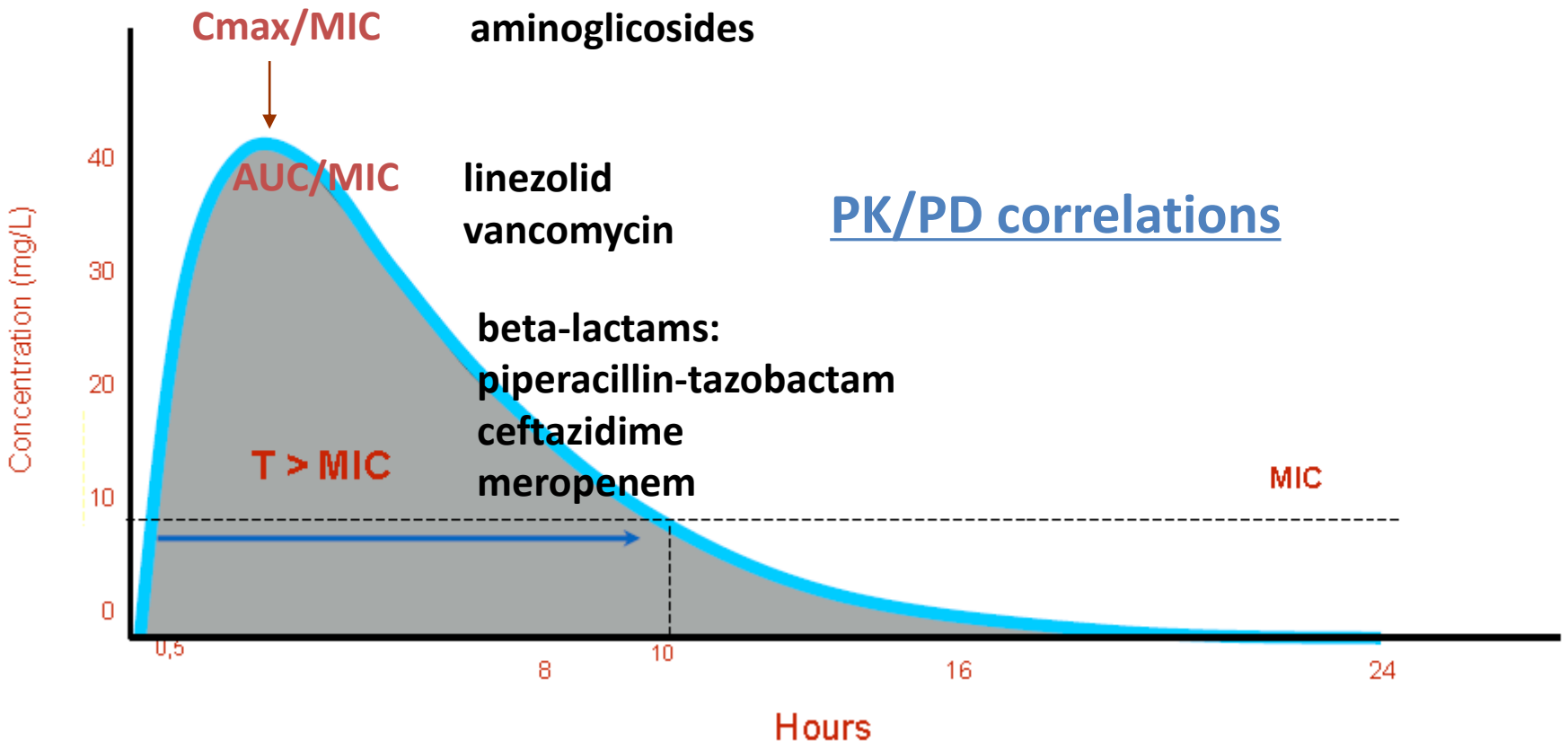
KPC

NDM

OXA-48

In the absence of/waiting for new drugs…

PK and PD relationships of antibiotics need to be explored in children



Andes D. Infect Dis Clin N Am 2003;17:635-49

NEJM 2003; 349: 1157-67

TDM in pediatrics: critical issues

- ✓ The criteria for monitoring drugs in children are the same as those for adults but several additional factors must be considered.
- ✓ Different clinical pharmacokinetic (ADME) and pharmacodynamic parameters from adults.
- ✓ Off-label use of drugs: approximately 10% of all drugs prescribed are for children
- ✓ Co-medications: the average number of drugs administered in neonatal intensive care units to premature infants < 1Kg is usually in the range of 15-20; infants <2,5 Kg usually receive 4 -10 drugs during their hospital stay.

Drug concentrations in many of these patients need to be monitored by the laboratory, and the possibility of drug interactions needs to be considered.

Availability of a reliable analytical method

"General" requirements

- ✓ High specificity
- ✓ High sensitivity
- ✓ High accuracy
- ✓ High reproducibility
- ✓ High throughput
- ✓ Fast turn Around Times
- ✓ Low costs

"Special" requirements for pediatrics

- **Higher sensitivity** due to the **low volume of available samples (especially for very low birth weight infants)**
- A **dynamic range** that can accommodate a wide range of analyte concentrations in a heterogeneous patient population that ranges from 0 to 18 years
- Sufficient **robustness** to withstand the non-standard matrix effects encountered in hemolytic, lipemic, icteric, and hyperuricemic samples
- Possibility to use "**non conventional matrices**": dried matrices, saliva, cerebrospinal fluid…

The answer is:

Microsampling coupled to LC-MS/MS!

VAMS- LC-MS/MS assay for the simultaneous quantification of four antibiotics in human blood: piperacillin-tazobactam, meropenem, linezolid and ceftazidime. Method development, validation and comparison with dried blood spot

Sebastiano Barco, Elio Castagnola, Andrea Moscatelli, James Rudge, Gino Tripodi and Giuliana Cangemi.

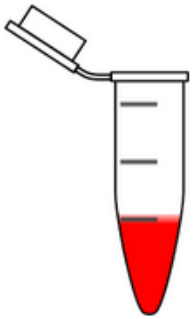
Submitted to Journal of Pharmaceutical and Biomedical Analysis

+ 50 µl H_2O

10 min 37° C

+ 200 µl MeOH

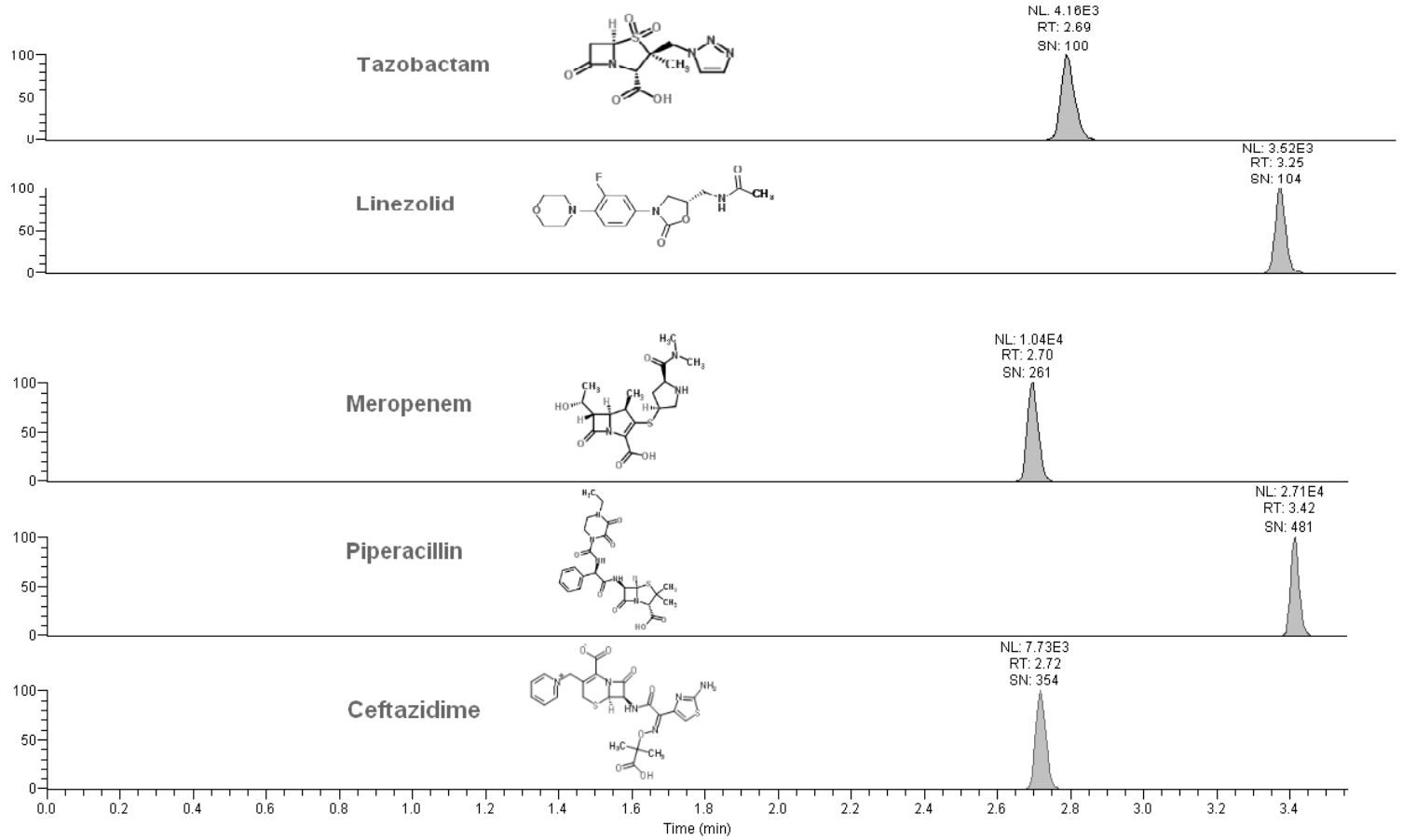
10 min sonication



30 min

Upper phase collection

Injection


Tazobactam
NL: 4.16E3
RT: 2.69
SN: 100
Linezolid
NL: 3.52E3
RT: 3.25
SN: 104
Meropenem
NL: 1.04E4
RT: 2.70
SN: 261
Piperacillin
NL: 2.71E4
RT: 3.42
SN: 481
Ceftazidime
NL: 7.73E3
RT: 2.72
SN: 354
100
50
0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
Time (min)

VAMS-LC-MS/MS method validation following EMA** and FDA*** guidelines

by using cal and QCs at HCT 45%

- ✓ Selectivity
- ✓ Carry over
- ✓ Matrix effect and extraction recoveries
- ✓ Linearity
- ✓ Precision and accuracy
- ✓ LLOQ
- ✓ Short term (72 h) and long term (one month)stability of QC_{low}, and QC_{high} 25° C, 4° C and -20° C
- ✓ Freeze-thaw sample stability (three freeze and thaw cycle)
- ✓ Stability of extract samples was calculated after maintaining extracts at 25° C for over 24 hours
- ✓ Stock solution stability

**Guidance for industry: Bioanalytical Method Validation. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), Rockville, USA (2013).
*** Guideline on bioanalytical method validation, European Medicines Agency, London, UK (2011).

Validation results (1)

Table 1. Results of matrix effect (ME) and extraction recovery (ER) experiments at HCT 45%

Drug	VAMS				DBS			
	QC_{low}		QC_{high}		QC_{low}		QC_{high}	
	ER%	ME%	ER%	ME%	ER%	ME%	ER%	ME%
Piperacillin	86	109	90	107	57	98	66	86
Tazobactam	88	101	83	110	59	95	61	90
Linezolid	94	111	83	95	76	106	75	94
Meropenem	86	95	78	109	50	107	43	93
Ceftazidime	103	104	86	97	55	110	55	86

Validation results (2)

Table 2. Results of accuracy and precision obtained for VAMS and DBS at HCT 45%. (CV=coefficient of variation, E%= mean relative error)

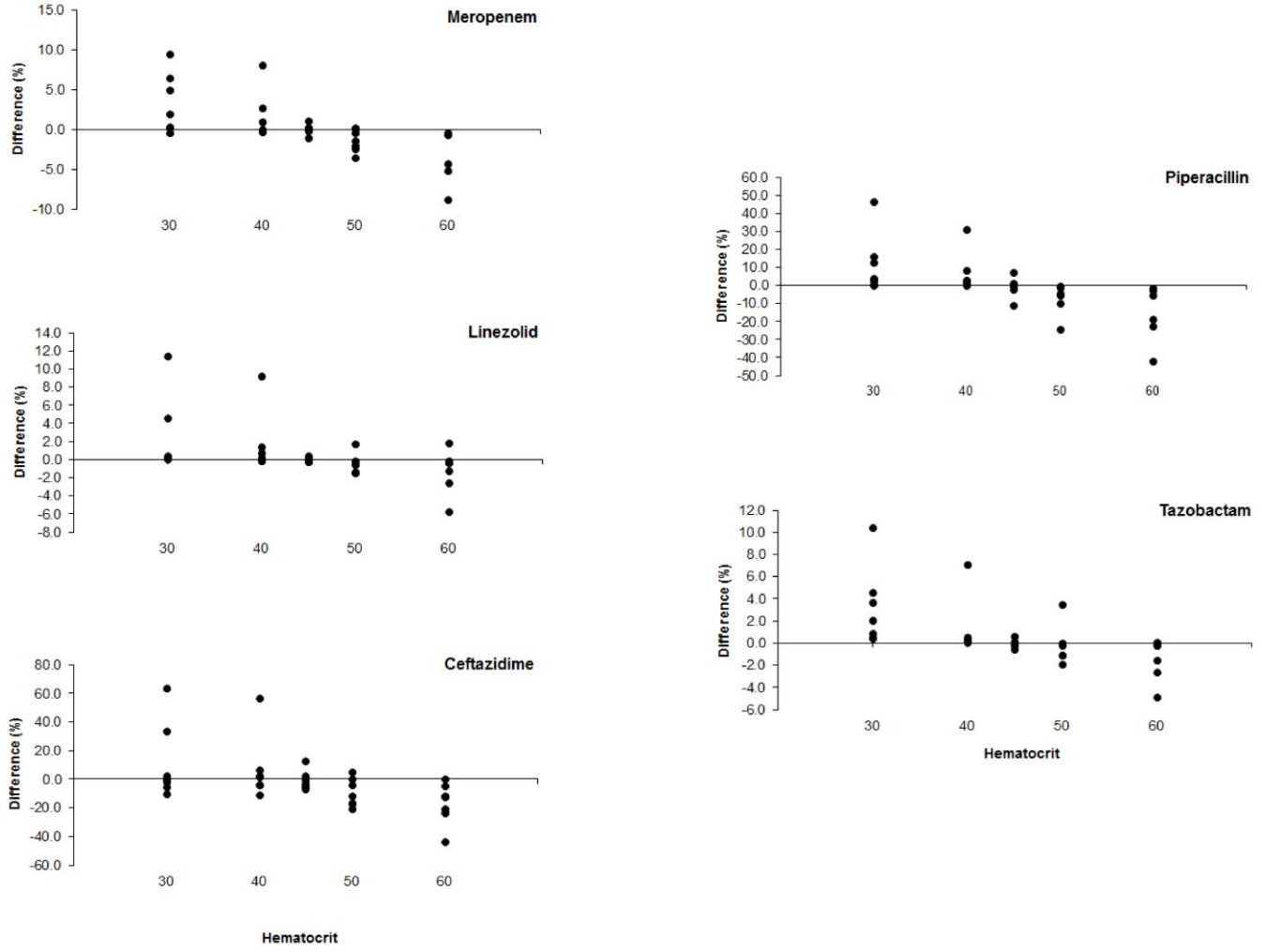
Drug	QC	VAMS				DBS			
		Intra-assay		Inter-assay		Intra-assay		Inter-assay	
		CV (%)	E (%)	CV (%)	E (%)	CV (%)	E (%)	CV (%)	E (%)
Piperacillin	LLOQ	5.3	97.9	4.2	106.0	4.2	109.5	5.6	111.1
	QC_{low}	2.9	101.7	3.2	102.8	3.3	105.1	3.4	109.5
	QC_{medium}	8.6	95.4	13.6	94.9	3.2	101.4	9.6	104.5
	QC_{high}	10.7	108.5	10.4	102.8	7.5	100.1	10.1	104.0
Tazobactam	LLOQ	4.7	117.3	7.5	115.7	12.3	111.2	4.3	98.1
	QC_{low}	6.6	92.1	8.2	103.6	12.8	110.8	5.1	106.2
	QC_{medium}	2.8	85.1	9.1	92.0	3.5	99.7	5.9	104.9
	QC_{high}	2.4	99.3	6.2	97.6	5.8	97.8	9.9	101.0
Linezolid	LLOQ	8.7	106.6	2.3	91.6	10.9	104.3	4.4	106.2
	QC_{low}	2.5	101.9	3.2	95.9	2.0	107.0	2.0	105.4
	QC_{medium}	6.1	94.1	7.7	97.0	2.7	96.2	7.0	100.1
	QC_{high}	4.9	107.3	9.5	109.3	5.1	95.0	8.2	97.8
Meropenem	LLOQ	16.7	108.9	10.6	104.2	6.7	84.0	9.0	82.7
	QC_{low}	6.7	106.4	9.1	93.5	1.3	88.4	4.5	90.9
	QC_{medium}	5.4	83.0	7.1	105.3	6.7	108.5	1.5	103.8
	QC_{high}	2.1	96.0	9.7	93.6	5.2	110.1	3.8	107.6
Ceftazidime	LLOQ	3.5	113.7	2.1	115.7	5.9	115.5	2.3	110.9
	QC_{low}	4.4	91.0	0.6	95.9	5.5	112.7	2.3	110.1
	QC_{medium}	4.8	78.6	5.3	95.2	1.5	99.5	9.9	103.7
	QC_{high}	3.4	96.1	3.2	97.8	7.1	96.9	3.3	99.5

Influence of HCT on accuracy

Table 3. Additional sets of calibrators at different HCT % (30, 40, 50 and 60) analyzed by using a calibration curve obtained at HCT 45 %. The percentage difference between nominal and calculated values of calibrators are shown.

Drug	VAMS					DBS				
	30	40	45	50	60	30	40	45	50	60
Piperacillin	5.3	2.4	0.7	5.6	9.8	0.9	5.9	7.7	22.5	54.5
	-6.2	-0.8	3.0	3.0	7.1	-14.8	-12.5	5.2	9.1	33.9
	0.3	0.0	-6.2	4.7	0.4	-20.9	-15.9	-6.8	13.6	22.6
	-1.8	4.0	-6.0	0.2	-2.0	-18.1	-7.4	-10.5	23.2	20.1
	4.3	2.0	-3.3	0.6	-2.4	-21.7	-14.2	0.9	20.1	34.8
	-2.3	3.8	-6.5	-10.4	-6.6	-18.2	0.8	4.4	14.1	35.4
	0.5	-0.7	2.8	2.9	0.5	-22.8	-16.3	-1.0	5.1	12.0
Tazobactam	-5.6	8.8	-0.8	-4.0	10.4	-11.5	0.4	8.7	27.1	50.9
	-2.4	-0.5	-9.5	11.0	10.9	-16.5	-25.2	7.4	9.9	28.3
	8.2	-15.5	-11.4	-6.3	-6.7	-27.2	-18.3	-5.4	-7.0	1.3
	10.5	-13.4	-10.5	-5.4	-9.2	-30.0	-16.6	-11.6	15.7	22.0
	11.9	-7.2	1.8	1.7	-3.0	-24.7	-12.5	-4.8	12.8	23.5
	4.9	1.0	4.2	-2.4	1.1	-17.9	-1.0	6.7	7.3	25.6
	-0.4	3.6	-0.8	4.4	4.9	-26.4	-14.2	-1.0	-4.3	4.8
Linezolid	12.0	10.3	8.9	5.6	10.2	-14.2	-2.0	0.5	21.4	32.1
	5.1	7.5	-4.3	7.2	7.2	-7.2	-10.1	0.3	15.9	32.8
	-6.4	-3.8	-2.8	1.5	-5.8	-9.5	-5.0	2.6	18.6	9.1
	-5.4	-3.9	-4.4	0.4	-2.6	-6.4	-1.2	-3.2	28.7	22.2
	-6.3	-0.4	2.0	2.7	-3.9	-10.8	-7.8	-1.8	16.2	22.2
	10.9	3.0	0.8	-2.8	-5.7	-12.1	-3.8	1.9	4.8	23.0
	3.3	3.0	-0.2	1.3	-1.2	-25.2	-20.1	-0.3	-3.1	-5.9
Meropenem	-4.0	-5.6	12.0	-0.8	-8.7	108.9	84.9	14.3	52.6	138.2
	8.0	-1.9	-10.3	1.1	-11.5	11.1	4.4	9.2	-6.2	26.3
	11.9	-2.4	-8.0	-7.0	4.0	6.7	-16.6	-11.9	-15.4	0.9
	-3.0	-8.8	-9.3	-1.1	-12.6	-18.2	-12.0	-13.7	39.4	-4.4
	10.0	-4.4	-1.9	-8.2	-12.4	-20.9	-13.7	-12.6	27.3	30.1
	13.0	7.1	5.5	-0.5	-8.1	-19.2	-6.6	11.0	11.9	36.0
	2.5	4.4	-0.8	-0.6	-4.8	-21.1	-15.8	-1.4	3.2	8.3
Ceftazidime	-0.2	12.6	10.4	1.4	8.2	-51.9	-17.4	11.5	25.9	42.4
	-10.4	4.8	-4.2	-2.4	1.5	-15.1	-36.6	9.2	11.8	70.5
	-4.0	-12.0	1.6	-0.8	-7.2	-14.0	-11.0	14.8	74.6	67.4
	-16.0	-4.0	4.0	-5.6	-4.0	-15.7	-20.3	8.0	60.6	59.4
	-6.6	-8.2	-6.2	-4.5	-9.9	-5.2	-0.2	3.2	30.3	37.5
	1.5	5.4	9.3	-3.9	-8.9	-17.4	-1.2	-3.5	12.7	34.7

Percentage difference between the concentrations obtained by analyzing calibrators in DBS and VAMS at HCT 30, 40, 45, 50 and 60 and calculated by using calibration curves at HCT45%.



Analysis of clinical samples

Table 4. Results obtained analysing clinical samples.

Sample	VAMS	DBS	Plasma	HCT	Drug
1	37.7	43.3	44.6	32.4	Ceftazidime
2	5.2; 4.8	7.2; 5.9	8.3; 2.2	39	Meropenem ; Linezolid
3	57.3	64.1	81.1	28.9	Ceftazidime
4	30.5	39.4	42.5	26.4	Ceftazidime
5	9.9	14.6	12.5	41	Linezolid
6	8.3	11.9	8.7	31.2	Linezolid
7	2.4	3.2	2.5	31.2	Linezolid
8	<0.6	<0.6	<0.6	36.9	Linezolid
9	102.4; 17.0	143.8; 23.7	190.0; 25.7	31.5	Piperacillin; Tazobactam
10	<0.6	<0.6	<0.6	39.8	Meropenem
11	0.8	1.5	1.9	38.4	Meropenem

Points of strenght

- ✓ Robustness: the method is robust for the quantification of antibiotics from 10 µL blood
- ✓ No influence of HCT on accuracy, recovery and matrix effect
- ✓ Unique analytical procedure for the measurement of four antibiotics
  (advantage for routine analysis in terms of TAT when different samples for different patients and drugs must be test, or in the case that some of these drugs are co-administered)
- ✓ Applicable to clinical samples
- ✓ Short term (72 hours) stability of the 5 drugs in VAMS at +4° C: management and shipment without dried ice (cost savings!).
- ✓ Short term (72 hours) stability of 3 drugs (excepted ceftazidime and piperacillin) in VAMS at RT
- ✓ Long term (1 month) stability of the 5 drugs in VAMS at -20° C

Pifalls

- ✓ Short term (72 hours) stability: not all antibiotics in VAMS are stable at RT! (delivery and storage at RT are not possible for piperacillin and ceftazidime!)
- ✓ Long term (one month) stability: only at -20 ° C…
- ✓ Very few clinical samples were analyzed
- ✓ No “real life” samples directly from heel or finger were obtained and analyzed

Conclusions

The applicability to a routine TDM setting requires further steps!

1) Whole blood vs plasma: It is necessary to interpret the drug concentration converting the information obtained in whole blood to plasma matrix on which the majority of reference ranges are established (so-called "HCT effect"?)

2) Capillary vs venous: A comprehensive clinical validation with analysis of paired clinical samples in VAMS, venous blood and plasma samples is necessary to assess any difference between capillary blood from finger or heel pricks and venous blood.

Nevertheless

✓ It is important to have fully validated methods for several molecules on alternative matrices

✓ The VAMS-LC-MS/MS method that we describe could represent a useful alternative sampling strategy to be used in neonatal intensive care units where samples could be more easily obtained from heel pricks and easily transported from remote sites to tertiary care centers able to perform analyses by LC-MS/MS.

Thanks to…

Dr. Sebastiano Barco for his precious support

Lab technicians in particular: Laura Barbagallo, Paola Bonifazio, Daniela Bugnone, Angelo Maffia and Marina Talio for their qualified expertise

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