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# Therapeutic Drug Monitoring

## news from the laboratory

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- 1. Introduction**
- 2. Analytical methods**
- 3. Testing on dry blood spots**
- 4. Measuring unbound vancomycin concentrations**
- 5. Antibiotic treatment in the era of personalized medicine**

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- a multi-disciplinary effort to individualize/optimize drug treatment (maximize efficacy/minimize side effects)
- Requires excellent communication with prescribers
- Requires competencies in pharmacology/toxicology
  - Pharmacokinetics
  - Pharmacodynamics
  - Pharmacogenetics
- Requires competencies in drug analysis
  - Preanalytical phase
  - Analytical phase (immunoassays, chromatographic methods, mass spectrometry, PCR, sequencing)

# How individualize drug treatment?

## *Example of immunosuppressive drugs*

### Adverse events

Nephro- , neurotoxicity  
Hypercholesterolemia  
Overimmunosuppression

### Treatment efficacy

Acute rejection  
Chronic rejection  
Tolerance

### Pharmacokinetics

Drug exposure  
Drug interactions  
Distribution  
Metabolism  
Elimination  
Pharmacogenetics  
(CYP3A5, P-gp,...),...



### Pharmacodynamics

Action on receptors  
IL2  
Lymphocytes CD+4  
Cylex assay  
Pharmacogenetics,  
Proteomic, metabolomics..

### Methods

Immunoassays  
LC-MSMS, RT-PCR,...  
Analytical performances (specificity, sensitivity,...)  
Dry spot analysis,...



- Narrow therapeutic index
- Serious consequences for under- or over-dosing
- Defined therapeutic range and toxic threshold
- Good relationship between [blood] and clinical/toxic effect
- Poor relationship between drug dose and [blood]
- Significant inter-individual variation
- Significant intra-individual variation (e.g. drug-drug interactions)
- Knowledge of the drug level influences management

## Common factors associated with altered pharmacokinetics of antibiotics in critically ill patients

### Increased $V_d$

Hypoalbuminaemia,  
leading to increased  
unbound drug  
Capillary leakage  
Fluid resuscitation

### Decreased CI

Renal hypoperfusion  
Acute kidney injury  
Renal/hepatic  
dysfunction

### Increased CI

Augmented renal  
clearance

### Variable changes in $V_d$ and/or CI

Extracorporeal  
interventions (e.g.  
RRT, ECMO)

$V_d$ : volume of distribution, CI: clearance, RRT: renal replacement therapy, ECMO: extracorporeal membrane oxygenation



- Most antibiotics ( $\beta$ -lactams, macrolides, quinolones) have a wide therapeutic index and do not require monitoring to avoid toxicity. In specific patient populations such as critically ill patients, TDM could be utilized to optimize PK/PD.
- Aminoglycosides (gentamicin, tobramycin, amikacin) and vancomycin have a narrow therapeutic indexes and toxicity may be severe or irreversible (e.g. nephrotoxic). The benefit of TDM for these antibiotics is well-established.
- There is also interest in TDM for some antifungals (e.g. voriconazole, posaconazole).

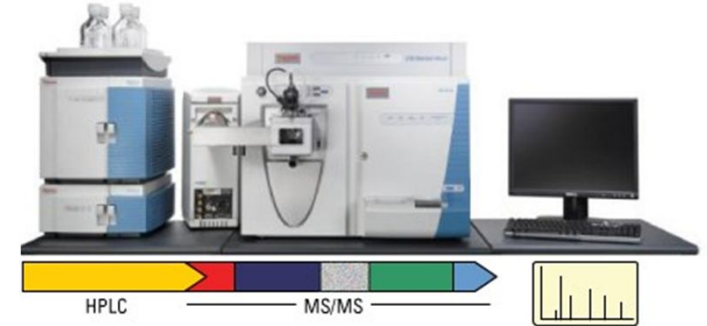


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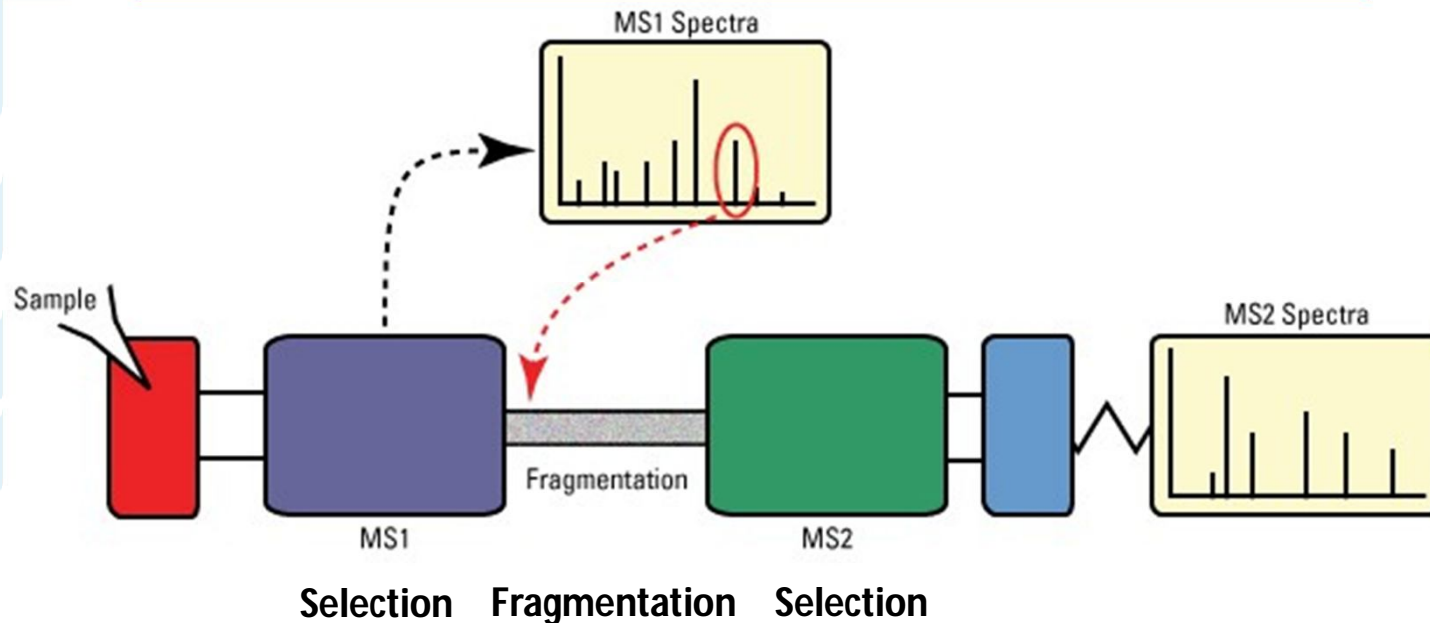
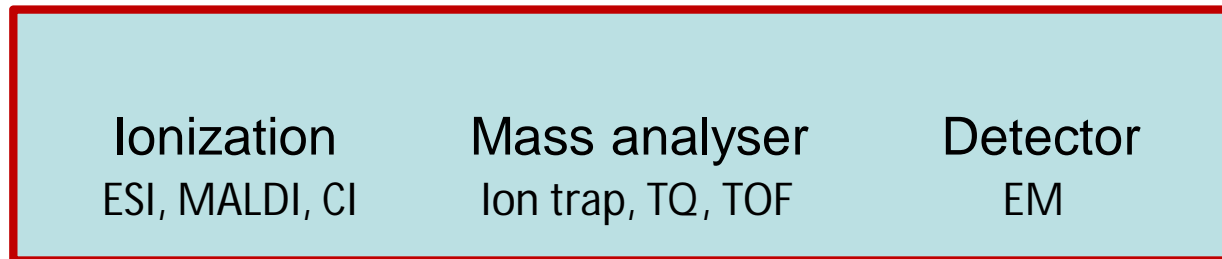
- Immunoassay (enzyme immunoassay (EIA), chemiluminiscent immunoassay (CLIA), radioimmunoassay (RIA))
- High performance liquid chromatography with UV detection (PLC-UV)
- Gas chromatography-mass spectrometry (GC-MS)
- Liquid chromatography tandem mass spectrometry (LC-MSMS)

- LC-MSMS is considered the gold standard for TDM.
- Immunoassays for vancomycin are most widely used and remain appropriate for dialy use.
- There is no data indicating the superiority of any of the immunoassay methods over others. However, bias due to lack of between-method standardization has been reported. This is not taken into account when developing guidelines or performing meta-analyses.
- Immunoassays are more prone to interference than LC-MSMS. Interference by cross-reacting substances such as vancomycin degradation products have been described for some immunoassays.

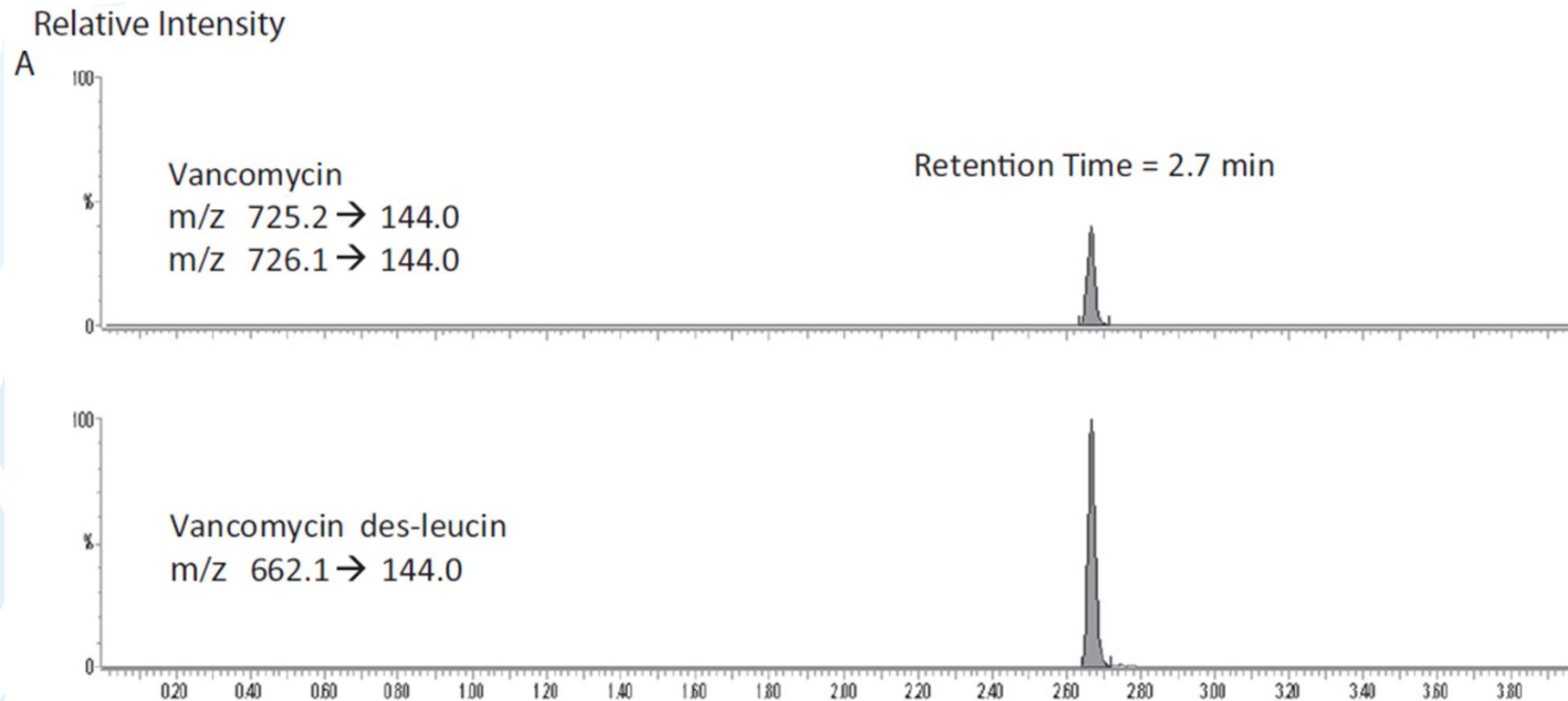
## Mass spectrometer



Separation



- LC-MSMS offers the advantage higher specificity compared to immunoassays.
- The use of a structurally closely related standard is preferred. Older methods use IS that are not closely structurally related such as teicoplanin, atenolol or kanamycin-B.



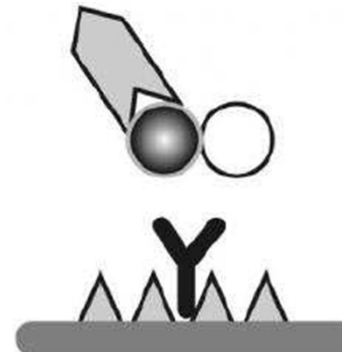
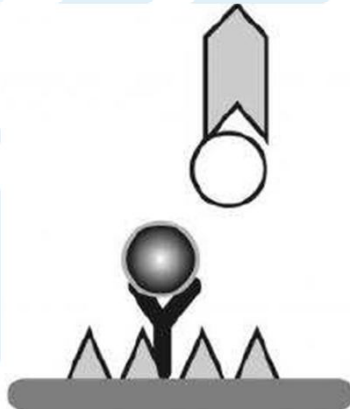
No interference



Heterophilic antibody  
(positive interference)



Cross-reactivity of an interfering substance with the capture or detector antibody (negative interference)



blocking protein  
for solid phase



analyte



interfering/cross  
reacting substance



capture antibody



heterophilic antibody



labeled detector  
antibody

## Case report

An 82-year-old patient, with Waldenström's disease, was admitted to the hospital with MRSA sepsis, treated with vancomycin. The TDM result the next day gave an extremely high result (>100 mg/L) on Architect i2000SR.

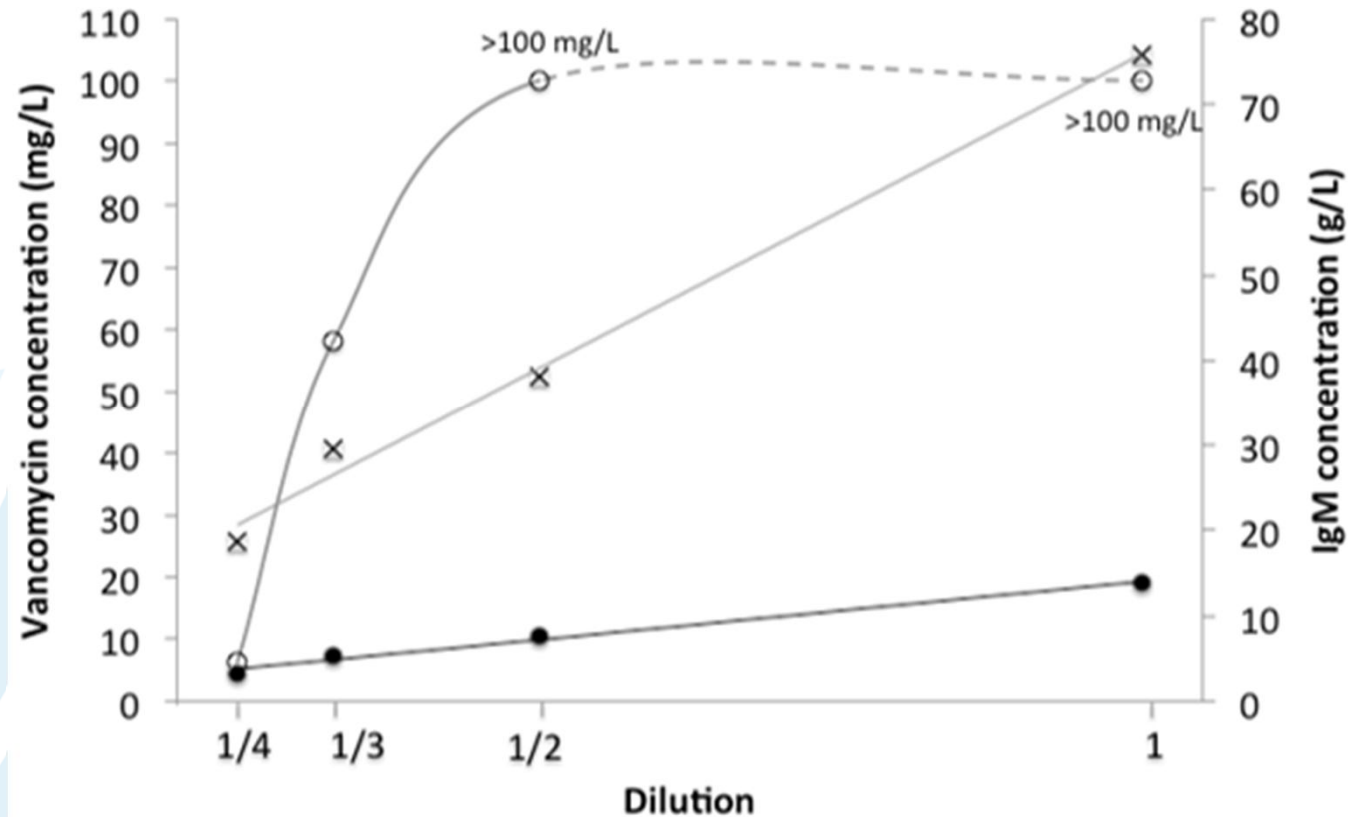
Day	<u>Total vancomycin (mg/L)</u>			<u>Unbound vancomycin (mg/L)**</u>	<u>Estimated total Vancomycin (mg/L) ***</u>	IgM (g/L)
	<u>LC-MSMS</u>	<u>Cobas c502</u>	<u>Architect i2000SR</u>			
0*	0.3	<1.7	70.3	<1.7	ND	76.7
1	19.5	18.6	>100	14.8	20.0	78.6
2	26.3	24.6	>100	18.5	25.3	73.9
3	28.2	27.2	>100	19.0	26.1	75.9

ND: not determined; \*: before vancomycin administration; \*\*: measured on Architect i2000SR; \*\*\*:

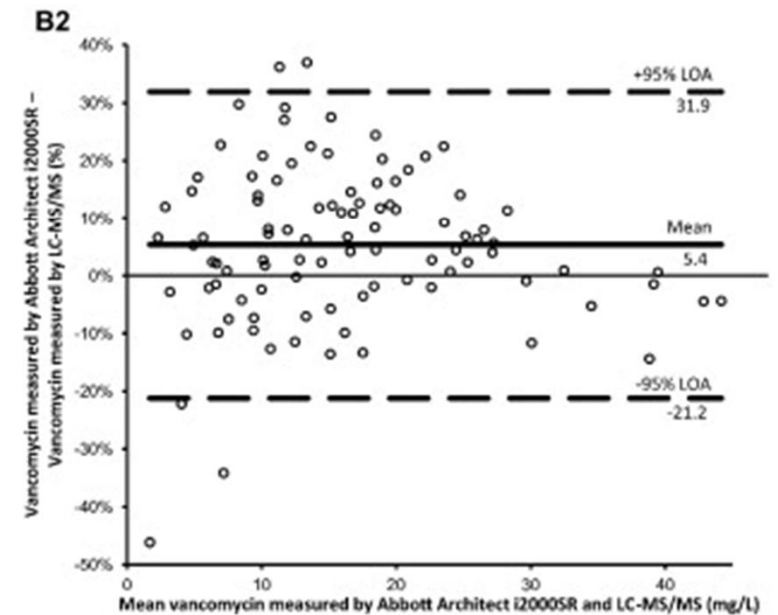
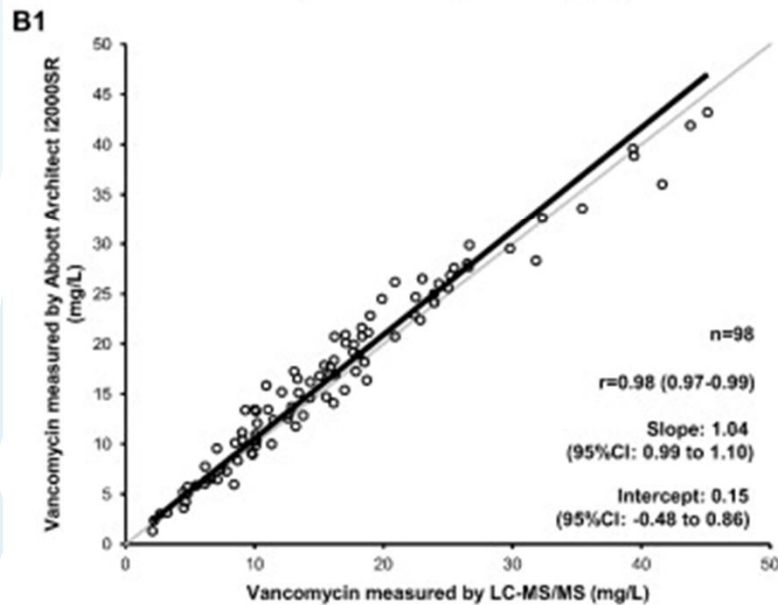
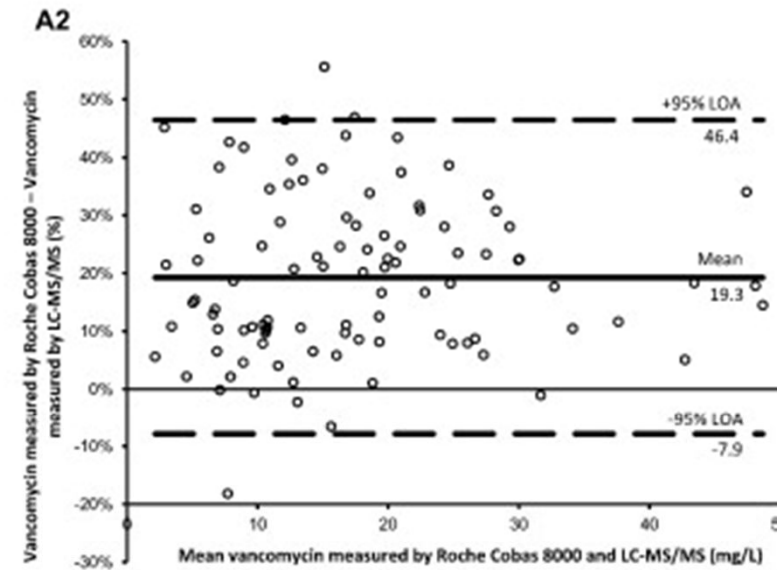
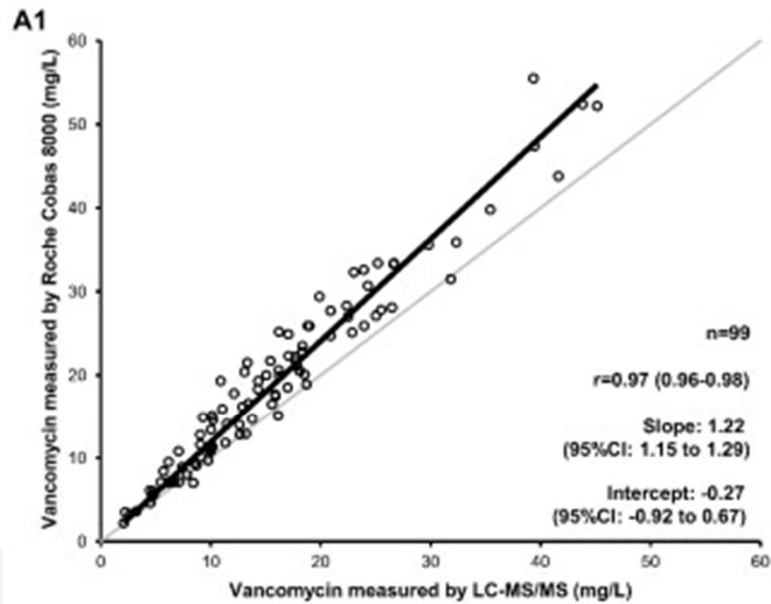
Formula: total vancomycin conc = (unbound vancomycin conc - 0.84) / 0.697

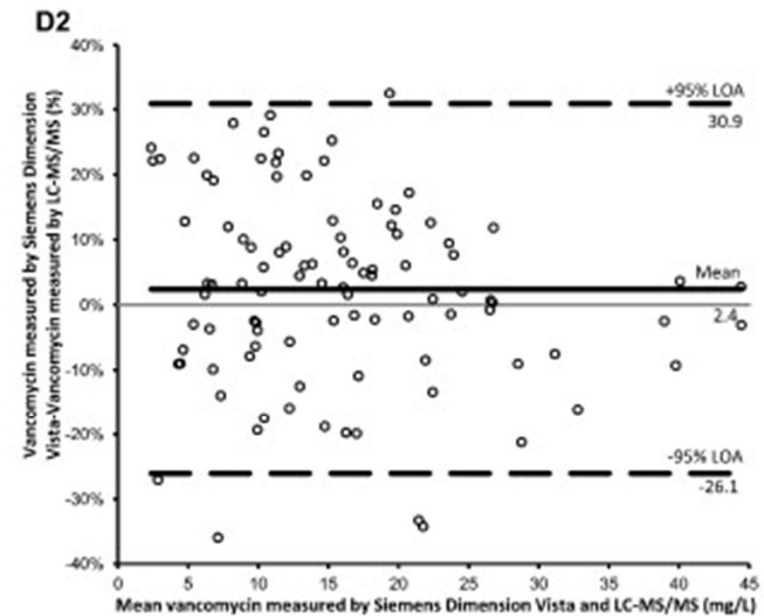
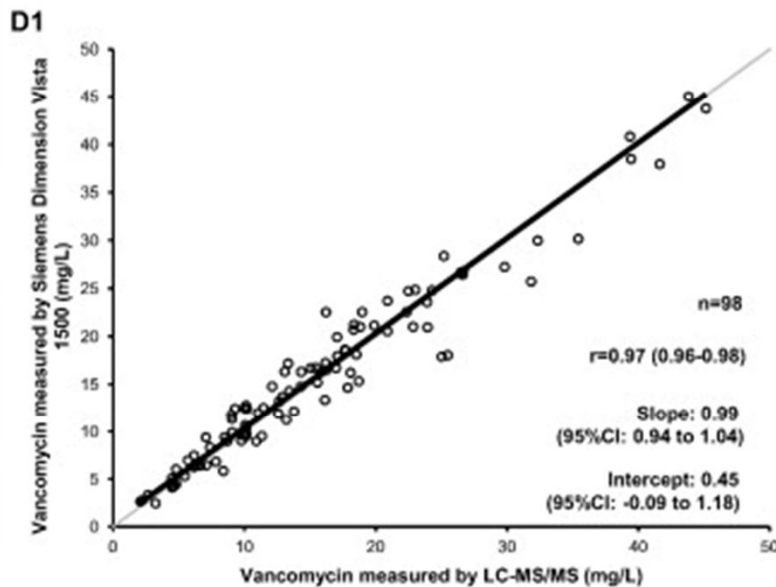
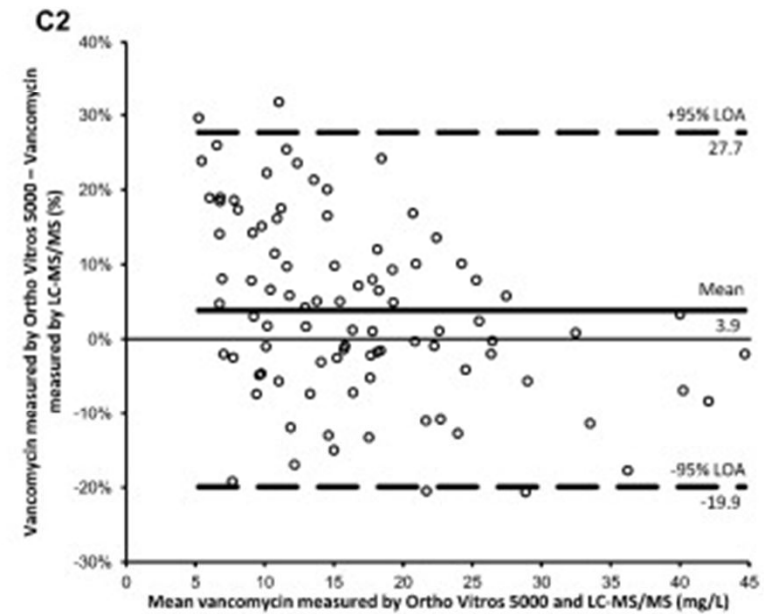
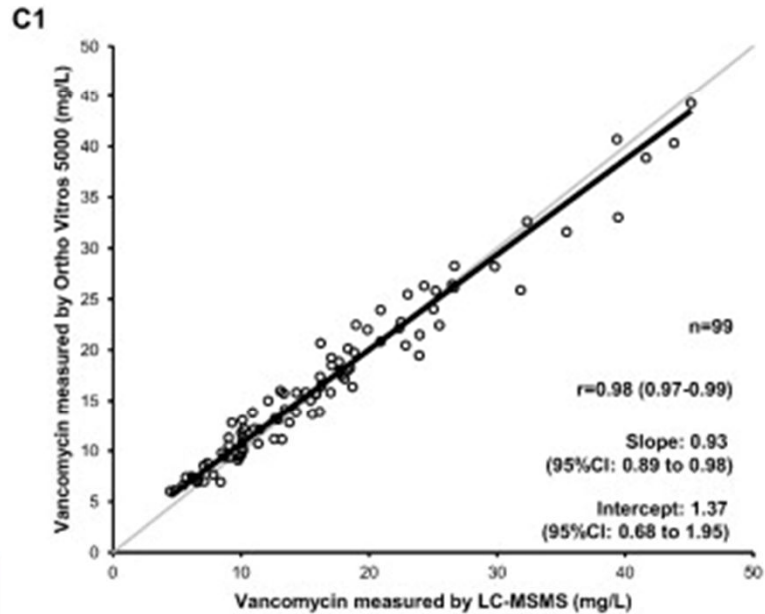


## Dilution experiment



Total (○) and unbound (●) Vancomycin concentrations in function of the respective dilution. A dilution of 1 is corresponding with undiluted serum.





## Clinical interpretation of the obtained concentrations with the different assays compared to LC–MS/MS

**A**

Discordance 22.2%		Roche Cobas 8000		
		<15 mg/L	15-25 mg/L	>25 mg/L
LC-MS/MS	<15 mg/L	43	10	0
	15-25 mg/L	0	20	12
	>25 mg/L	0	0	14

**B**

Discordance 12.2%		Abbott Architect i2000SR		
		<15 mg/L	15-25 mg/L	>25 mg/L
LC-MS/MS	<15 mg/L	45	7	0
	15-25 mg/L	2	27	3
	>25 mg/L	0	0	14

**C**

Discordance 8.1%		Ortho Vitros 5000		
		<15 mg/L	15-25 mg/L	>25 mg/L
LC-MS/MS	<15 mg/L	50	3	0
	15-25 mg/L	2	28	2
	>25 mg/L	0	1	13

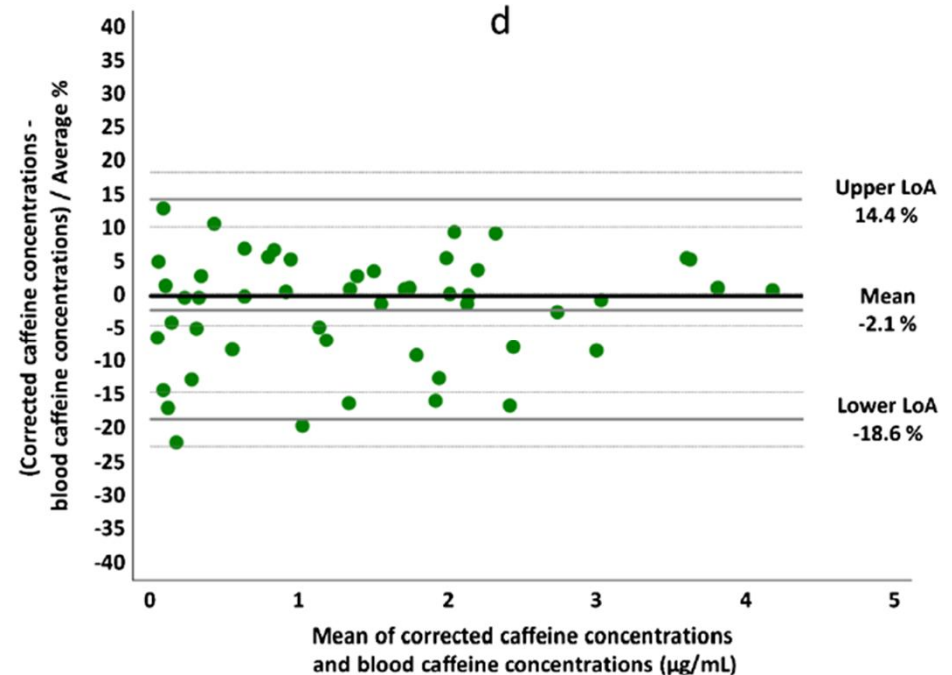
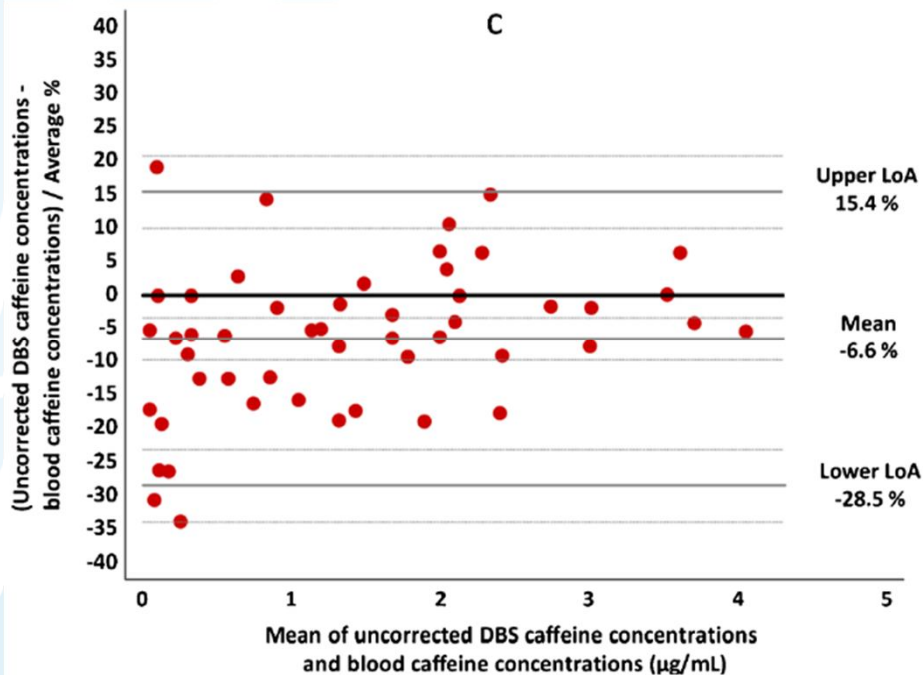
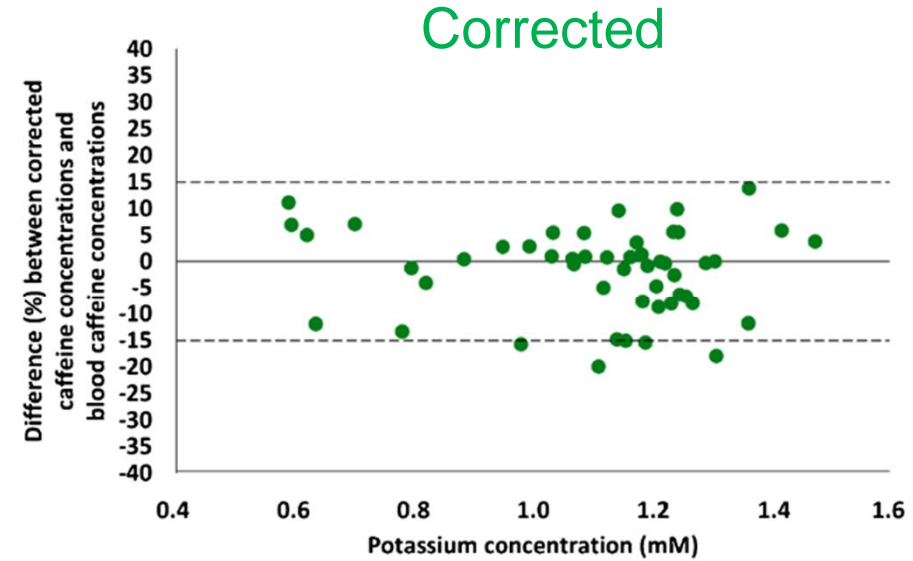
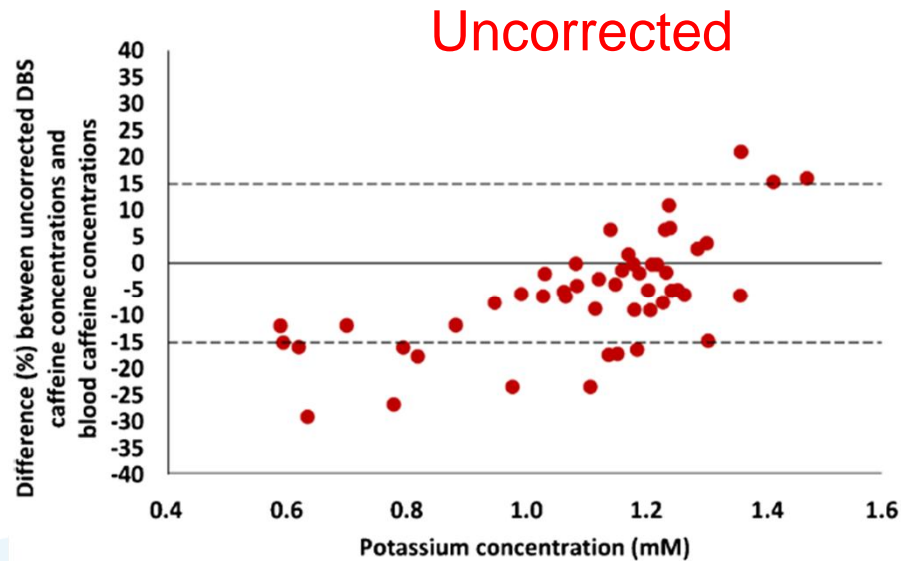
**D**

Discordance 6.1%		Siemens Dimension Vista 1500		
		<15 mg/L	15-25 mg/L	>25 mg/L
LC-MS/MS	<15 mg/L	50	3	0
	15-25 mg/L	2	30	2
	>25 mg/L	0	1	13

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- The potential of performing tests on capillary blood collected via dried blood spots (DBS) as an alternative for classical venous blood sampling was described more than a century ago by Ivar Bang.
- DBS sampling has been used for almost 50 years for neonatal screening, but is only recently investigated for TDM and toxicology
- The advantages of DBS are low blood volume requirement, transportation and storage without special treatment, good analyte stability and enhanced clinical cooperation
- DBS are currently used for the monitoring of immunosuppressants in clinical trials. There is evidence for the use of DBS for TDM for gentamicin, linezolid, voriconazole and posaconazole.
- The impact of hematocrit on DBS results is an important factor limiting the breakthrough of DBS for routine bioanalysis. Measuring potassium in DBS can help correct for this problem.



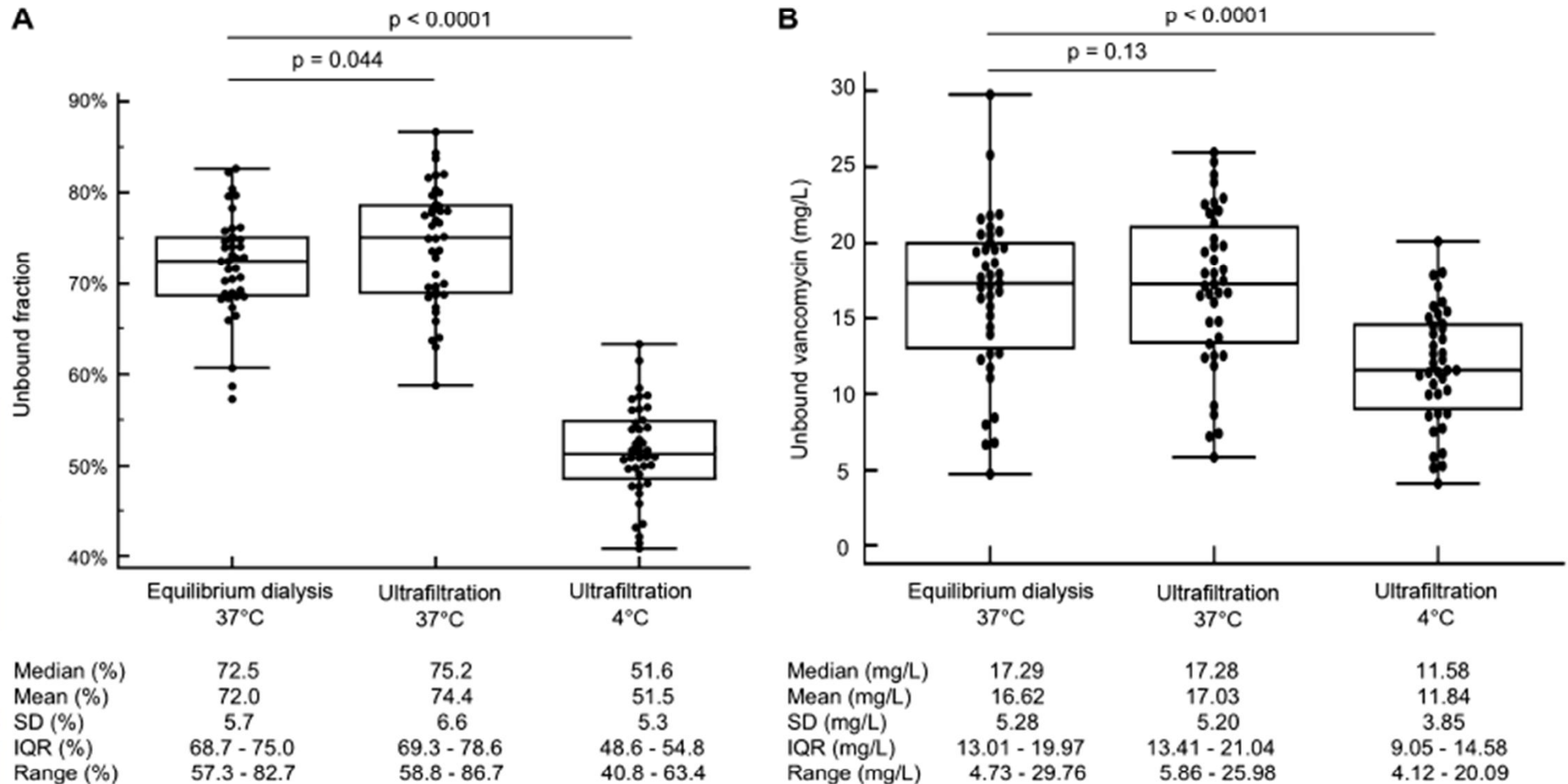




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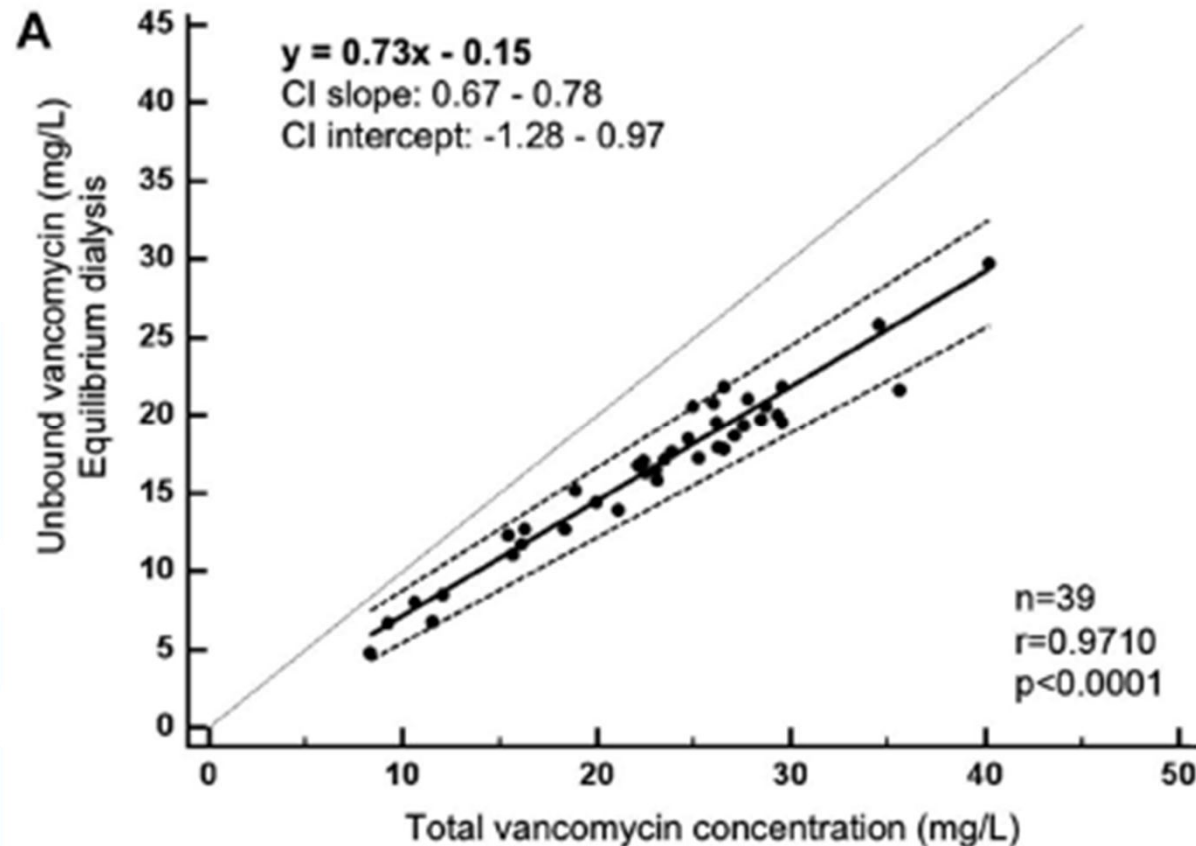


- Vancomycin is generally considered as a moderately protein bound antibiotic (30-60%), with albumin being an important binding protein.
- A estimated protein binding of 50% is often used to calculate unbound vancomycin concentrations. However, protein binding of vancomycin shows considerable variability across studies (ranging from almost 0 up to 90%).
- Great controversy also exists about the correlation between unbound and total vancomycin concentrations.
- The highly variable results reported by studies measuring unbound vancomycin are at least partially explained by differences in the procedure used to isolate unbound vancomycin in serum/plasma.



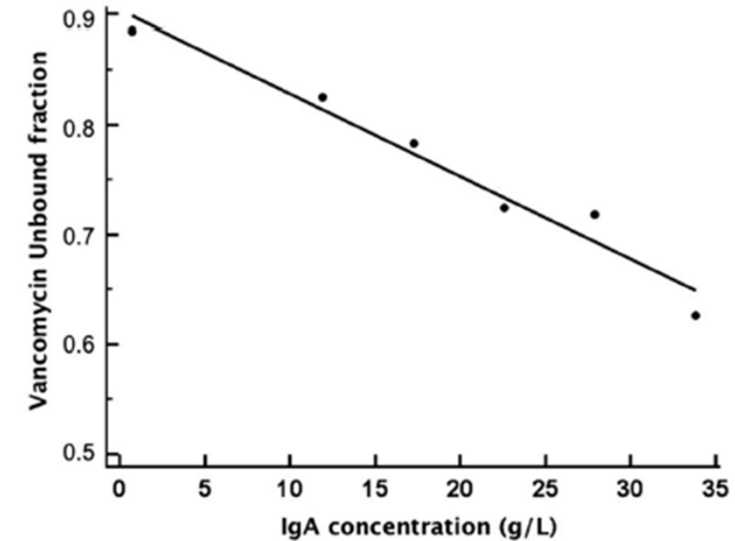
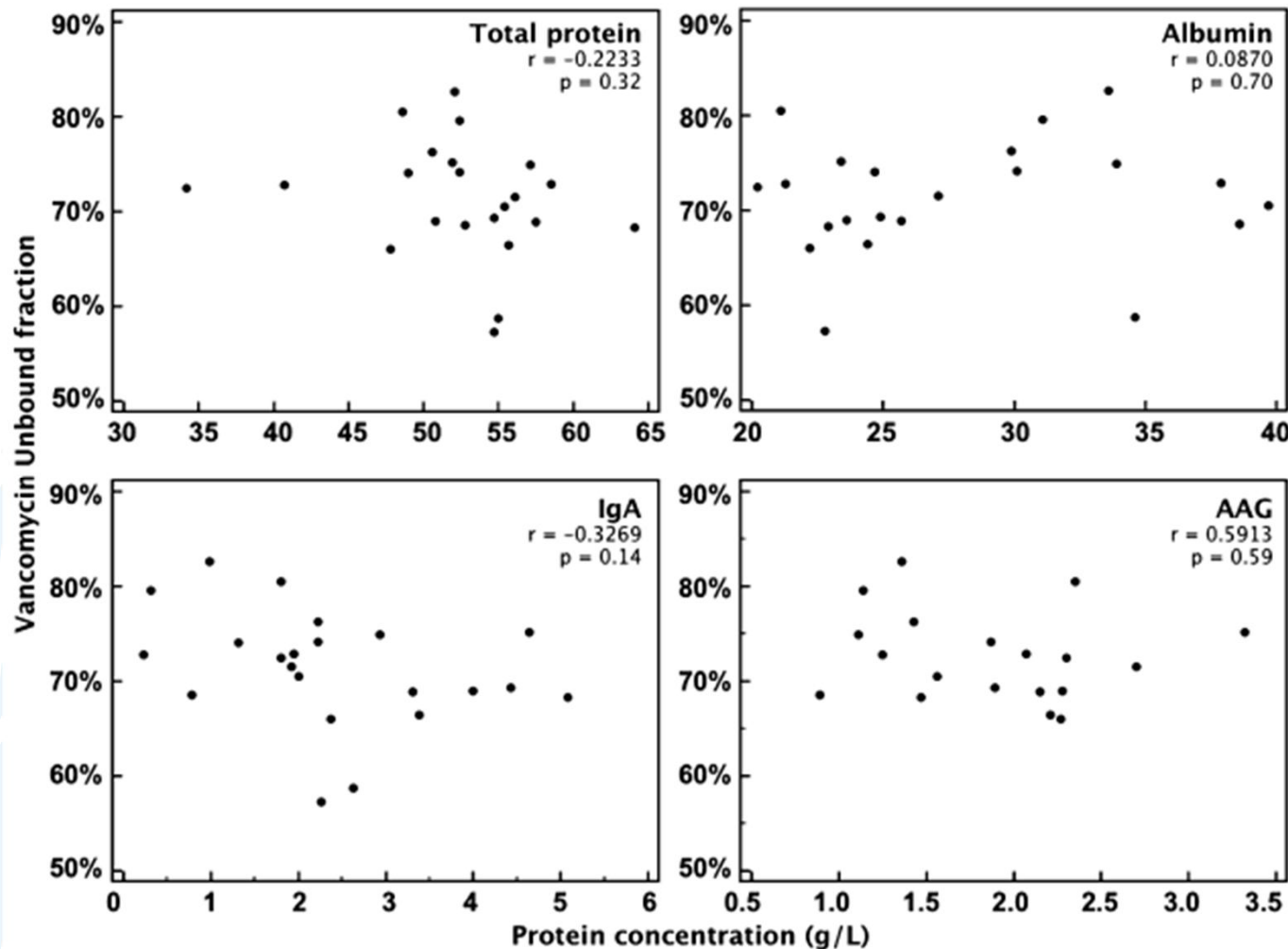
**FIGURE 1.** Unbound vancomycin fraction (A) and concentration (B) obtained after ED (37°C) and UF (4°C and 37°C) of 39 serum/plasma patient samples. The middle line represents the median, and the central box represents the values from the lower to upper quartile (25–75 percentile). The vertical line of the whiskers extends from the minimum to the maximum value, excluding outside and far outside values, which are displayed as separate points.

The study found good correlation between unbound and total vancomycin concentration

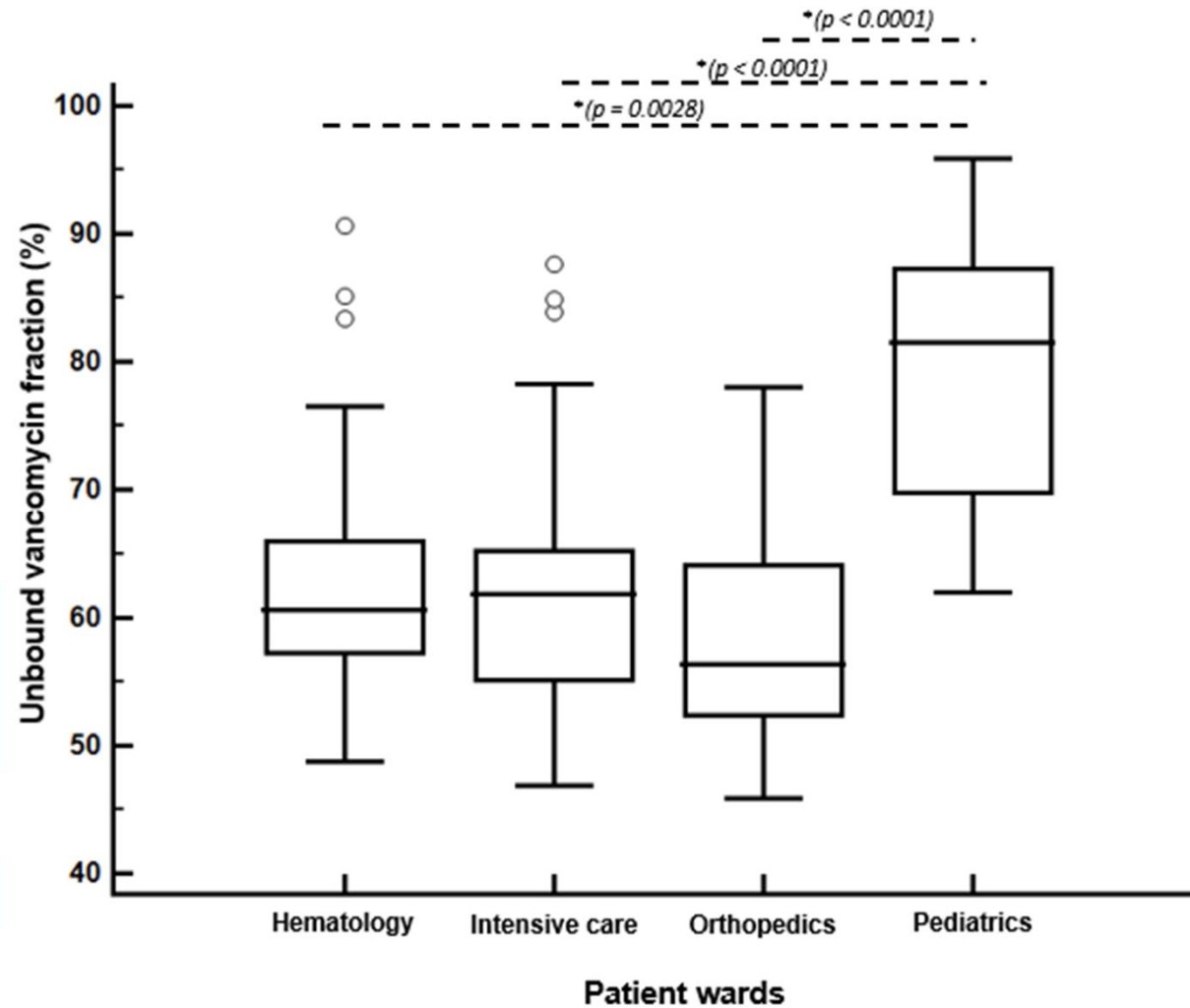


$$[\text{unbound vancomycin (UF, 37}^\circ\text{C)}] = 0.697 \cdot [\text{total vancomycin}] + 0.840$$

Including albumin, immunoglobulin A (IgA) or alpha-1-acid glycoprotein (AAG) dit not improve the model



## An evaluation of unbound vancomycin concentrations in UZ Leuven



- In UZ Leuven, we found lower unbound vancomycin concentrations 63% vs. 74% than the study by V. Stove et al.. Along with analytical issues (immunoassay vs. LC-MSMS), this could be related to the fact that in Gent most patients received continuous infusion while most patients in UZ Leuven receive intermittent dosing except for the ICU.
- Our study confirmed the negative correlation between serum IgA and unbound vancomycin concentration.
- Total vancomycin was the strongest predictor of unbound vancomycin in adults, but albumin and serum IgA were also identified as significant predictors.
- Our study was the first to study pediatric patients. Although the group was small (n=11), a significant difference was observed. This was not surprising as infants have lower IgA.



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## Personalized Medicine = Tailored Medicine

- Medical decisions and treatment strategies are tailored to the individual patient
- The term was first coined in the context of genetics, but applies to any strategy that tailors treatment to the disease AND the patient
- “The **right medicine** to the **right patient** at the **right dose** at the **right time**”

Theranostics, a portmanteau of diagnosis and treatment, aims to improve individual patient outcome via:

1. **Early and specific diagnosis**
2. **Predict disease response to treatment (prognosis, efficacy)**
3. **Predict patient response to treatment (PK/PD, safety)**
4. **Monitor effect of treatment**

## Patient Response to treatment (PK/PD, safety)

- Adverse drug reactions are a major problem with traditional drugs (US: est. 3,5 billion \$)
- PK/PD, safety and side effects are an important part of ongoing clinical research
- “Pharmacogenomics” is hot (FDA recommendations Jan 2013)
- After approval, however, companies are often not so interested in optimizing drug dosing

Who wants to sell a drug that requires pharmacogenomics or TDM?

- Despite our rapidly expanding knowledge of pharmaco-genomics, the clinical applicability is still limited to a few medications such as cyclosporine and tacrolimus.
- The clinical utility is, however, likely to change in the near future.
- Currently there are no genotype-based dosing guidelines for vancomycin.
- In 2012, researchers from Vanderbilt University in Nashville performed a genome wide association which found 2 novel associations of loci (at 1q41 and 5q14.3), possibly identifying mechanisms underlying vancomycin pharmacokinetic variability (*S. L. Van Driest et al., presented at ASHG 2012*).

- The interest in therapeutic drug monitoring for antimicrobial agents to individualize/optimize drug treatment is increasing
- Immunoassays which are traditionally used for TDM are more prone to interference than LC-MSMS
- TDM on dry blood spots is being investigated as an alternative
- The clinical utility of measuring unbound vancomycin is being investigated
- The clinical utility of pharmacogenomics for antimicrobial agents could increase in the coming years